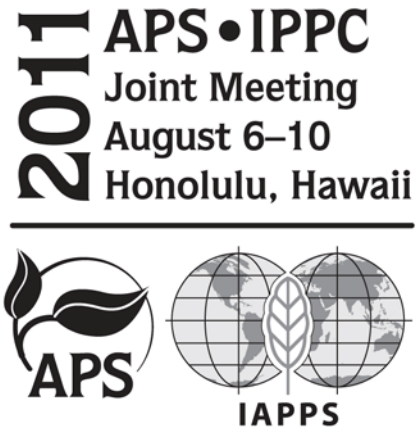

2011 APS-IPPC Joint Meeting
Abstracts of Presentations

Abstracts submitted for presentation at the APS-IPPC 2011 Joint Meeting in Honolulu, Hawaii, August 6–10, 2011 (including abstracts submitted for presentation at the 2011 APS Pacific Division Meeting). The abstracts are arranged alphabetically by the first author's name.

Prioritizing cover crops for improving root health and yield of vegetables in the Northeast

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Phytopathology 101:S1

Cover crops are used increasingly by growers to improve soil quality, prevent erosion, increase organic matter, and suppress root pathogens and pests. However, limited information is available on their use for suppressing pathogens (Rhizoctonia, Pythium, Fusarium, Thieloviopsis, Pratylenchus, and Meloidogyne) of vegetables grown in the Northeast. Thus, a collaborative project was initiated in 2009 to assess the efficacy of selected cover crops in suppressing root pathogens of vegetables and improving soil health in research and on-farm field trials in New York, Pennsylvania and Connecticut. In NY, strips (4.5 X 60 M) of 9 cover crops (rye grain + hairy vetch, oat, sudex, forage radish, red clover, rapeseed, buchwheat, wheat, and a fallow check) were randomized in 4 fields with 3 replications (3.2 ha total). The fields had different management histories resulting in varied levels of pathogen pressure and soil quality. In 2010, cover crop biomass was measured and collected soil samples were assessed for root health (greenhouse bean bioassay), nematode diversity and density, and selected soil health parameters (Cornell Soil Health Test). In general, root rot severity was lowest and yield of snap bean was highest in the field with the highest soil quality. After one year, the cover crops greatly affected root health and bean yield in this trial as well as the microplots and/or on-farm trials conducted in CT and PA. Another cycle of evaluations is in progress.

Reduction of aflatoxins, cyclopiazonic acid and fumonisins in corn by biocontrol strains of non-aflatoxigenic *Aspergillus flavus*

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Phytopathology 101:S1

Non-aflatoxigenic biocontrol strains of Aspergillus flavus were examined for ability to reduce, production in corn of aflatoxins and cyclopiazonic acid (CPA) by A. flavus and fumonisins (FBs) by Fusarium verticillioides. The ability of non-aflatoxigenic strains to prevent aflatoxin production by subsequent challenge with toxigenic A. flavus strains was assessed in 4 experiments. Non-aflatoxigenic strain K49 effectively prevented toxin production at various inoculation levels in 3 experiments. K49 also was evaluated alongside the widely used biocontrol strains NRRL 21882 (Afla-Guard®) and AF36 for prevention of aflatoxin and CPA production by strains K54 and F3W4. K49 and NRRL 21882 were superior to AF36 in reducing aflatoxins. K49 and NRRL 21882 produced no CPA, and reduced CPA and aflatoxin production in a subsequent challenge with F3W4 and K54 by 84–97% and 83–98%, respectively. In contrast, AF36 inoculation and subsequent challenge with F3W4 reduced aflatoxins by 20% and 93% with K54, but showed no CPA reduction with F3W4 and only 62% CPA reduction with K54. Because AF36 produces CPA, high CPA accumulated in corn with AF36 alone. Pin-bar wounding and pin-bar inoculation with F. verticillioides NS-2 resulted in FBs levels of 253 and 1087 ppm, respectively. Inoculation with K49 alone or a mixture of K49 and NS-2 reduced FBs level to 0.1 and 27 ppm, respectively. AF36 and NRRL 21882 showed similar FBs reduction trends to K49. NRRL 21882 and K49 are effective in reducing aflatoxins, CPA and FBs in corn.

Managing potato scab and enhancing tuber yield with low rates of fish emulsion applied as a pre-plant soil amendment

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Phytopathology 101:S1

Fish emulsion (FE) is an excellent organic soil amendment to enrich soil microbes and generate disease suppressive conditions against soil-borne diseases such as seedling damping-off, potato scab, and verticillium wilt. However, the rates (20,000 L/ha) of FE that provided effective control of potato scab can be too costly for commercial use. The aim of this 3-year field study was to see if much lower rates of FE could suppress potato scab and increase tuber yield. Diluted FE (1000 and 2000 L/ha or 0.05 and 0.1%) was applied to the field plots twice a year before planting and after harvesting potatoes starting in fall of 2007. The high rate of FE (2000 L/ha) consistently reduced scab severity by 42% in 2008, 57% in 2009, and 44% in 2010; reduced the percentage of tubers with deep-pitted scab by 30% in 2008, 51% in 2009, and 66% in 2010; and increased the percentage of marketable tubers by 21% in 2008, 55% in 2009, and 12% in 2010. Both rates of FE increased total tuber yield by 16–19% in 2008, 14–20% in 2009, and 7–11% in 2010. FE soil amendment enhanced the numbers of soil bacteria including those of potential bio-control agents belonging to the genera *Pseudomonas* and *Bacillus*. These results suggest that economically feasible rates of FE applied more frequently can provide disease suppression and enhance tuber yield. Next step is to monitor the lasting impact of these disease suppressive conditions on continuous potatoes without any further FE application.

Effect of arbuscular mycorrhizae on aphid infestation of wheat

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Phytopathology 101:S2

In greenhouse-grown pots of wheat serendipitously infested with bird cherry oat aphid, *Rhopalosiphum padi*, the aphids preferred non-mycorrhizal treatments over treatments with arbuscular mycorrhizal (AM) fungi. Therefore, studies were developed to determine if mycorrhizal infection reduced ability of wheat to support a population of *R. padi*. Two AM fungi [*Glomus intraradices* (*Gi*), *Gigaspora margarita* (*Gm*)] were propagated on sorghum, and non-mycorrhizal sorghum served as a control. After three months, vegetative growth of sorghum was removed, and cultures, both with and without AM, were mixed 1:1 with growing medium. Wheat (*Triticum aestivum* Pioneer '26R22') seed (10/pot) were planted and allowed to grow in the greenhouse for three weeks. Twenty apterous aphids were transferred to each plant. Three pots/treatment were used in Trial 1; six pots/treatment were used in Trial 2. After 5 days, aphids were counted on each plant. Mycorrhizal treatment had no impact on germination and survival of wheat plants. Number of aphids/plant was approximately 2.5 times greater on nonmycorrhizal plants than on *Gm*-colonized plants [Trial 1 (P = 0.0912); Trial 2 (P = 0.0955)]; numbers of *R. padi* on *Gi*-colonized plants were intermediate and not different from the other treatments.

Multiplex PCR for four Sclerotinia species

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Phytopathology 101:S2

Sclerotinia homeocarpa, *S. minor*, *S. sclerotiorum*, and *S. trifoliorum* are common species within the genus *Sclerotinia*, where the morphological identification is challenging, especially when one crop hosts multiple species. The objective of this study was to design species specific primers compatible with multiplexing, for rapid and accurate species identification. Highly specific primers working under similar PCR conditions were designed for the aspartyl protease gene of *S. sclerotiorum*, the calmodulin gene of, *S. trifoliorum*, the elongation factor-1 alpha gene of *S. homeocarpa*, and the laccase 2 gene of *S. minor*. The specificity and sensitivity of primers were tested individually and in multiplex against isolates of each species and each consistently amplified DNA of their target species only. Four DNA fragments of different sizes were amplified: a 264-bp PCR product for *S. minor*, a 218-bp product for *S. homeocarpa*, a 171-bp product for *S. sclerotiorum*, and a 97-bp product for *S. trifoliorum*. Primer sets differed in their lower sensitivity limits: SMlac2 = 1pg/µL; SHelf1 = 0.1pg/µL; SSaspr, and STCad = 10pg/µL. These primer sets can be used individually for verifying the identity of isolates of a particular species or as a multiplex assay. This multiplex assay is accurate and rapid tool to differentiate between these plant pathogenic *Sclerotinia* species in a single PCR reaction.

Sensitivity of tomato early blight isolates (*Alternaria solani*) from Jordan to mancozeb, chlorothalonil and azoxystrobin fungicides

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Phytopathology 101:S2

The in vitro sensitivity of tomato early blight pathogen (*Alternaria solani*) isolates from Jordan were evaluated to the fungicides mancozeb, chlorothalonil, and azoxystrobin. Sensitivity of sixty single conidium isolates of *A. solani* to mancozeb and chlorothalonil was assessed using the inhibition of radial mycelial growth (RG) method, using fungicide concentrations of 0, 1.0, 10, 100, 500, and 1000 µg a.i. ml^{-1} medium. The sensitivity of each isolate to azoxystrobin was measured by assessing the conidial germination inhibition of each isolate on water agar plates amended with 0.0, 0.001, 0.01, 0.1, 1.0, and 10 µg a.i $a.i.ml^{-1}$ medium. The EC50 (concentration of the fungicide causing 50% relative reduction of radial mycelia growth or spore germination) values of different *A. solani* isolates to mancozeb ranged from 9.05 µg/ml to 712.65 µg/ml, and had a mean of 187.12 µg/ml. EC50 values of different isolates to chlorothalonil ranged from 4.25 µg/ml to 849.4 µg/ml and had a mean of 153.65 µg/ml. Forty three isolates, and thirty isolates of *A. solani* exhibited reduced sensitivity to mancozeb and chlorothalonil respectively, with EC50 values more than 100 µg/ml. The EC50 values of different isolates to azoxystrobin ranged from 0.040 µg/ml to 1.09 µg/ml and had a mean of 0.29 µg/ml. Forty six isolates of *A. solani* exhibited reduced sensitivity to azoxystrobin with EC50 values more than 0.1 µg/ml.

Comparison of endophytic *Undifilum* DNA and swainsonine content on locoweeds

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Phytopathology 101:S2

Locoweeds are toxic species of *Astragalus* and *Oxytropis*, associated with the endophytic fungus *Undifilum oxytropis*. The fungus produces swainsonine, an α-mannosidase-inhibiting alkaloid which causes cell damage to mammals when ingested. We used real-time PCR to quantify *Undifilum* DNA (UD) from field samples and *in vitro* grown plants. Amplification of the ribosomal ITS allowed reliable quantification of UD in plant tissues. UD in *in vitro O. sericea* increased between the first and third week of growth, whereas swainsonine concentration started increasing between the second and fourth week. A strong correlation existed between the amount of UD and the swainsonine concentration in plants grown on culture medium during the first four weeks. Addition of polyethylene glycol to the medium significantly impaired the plant development, accelerated endophyte colonization (40 pg UD/ng total DNA at Week 2), and increased the swainsonine concentration. Acidification of the medium resulted in increased plant growth and minimal swainsonine content. UD ranges were estimated in locoweed populations in New Mexico and Colorado and annual cycles were described for swainsonine content. Both UD and swainsonine content coincided at their lowest, while swainsonine concentration was variable when medium or high amounts of endophyte DNA were found. Our findings highlight the reliability of qPCR for studying endophyte colonization and show environmental cues affecting the swainsonine synthesis *in planta*.

Kasugamycin in combination with copper or mancozeb for management of walnut blight in California

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Phytopathology 101:S2

Walnut blight caused by *Xanthomonas arboricola* pv. *juglandis* (*Xaj*) is one of the most economically important diseases of walnut in California and worldwide. Copper resistance has developed in pathogen populations and is widespread throughout CA. Screening of new bactericides has led to the identification and development of kasugamycin for management of fire blight and other bacterial diseases. Baseline sensitivity studies were conducted to determine the range of toxicity to *Xaj* and to aid in monitoring antibiotic sensitivities in field populations. *Xaj* exhibited a relatively high and continuous range of sensitivities to kasugamycin (LIC 36 to 44 µg/ml, MIC 44 to 70 µg/ml) as compared to other bacterial plant pathogens such as *Erwinia* and *Pseudomonas* spp. In 2008, 2009, and 2010, kasugamycin significantly reduced blight incidence by 50% to 75% as compared to the untreated control. In combination with copper or mancozeb, however, the efficacy of the antibiotic was significantly improved and incidence was reduced by more than 85% in orchards with copper-resistant pathogen populations. In orchards with no copper resistance, the efficacy of the antibiotic was higher when used alone or in the combinations. Thus, kasugamycin in combination with copper or mancozeb and in rotation with copper-mancozeb mixtures should reduce the selection for resistance to the antibiotic and reduce the overall usage of mancozeb and copper as the only highly effective treatments available.

The filamentous phage phiRSS1 enhances virulence of *Ralstonia solanacearum*

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Phytopathology 101:S2

The RSS1 is a filamentous phage infecting *R. solanacearum*, the causative agent of bacterial wilt. This phage was frequently integrated in the host genome. This study was conducted to understand the effects of phiRSS1 infection on *R. solanacearum* cells in their interaction with host plant. Upon infection, phiRSS1 affects the physiological state, pathogenicity, and behavior of the host cells. The major changes include 3.53 fold increase of extracellular polysaccharides, increase (28.46%) of endoglucanase activity and 1.46 times faster of twitching motility compared with uninfected cells. These changes also affected the virulence and pathogenicity. Tomato plants inoculated with phiRSS1-infected bacteria killed 2 to 3 days earlier than those with uninfected bacteria. These results clearly showed that phiRSS1 infection enhances host virulence.

Foamy bark rot of Fukumoto navel: A condition with etiology not yet understood

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Phytopathology 101:S2

Fukumoto navel orange, which was introduced into California in 1983 has many desirable attributes but foamy bark rot condition is a serious concern for the variety. Symptoms include splitting of the bark of the trunk and branches that leads to the release of gum exudates and at times production of whitish foamy substance that smells like a beer brewery. Samples were collected from infected Fukumoto trees in Tulare, Fresno, and Kern counties, where the variety is commonly grown in California. Screening was done using morphology and molecular detection methods for *Phytophthora* spp., fungi, and bacteria. Some of the frequently isolated organisms include *Phytophthora citrophthora*, four bacterial genera (*Bacillus* spp., *Pantoea* sp., *Pseudomonas* spp. and *Acinetobacter*), and several fungi (*Alternaria citri*, *Botryosphaeria* spp., *Fusarium solani*, *F. oxysporum*, and *F. equiseti*). Although probing was done with *Xanthomonas* spp. - specific primers, none has been found so far. Pathogenecity tests are being conducted with Fukumoto trees propagated with disease-tested budwood onto Carrizo and Volkameriana rootstocks supplied by the California Citrus Clonal Protection Program. Preliminary data indicate that a combination of two or more of these organisms may play a role in the foamy bark rot condition.

First report of *Tomato mosaic virus* on eggplant in Iran

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Phytopathology 101:S3

During the June 2009 to August 2010, symptoms of leaves mosaic, interveinal necrosis and fruits discoloration were observed in eggplant fields in Golestan Province, Iran. A total number of 20 leaf samples collected from symptomatic eggplants and tested for the presence of Tobacco mosaic virus (TMV), Tomato mosaic virus (ToMV), Arabis mosaic virus (ArMV) and Cucumber mosaic virus (CMV) by DAS-ELISA using commercial specific antibodies (Agdia, Elkhart, IN, U.S.A.) according to the manufacturer's instructions. Among the samples, ToMV was detected in 55% of the samples. ArMV and TMV were not detected in any of the tested samples. Total RNA was extracted from each sample and analysed by RT-PCR using a pair of primers that flank the coat protein gene (CP) of Tomato mosaic virus (ToMV). The 750-bp ToMV-specific DNA fragment was amplified in all tomato samples collected from 3 fields in Karaj district and all mechanically inoculated tobacco plants. Amplicons were not produced from healthy plants or the water used as negative controls. RT-PCR products were purified and directly sequenced. BLAST analysis of ToMV (GenBank Accession No. HQ593624) sequence showed 98% nucleotide identity with reference sequences (AJ429083, AJ429086, AF012917, AF378152, AF067233, AF067231) deposited in the NCBI database. The experimental results confirm the observed symptoms were caused by ToMV in the eggplant in Gorgan region. ToMV has previously been reported from IraN, but this is the first report of ToMV in eggplant in Iran.

Cross-infection of *Colletotrichum* species on tropical fruit in postharvest

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Phytopathology 101:S3

The genus Colletotrichum is considered one of the major fungal pathogens of plants, that pathogen is distributed worldwide and affects different crops before harvest and postharvest. In the present study were isolated and identified morphologically and molecularly eight isolates of Colletotrichum sp, isolated of banana, papaya, starfruit, chile manzano, avocado, mango manila and mango ataulfo. These were inoculated on each of the fruits to assess the phenomenon of cross-infection. Diameter of injury was measured daily for seven days. The results showed that some isolates of C. gloeosporioides are more aggressive when inoculated on their original host, but cause less damage in alternate hosts, and other isolates of this species have more aggressive when inoculated in alternate hosts than on their original host. On the other hand C. musae having a smaller number of hosts. The fruits of starfruit were resistant to different isolates of C. gloeosporioides and C. musae. The fruits showed higher susceptibility were banana and papaya, as they were affected by all isolates.

Red palm weevil, *Rhynchophorus ferrugineus* (Olivier), the worst invasive pest of palms

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Phytopathology 101:S3

Red Palm Weevil (RPW), *Rhynchophorus ferrugineus* (Olivier) has been identified by the FAO of the United Nations as a 'category-1' insect pest of date palm, *Phoenix dactylifera*, and currently 18 other palms are reported as hosts of this pest. RPW was first described as a serious pest of the coconut palm, *Cocos nucifera*, in 1906, while in 1917 it was described as a serious pest of the date palm in the Punjab, India. In the mid of 1980's, it is invaded the Gulf Countries of the Middle-Eastern region from where it moved into Africa (Egypt) in the early 1990's and subsequently into Europe (Spain) by transporting of infested offshoots. Lately, it is pest of *Phoenix canariensis* in the Mediterranean basin. In 2005, 2006, 2007 RPW is reported from Greece, Turkey, Canary Islands, Cyprus, France, Italy, Portugal and Malta. While in 2008, 2009 and 2010, it is reported from Morocco, Albania, Republic of Georgia, Caribbean (Curacao island/Netherland Antilles), Libya, Lebanon and U.S.A. Where it arrived through infested palms. RPW mostly infests young palms less than 20 years old with a single female laying about 300 eggs, which hatch into the most damage stage. All stages (egg, larva, pupa and adult) are inside the palm itself. Early infestation of RPW is difficult to detect. RPW is currently managed through a pheromone based Integrated Pest Management (IPM) strategy. Field sanitation and cultural practices are one of the important components to prevent weevil infestation. No effective biological agent has been found.

Using the tomato spotted wilt virus nucleocapsid protein gene for pathogen-derived resistance in lettuce

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Phytopathology 101:S3

Tomato spotted wilt virus (TSWV) is the most important viral disease affecting Hawaii lettuce growers. Earlier work by others showed that the transgenic introduction of the TSWV nucleocapsid protein (N) gene into lettuce can confer pathogen-derived protection on those plants. In this work, four constructs were created using the N-gene in various expression contexts and inserted into lettuce cv. Grand Rapids via Agrobacterium-mediated transformation. At maturity, the primary transformants exhibited poor seed set which may have been due to environmental conditions. However, R_1 plants exhibited good seed set with segregation ratios indicating the presence of a single transgene. R_2 plants were challenged with TSWV obtained from field-collected infected tomato. One line expected to express the entire N gene in plus-sense showed good resistance. Twenty-four transgenic plants were challenged. At ten days post inoculation, six plants showed good resistance with no lesions, seven showed moderate resistance as measured by lesion number and nine showed limited or no resistance. Non-transgenic control plants were showing symptoms of systemic infection; numerous and confluent lesions were found on the inoculated leaves, and lesions as well as some veinal necrosis appeared on uninoculated, systemically infected leaves. Inoculation challenges of the remaining transgene constructs in lettuce are underway, and this set of transgene constructs is also being used for the transformation of Romaine lettuce.

Relationships between nematode distribution in pine stem and development of xylem embolism observed with a compact MRI in pine wilt disease

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Phytopathology 101:S3

Pine wilt disease, caused by pinewood nematode (Bursaphelenchus xylophilus) introduced from North America, is one of the most serious tree diseases in the north temperate zone. This disease is known to induce xylem embolism caused by migration and feeding activity of the nematode in infected pines. Previous studies have shown that a number of nematodes grow around the infection site at the early stage, and then start rapidly moving and multiplying through the tree at the later stages of this disease symptom. It was unclear whether the distribution of nematode corresponds to the development of xylem embolism. In this study, development of xylem embolism was monitored nondestructively and 3-dimensionally with multi-cross-sectional slices taken by a compact MRI in Japanese black pines and compared to the distribution of the nematodes with staining nematodes with F-WGA. In a seedling in which embolism occurred in +3 cm - –10 cm from the inoculation point, there were many nematodes in xylem resin canals, cortex resin canals and cambium near the inoculation point, while there were a few nematodes beyond ±3 cm. In a seedling with its total length of observed xylem was embolized, nematodes reached even the opposite side of inoculation point throughout the stem length. These result suggested that development of xylem embolism was strongly related to nematode distribution.

Identification of small molecule inhibitors against SecA of *Candidatus* Liberibacter asiaticus by molecular modeling studies

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Phytopathology 101:S4

Plant pathogenic bacteria are responsible for overwhelming losses in citrus agriculture. Huanglongbing (HLB) is one of the most devastating diseases of citrus crop in many countries. *Candidatus* Liberibacter asiaticus (*Ca*. L. asiaticus) bacteria are causal agent of HLB disease. Treating *Ca*. L. asiaticus infected citrus trees is one attractive goal due to the great value of citrus trees and the high cost of citrus tree removal and replanting. Identification of small molecule compounds which inhibit the activity *Ca*. L. asiaticus is an alternate approach to control the HLB disease. SecA is an ATPase translocase protein of *Ca*. L. asiaticus which involves in pre-protein translocation between the membrane proteins of the bacteria. Interrupting the ATP-hydrolysis process of SecA by small molecule inhibitor would disrupt the pre-protein translocation and this could lead to the possible antimicrobial compound. In the current study, homology modeling, structure based virtual screening & molecular docking studies have been used to identify the small molecule inhibitors. Finally we found twenty compounds at micro to nano molar activity against SecA. Optimization of the high activity compounds will lead to potential antimicrobial candidates that could be used to control the HLB disease. All the molecular modeling studies have been performed on Linux system by using Maestro module of Schrodinger suite softwares. ATPase assay kit and purified SecA enzyme was used for biological testing.

Detection of Grapevine leafroll-associated virus 7 using Real-time® qRT-PCR and conventional RT-PCR

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Phytopathology 101:S4

Grapevine leafroll-associated virus 7 (GLRaV-7) is an unassigned member in the *Closteroviridae* family that was first recorded in an asymptomatic white-berried grapevine cultivar from Albania. In California, the virus has been detected in several cultivars including Chardonnay, Merlot, Pinot Noir, Emperor, Black Seedless and Sauvignon Blanc. An ELISA test is available for GLRaV-7, but that assay has been described as unsuitable for field use. In order to improve field detection of this virus, sequences in the coat protein gene (CP) and the heat shock protein homolog gene (hHSP70) from nine Californian GLRaV-7 isolates were compared. Consensus sequences derived from those comparisons were used to design both RT-PCR primers and qRT-PCR assays. When 77 grapevine samples were screened with these two detection methods, the qRT-PCR assay based on the hHSP70 sequence identified more positives (13.86%) than the one based on the CP sequence (9.24%). Both qRT-PCR assays (CP and hHSP70) appeared to be more sensitive than the RT-PCR assay which detected only 2.31%.

***Grapevine leafroll-associated virus 1* occurs as genetically diverse populations in wine grape cultivars**

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Phytopathology 101:S4

We studied the genetic diversity of thirty-four field isolates of *Grapevine leafroll-associated virus 1* (GLRaV-1) from different wine grape cultivars in California, New York and Washington States in the U.S. Genetic segments of the heat-shock protein-70 homolog (HSP70h), coat protein (CP), coat protein duplicate 2 (CPd2) and ORF9 (p24) were amplified by RT-PCR, cloned and sequenced. A pairwise comparison of nucleotide sequences revealed intra- and inter-isolate sequence diversity, with CPd2 being the most diverse among the four genomic regions. An estimation of dN/dS values indicated different selection pressures acting on each of the four genomic regions. A global phylogenetic analysis of the HSP70h and p24 gene sequences revealed segregation of GLRaV-1 isolates into three major lineages with isolates from the U.S. distributed in all three lineages, indicating a lack of clustering by geographical origin. Such a segregation of GLRaV-1 isolates into three lineages was not apparent when the CP and CPd2 gene sequences were used for phylogenetic analyses. Topological incongruence in phylogenetic trees and putative recombination events among the four genomic regions further revealed a higher degree of natural variation in GLRaV-1. The genetic landscape of GLRaV-1 will provide a foundation for better understanding the epidemiology of grapevine leafroll disease across grape-growing regions in the U.S.

Impacts of grapevine leafroll disease on an own-rooted wine grape cultivar

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Phytopathology 101:S4

We conducted studies in an own-rooted Merlot block to measure impacts of grapevine leafroll disease (GLRD) on grapevine (*Vitis vinifera*) performance, fruit yield, berry and wine quality. For this purpose, grapevines were identified in a commercial vineyard in such a way that individual grapevines exhibiting typical GLRD symptoms and tested positive for *Grapevine leafroll-associated virus 3* were adjacent to healthy grapevines in a given row to minimize error in sampling and experimental results due to variations in growing conditions. Data collected during 2010 season showed differences in leaf photosynthesis and chlorophyll fluorescence between GLRD-affected and unaffected leaves only after *véraison*. Fruit maturity indices (soluble solids and fruit acidity) and total anthocyanins measured at various stages of berry development showed significant differences between berries produced by GLRD-affected and unaffected grapevines. Fruit yield measured at the time of commercial harvest showed significant reduction in GLRD-affected grapevines. Small-lot wines made from grapes harvested from GLRD-affected Merlot grapevines showed significantly less amounts of pigments (anthocyanins, small- and large-polymeric pigments), tannins and alcohol than in wine made from fruit harvested from unaffected grapevines. These results demonstrated that GLRD affects vine performance and impacts fruit and wine quality in own-rooted Merlot grapevines grown under cool-climate conditions.

Determination of presumptive vegetative compatibility groups of *Verticillium dahliae* occurring on sunflower using molecular markers

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Phytopathology 101:S4

Verticillium wilt on sunflower is caused by *Verticillium dahliae*, and was managed by the dominant gene V1 for the last two decades. A new strain in North America (NA-Vd2) is able to overcome the V1 resistance gene. In Argentina, where Verticillium wilt is endemic, a strain different from that in North America is hypothesized. However, the VCGs of *V. dahliae* sunflower isolates have never been characterized from any country. Thus, characterizing sunflower *V. dahliae* VCGs, knowing their relative aggressiveness, and studying their genetic diversity are important in breeding programs and in screening the resistant lines efficiently. A total of 900 *V. dahliae* isolates were collected from sunflower and other hosts from the U.S., Canada, Australia and Argentina. DNA was extracted from all *V. dahliae* isolates and six polymerase chain reactions and AFLP analysis were used to study population variability. Five PCR patterns have been found and are designed A, B, C, or D. In some instances there were no amplifications (NA). PCR patterns varied based on host and geographic origin. Preliminary results of North American isolates showed that most *V. dahliae* isolates recovered from sunflower belong to VCG-2A.

Profile of *Pythium* spp. in certified organic fields for vegetable production in central Washington

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Phytopathology 101:S4

Damping-off is a common disease of many crops, including vegetables. The disease is exacerbated in organic production by the lack of highly effective organic seed treatments. *Pythium* is the major pathogen typically associated with damping-off in early season plantings in temperate regions, when soil and irrigation water are cold. The objective of this study was to identify *Pythium* species associated with damping-off in organic vegetable production in the semi-arid Columbia Basin of central Washington, particularly in pea and sweet corn crops. In October 2009, soil samples were collected from 37 certified organic fields with a history of pea and/or sweet corn production. *Pythium* spp. were baited from the soils using grass leaves and root baiting with pea and sweet corn seeds. Approximately 300 isolates were obtained and identified to species by sequencing the internal transcribed spacer (ITS) region of the rDNA. Nineteen *Pythium* species were identified, with *P. irregulare*, *P. torulosum*, and *P. ultimum* the most prevalent. Pathogenicity of isolates of each species was tested on pea planted into inoculated soil under cool and wet conditions in a growth chamber. At least six species (*P. abappressorium*, *P.*

dissotocum, *P. irregulare*, *P. sylvaticum*, *P. ultimum*, and *P. violae*) caused damping-off, with differences in aggressiveness detected among isolates of each species. Inoculum levels of these species in the sampled soils will be quantified using real-time PCR assays.

Aggressiveness of *Sclerotinia sclerotiorum* from the north central United States on multiple crops

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Phytopathology 101:S5

Sclerotinia sclerotiorum is a pathogen of many crops yet little is known about aggressiveness within a diverse population from multiple crops. A collection of isolates from the North Central states were evaluated for aggressiveness as measured by lesion length. Thirty isolates from six mycelial compatibility groups (MCG) with five isolates in each MCG were evaluated on six crops: drybean, canola, lentil, pea, soybean and sunflower. Another 67 isolates, representing 31 MCG's, were evaluated only on dry bean and sunflower. Plants were inoculated 5 to 7 weeks after planting using the cut-stem technique, placing a straw or pipette tip with agar and mycelium over the cut end of the stem, then misting plants for three days. In the experiments with the 30 isolates, all isolates were pathogenic and crop was a significant factor with the longest lesions on dry bean and the shortest on pea. However, MCG, isolate within MCG, and the interactions of crop x MCG, and crop x MCG x isolate were not significant factors. All of the 67 isolates were pathogenic on both crops and isolate was a significant factor, but crop was not. There were significant interactions of experiment by crop and crop by isolate. In all experiments, performance of aggressive isolates was consistent across crops. The data showed that within an MCG there were significant differences among isolates in aggressiveness, suggesting that MCG may not be related to pathogenicity factors.

The role of rice rhizobacteria in defense against *Magnaporthe oryzae* infection

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Phytopathology 101:S5

Rice blast disease, caused by the fungal pathogen *Magnaporthe oryzae*, infects all foliar parts of the rice plant. Exploitation of the natural alliance between soil microbes and rice roots can increase protection against rice blast through induction of systemic resistance (ISR). ISR is mediated through intra-plant signaling and can be triggered by direct bacterial root colonization of plant growth promoting bacteria or through microbial volatile components. Bacteria isolated from California field grown M104 (temperate japonica variety) were tested against *M. oryzae* strain 70-15 *in vitro* and *in planta*. The volatile components of a *Pseudomonas* isolate, EA105, indirectly inhibited 70-15 growth. Five days post co-inoculation, the fungal diameter averaged 55 percent of the control. To see if this inhibition was due to hydrogen cyanide production, a known cyanide producer *P. fluorescens* CHAO was tested. CHAO averaged 59 percent of the control diameter, yet exhibited more aerial hyphae than EA105, indicating a better living environment for 70-15 compared to an EA105 volatile exposed one. Gas chromatography-mass spectrometry method will be used to determine the volatile components. EA105 root treated rice plants were not harmed by the inoculum and priming of roots with EA105 for 24 hours helped to reduce blast lesion formation when infected with 70-15 spores. However, additional biological replicates must be performed to confirm this ISR-like response to pathogen infection.

Incidence of Fig leaf mottle-associated virus and Fig mosaic virus in Eastern Province of Saudi Arabia

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Phytopathology 101:S5

Fig plant, Ficus carica L., is grown in Saudi Arabia and is being affected by fig plant mosaic diseases (Fig leaf mottle-associated virus, Fig mosaic virus). The main symptoms are chlorotic mottling, blotching and various types of leaf deformation. Samples were collected, with consideration of the economically importance and distribution of the cultivars, from different areas of the East Province of Saudi Arabia. Each sample was consisted of 10–15 leaves. Samples were labelled and stored in plastic bags at 4°C; then transferred to the laboratory. For total nucleic acids (TNAs) extraction. One hundred mg of leaf veins and or cortical scrapings were used for extraction. Samples were macerated in 1 ml of grinding buffer. TNAs were recovered with a silica-capture procedure and stored at –20°C till used. 8–10 µl of TNA extracts were mixed with 1 µl random hexamer primer, (Boehringer Mannheim, GbmH) (0.5 µg/µl). RT-PCR assay of leaves extracts of infected fig accession using specific primers gave positive results and non with FLMaV-2. Mixed infection of FLMaV-1 and FMV were found. To our knowledge this is the first record and identification of FLMaV-1 and FMV in Saudi Arabia. Further studies are needed to investigate the fig mosaic disease throughout the country.

Virulence variability and genetic diversity among *Cochliobolus sativus* isolates recovered from barley and wheat in North Dakota

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Phytopathology 101:S5

Cochliobolous sativus (anamorph: *Bipolaris sorokiniana*) causes economically important diseases including common root rot and foliar spot blotch in cereals. In North Dakota, the diseases occur in both barley and wheat which are concurrently grown in the region. However, the relationship between the pathogen populations recovered from the two crops is poorly understood in terms of virulence and host specificity. In this study, 30 *C. sativus* isolates recovered from barley and 30 *C. sativus* isolates recovered from wheat were inoculated on two-leaf-stage seedlings from three barley genotypes (Bowman, ND5883, and NDB112) and four wheat genotypes (Grandin, Chris, PI 644122, and PI411132) in the greenhouse. The results indicate that the majority of the wheat isolates were more virulent on the wheat genotypes than the barley isolates. In contrast, the barley isolates were more virulent on the barley genotypes as compared to the wheat isolates. However, variability in virulence was observed among the isolates derived from the same host. Amplified fragment length polymorphism (AFLP) analysis was performed on the 60 isolates tested for virulence and a high level of polymorphism was revealed using four primer combinations (E-AT/M-CT, E-AC/M-CC, E-AG/M-CA, and E-AA/M-CA). The relationship between phenotypic variation and DNA polymorphism in the pathogen population will be presented.

Maintaining Maturity Group IV soybean seed quality: Perspectives from Mississippi, 2009 and 2010

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Phytopathology 101:S5

Numerous factors can reduce Maturity Group IV soybean seed quality. Over the past two years, research in Mississippi has attempted to determine strategies to reduce losses attributed to poor seed quality. Environmental conditions during the 2009 season resulted in severe quality losses that could be attributed to mold, stink bugs, and additional environmental factors influencing harvested seed quality. Foliar applications of the fungicide azoxystrobin (291 ml/ha of Quadris) and insecticide bifenthrin (380 ml/ha of Brigade) were made at 3 locations at R3, R5, and R3 + R5, alone and tank mixed. Locations sustained different environments resulting in mild (Stoneville), moderate (Raymond), and severe (Starkville) seed quality damage. Return-on-investments (ROI) were calculated and while some applications did reduce observable percent mold there were still excessive ROI losses. In addition, secondary trials were conducted whereby azoxystrobin applications were made at numerous timings (R1, R3, R5, R6) and final concentrations (0, 291, 437, 582, 876, 1168 ml/ha). At Raymond, number of applications (R^2 = 0.4587; p = 0.025) as well as final fungicide concentrations (R^2 = 0.507; $p < 0.0001$) significantly reduced percent mold. Additionally, the number of insecticide applications significantly reduced percent mold (R^2 = 0.620; p = 0.003). Optimal conditions during 2010, resulting in no seed quality losses, made assessing treatments and their overall effect on seed quality difficult.

Identification and characterization of promoter elements from plant pararetroviruses from dahlia (*Dahlia variabilis*)

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Phytopathology 101:S5

Dahlia mosaic virus (DMV), Dahlia common mosaic virus (DCMV) and an endogenous plant pararetrovirus (DMV-D10) are three distinct dahlia-associated caulimoviruses whose promoters have not been characterized. Based on sequence comparisons and promoter prediction programs, the putative 35S promoter region from these three viruses was identified. The promoter regions were independently cloned into pCAMBIA1281Z. All constructs were delivered into *Agrobacterium tumefaciens* by electroporation, and agroinfiltrations were done into *Nicotiana benthamiana*. The activity of the 35S promoter homologs was determined by transient expression of the beta-glucuronidase gene (GUS). The length of the promoter regions corresponded to 438 bp for DMV, 439 bp for DCMV and 256 bp for DMV-D10. Quantitative GUS assays demonstrated that the promoter activity of 35 homologs from DMV and DCMV was similar to that of *Cauliflower mosaic*

virus 35S promoter in *N. benthamiana* leaf tissue, whereas significantly lower promoter activity was observed for DMV-D10. Qualitative GUS assays were consistent with quantitative GUS results.

Rapid immuno-test combined with magnetic bead technology for on-site detection of potato leafroll virus
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Phytopathology 101:S6

The rapid on-site detection of plant pathogens is relevant for growers and inspectors. Lateral-flow tests for recognizing five of the six most important potato viruses are available, but the detection of potato leafroll virus (PLRV) has not been possible with the conventional lateral-flow format, presumably due to low virus titer. In order to overcome this shortcoming, the PLRV AgriStrip-magnetic was developed. This lateral-flow test is based on antigen-antibody reaction combined with magnetic bead technology, enabling the preceding enrichment of target antigen (PLRV). Potato leaf extracts are first incubated with magnetic beads coated with antibodies specific for PLRV. The beads bind the viral antigen and are then separated from the extract with a magnet and resuspended in a small volume of running buffer. After inserting the strip into the enriched solution, the concentrated beads migrate upwards and colored test- and control- lines become visible, indicating the health status of test samples. The PLRV AgriStrip-magnetic assay employs mono- and polyclonal antibodies and reacts specifically with PLRV. No cross-reaction is observed with other potato viruses. The sensitivity attained with the AgriStrip-magnetic test is comparable to the sensitivity in the DAS-ELISA method. With the availability of the AgriStrip-magnetic, potato sample extracts can be analyzed in parallel for the presence of the six most important potato viruses PVA, PVM, PVS, PVX, PVY and PLRV.

Resistance of *Brachiaria* genotypes to *Rhizoctonia* spp.
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Phytopathology 101:S6

Foliar blight, caused by *Rhizoctonia* spp., is an important production limitation to *Brachiaria* pasture-based livestock in the Tropics. The potential for increasing genetic host-plant resistance to foliar blight will depend on the genetic variation for pathogenicity and virulence in the fungal populations, in the genetic variation in resistance in the host plant, and in the possible interaction between pathogen and host-plant variability. The present study sought to initiate characterization of pathogen variability encountered within Colombia and the interactions between this variation and relevant host-plant variability. We obtained 147 *Rhizoctonia* isolates collected from different *Brachiaria* genotypes in five Departments of Colombia. Isolates were morphologically characterized and their pathogenicity was evaluated in artificially inoculated trials under greenhouse conditions. Isolates differed both in their morphology and their aggressiveness. Isolates from Meta, Casanare, Córdoba, and Caquetá were more pathogenic than those from Cauca. Restriction fragment length polymorphism analysis classified the isolates into two groups: *R. solani* anastomosis group AG-1 I-A and *Rhizoctonia* spp. AG-D. A study of virulence showed that multinucleated *Rhizoctonia* isolates had the highest virulence. Pathogen isolate, host genotype, and isolate-genotype interactions were all significant sources of variation ($P > 0.0001$).

A new *Phytophthora* sp. causing basal rot on Japanese iris
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Phytopathology 101:S6

Japanese iris (*Iris ensata* var. ensata) is a popular flowering plant that is widely cultivated in Japan. Recently, a disease causing basal rot accompanying initial yellowing of a central leaf on the plants, has occurred in many iris gardens. These symptoms are visible from the early growing season until the flowering stage in wet cultivation conditions. Homothallic *Phytophthora* sp. was first isolated with high frequency from diseased plants collected at Suigou Sawara Aquatic Botanical Garden in Chiba prefecture, Japan. Typical symptoms developed on the plants when inoculated by root dipping in water containing cultured agar pieces of the fungus for 20–24 hr before transplantation to soil. The same fungus was recovered from the diseased tissues. The fungus formed oogonia with paragynous antheridia, oospores turning golden brown when aging, and non-papillate zoosporangia. Sequence analyses of rDNA-ITS region, beta-tubulin gene, and elongation factor 1 alpha gene revealed that the isolate showed similar homology with *Phytophthora europaea*. Its morphological and culturing characteristics (Jung *et al*., 2002) were almost coincident with those of the iris isolate. The isolate, however, were clearly distinct from *P. europaea* in phylogenetic trees. It was also found that the same disease caused by the clonal fungus was widely developed in Japan. We concluded that *Phytophthora* sp. isolated from Japanese iris differs from other known species in genetic characters and host plants.

Localization of *Candidatus* Liberibacter asiaticus associated with huanglongbing in various organs of its psyllid vector using FISH and Q-PCR
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Phytopathology 101:S6

Candidatus Liberibacter asiaticus (Las) has been associated with huanglongbing (HLB), or citrus greening, which is currently the most devastating citrus disease worldwide. HLB is transmitted by the Asian citrus psyllid *Diaphorina citri* (Hemiptera, Psyllidae) in a persistent manner, but its vector interactions, particularly at the organ and cellular levels, are poorly understood. We used fluorescent *in situ* hybridization (FISH) and quantitative PCR (Q-PCR) for the localization of Las in *D. citri*. Las was detected by FISH in the hemolymph, filter chamber, midgut and salivary glands of HLB-infected *D. citri* and in the phloem of infected citrus leaves. Additionally, Q-PCR detected Las in dissected organs of individual *D. citri* adults collected from field-infected trees or a laboratory-infected colony. The proportion of Las-infected (PCR-positive) salivary glands (47–70%) was significantly lower than that in other body parts (79–98%). The relative titer of Las, compared to psyllid genomic DNA in each sample, was significantly higher in both the salivary gland and alimentary canal compared to that in the rest of the body. These results provide the first molecular localization of Las in the hemolymph, alimentary canal and salivary glands of *D. citri*. They also strongly suggest that the salivary glands constitute an important infection and/or transmission barrier to Las in the psyllid vector, and that Las may replicate or accumulate in both the alimentary canal and salivary glands of *D. citri*.

A new detached-leaf assay to test the inoculativity of psyllids with *Candidatus* Liberibacter asiaticus associated with huanglongbing disease
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Phytopathology 101:S6

To test the inoculativity of the Asian citrus psyllid (ACP) *Diaphorina citri* with *Candidatus* Liberibacter asiaticus (Las), associated with huanglongbing (HLB) or citrus greening disease, psyllids are usually fed singly or in small groups on citrus seedlings. These seedlings are then tested by polymerase chain reaction (PCR) normally 3–12 months later because of the long incubation period of Las\HLB in whole citrus plants. Here, we developed a new 'detached-leaf assay' method in which ACP adults from a HLB-infected colony were fed for 1 week on detached healthy sweet orange leaves placed in 50-ml polypropylene tubes. One week later, these leaves were processed for quantitative PCR (Q-PCR) using two Las primers (Li and LJ900). When feeding 10, 5 or 1 ACP/leaf/week the percentages of Las-positive leaves were 40, 18.8 and 4.4%, respectively, using Li primers, and 60, 40.6 and 11.1%, respectively, using LJ900 primers. Ct values for Q-PCR using LJ900 primers were much lower than those using Li primers. Our results, using the more commonly used but less sensitive Li primers, are largely comparable to those obtained by previous workers using whole citrus seedlings for Las-inoculation by ACP. However, using more sensitive primers can increase the usefulness of this method. We suggest that this new 'detached-leaf assay' method can potentially speed up Las-inoculativity tests on ACP from 3–12 months to only 2–3 weeks, which can greatly enhance pathogen-vector relation studies on Las and ACP.

Genetic diversity and DNA fingerprinting of *Xanthomonas oryzae* pv. *oryzae* isolates from east and central Africa
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Phytopathology 101:S6

Genetic diversity and DNA fingerprinting of *Xanthomonas oryzae pv oryzae* (*Xoo*) isolates from east and central Africa was carried out using a newly developed PCR technique that combines molecular diagnostic and DNA fingerprinting for *Xoo* identification and differentiation. Molecular PCR diagnostic showed that the presence of at least a band indicates positive detection of *Xoo* pathogen and absence of a band indicates negative for no *Xoo* pathogen detected, while in the same PCR assay the presence of one or more band at different position revealed the DNA fingerprint of each *Xoo*

isolates. Molecular diagnostic revealed that all the 28 *Xoo* isolates from Uganda, Mozambique and Rwanda including control from IRRI Philippines were *Xoo* pathogens. DNA fingerprinting of the 28 *Xoo* isolates revealed two major *Xoo* genotypes (*XooG1* and *XooG2*) in Uganda, Mozambique and Rwanda. Interestingly, *XooG2* genotype is typical of Uganda origin only while *XooG1* genotype constitutes *Xoo* isolates from Uganda, Mozambique and Rwanda. The study revealed *Xoo* pathogen population structure and evidence of *Xoo* pathogen migration and movement within the three countries which could be due to poor seed health germplasm exchange across the region. Development of a reliable molecular technique for *Xoo* identification and differentiation is a prerequisite into understanding the genetics of *Xoo* population structure in east and central Africa and deployment of durable resistance cultivars. Key words: *Xanthomonas oryzae* pv. *oryzae*, Molecular diagnostic, DNA fingerprinting, PCR technique, Genotype, Africa.

Influences from long-term crop rotation, soil tillage and fertility on the severity of rice grain smuts

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Phytopathology 101:S7

False smut (*Ustilaginoidea virens*) and kernel smut (*Neovossia horrida*) are diseases of rice (*Oryza sativa*) that reduce both grain yield and quality. Susceptible rice varieties are in widespread use on production acreage in the United States, and the effects from crop management practices on smut control are poorly understood. We studied the long-term effects of crop rotation, soil tillage and fertility level on rice-smut severity. The highest levels of false smut observed in this study were on varieties grown in rotation with soybeans, on traditionally tilled soils, with high fertilizer treatments. The highest levels of kernel smut were observed in a rice-soybean rotation with winter wheat grown between summer crops. These rotations are commonly used in rice growing regions of the southern U.S. Using combinations of crop rotation, soil tillage and fertility rate, several alternative crop management practices were identified that provided effective control of smuts in susceptible rice varieties. The most effective method for controlling both false smut and kernel smut was in three-year rotations of rice, soybeans and corn. Regardless of rotation order, or tillage and fertility treatments within the rotations, rotating out of rice for two years was the most effective approach for smut control.

Development of mt*COI* PCR primers with 5′ AT-rich flaps for rapid identification of high consequence *Bemisia tabaci*

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Phytopathology 101:S7

Whiteflies (Hemiptera; Aleyrodidae) are globally distributed agricultural pests. The *Bemisia tabaci* (Gennadius) sibling species group is a cryptic species composed of morphologically identical but genetically distinct biotypes (well-characterized) and haplotypes. Biotypes differ with respect to host colonization, fecundity, virus transmission efficiency, pesticide resistance, and invasiveness. Rapid identification of *B. tabaci* variants is necessary to facilitate interventions that minimize the likelihood of exotic whitefly and plant virus introductions. Haplotype-specific mt*COI* endpoint PCR primers were designed to differentiate the high consequence B biotype of *B. tabaci* from other variants. In our PCR strategy 5′ AT-rich flaps also were incorporated into the primers for increased PCR sensitivity and increased yield of the amplicon. The limit of detection by primers with and without flaps was evaluated by performing a sensitivity assay. B biotype primers with and without flaps amplified B biotype DNA. Primer sensitivity was increased by adding 5′ AT-rich flaps when a higher DNA concentration was present in the reaction and when using a higher annealing temperature. The use of these primers in an endpoint PCR reaction followed by gel electrophoresis enabled rapid and sensitive identification of the B biotype, an approach that can now be extended to other economically important haplotypes.

Custom transcription factors for manipulation of gene expression in *Phytophthora infestans*

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Phytopathology 101:S7

Improvements to the genetic tools available for manipulating genes in oomycetes would enhance studies of gene function in these organisms. To help accomplish this we are testing the effectiveness of custom-engineered transcription factors based on two classes of DNA binding proteins with predictable DNA-binding specificity: zinc finger proteins (ZFPs) and transcription activator–like effectors (TALEs). Both ZFPs and TALEs have modular DNA-binding regions, each conferring recognition of one or three DNA base pairs respectively. Combining modules allows the assembly of DNA-binding domains with predictable binding specificity. In a variety of transgenic organisms (plants, yeast and mammalian cells), such custom ZFPs and TALEs fused to activation domains activate transcription of endogenous genes, whereas fusions to repression domains inhibit transcription. ZFP and TALE DNA binding domains were designed to target sequences in the native *INF1* promoter as well as to a minimal promoter (*NifS*) fused to a *GUS* transgene. Each of the DNA binding domains will be fused to a SID transcriptional repressor as well as to a VP16 activation domain, transformed into protoplasts of *P. infestans*, and the resulting transformants will be assayed for the expression of the TALE-SID/VP16 chimera, ZF-SID chimera, INF1, and GUS. Additionally, experiments will be performed with ZFPs and TALEs fused to the nuclease domain of *FokI*, which will be designed to target mutations to the *INF1* coding sequences.

Systemic infection of coffee plants (*Coffea arabica* L.) by *Tobacco mosaic virus*

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Phytopathology 101:S7

Tobacco mosaic virus (TMV) has proven to be a versatile gene expression vector in many different *Nicotiana* species. In this study we investigated the capacity of TMV to infect coffee (*Coffea arabica* L.) systemically, to determine if it could be used as a genetic tool for further studies in coffee. Previous studies have shown that coffee is host for few viruses. Coffee is naturally infected by the nucleorhabdovirus *Coffee ring spot virus* in Brazil and Costa Rica, whereas inoculation of different viruses including TMV resulted in only local infections. We inoculated TMV strain U1 virions onto leaves of sixteen 3-month old and sixteen 5-month old *C. arabica* cv. Caturra plants. Although no clear symptoms were observed in coffee leaves, ELISA tests of samples from inoculated leaves showed that all 32 plants were positive for TMV infection at 14 dpi. We found that TMV accumulates in coffee to a concentration that is approximately 13% the level found in *N. benthamiana* at 7 dpi, and that its titer increased in coffee over the next two weeks. We also detected TMV in upper non inoculated coffee leaves by ELISA, and visualized virions in both types of tissues by electron microscopy. Furthermore, TMV virions purified from coffee were still infective after two passages through coffee. We are currently evaluating the capacity of TMV to serve as an expression vector in coffee. Project Funded by the Colombian Ministry of Agriculture.

Abundance and diversity of fungal endophytic community in an Italian beech forest: Pyrosequencing vs isolation method

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Phytopathology 101:S7

Since the mid-1980s, a decline of beech (Fagus sylvatica), one of the most important forest tree species in Italy, has been reported with symptoms consisting on general suffering of trees and tree death. Some endophytic fungi, as B. nummularia, are known to lead to the onset of symptoms that arise with favourable ecological or physiological conditions. In this study the diversity of the fungal endophytic community in ten trees samples from an Italian beech forest has been assessed by cultivation method and high-throughput tag-encoded FLX amplicon pyrosequencing. A total of 149 cultures were isolated and identified on the base of morphological and molecular analysis. Pyrosequencing was carried out on amplicons derived from the ITS rDNA region. A total of 31.067 reads were obtained. Trough phylogenetic assignment using BLASTN, taxa were identified. The values of abundance and diversity of fungi in the samples are linked to the method of detection used. A few fungal taxa account for most of the species' abundance, whereas the majority of species are only rarely retrieved. Pyrosequencing resulted to be a reliable technique for investigating fungal communities in forest ecosystem.

Antimicrobial activity of essential oils of various plants against brown blotch disease on *Agaricus bisporus*

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Phytopathology 101:S7

Antimicrobial properties of essential oils of *Mentha Piperita*, *Eucalyptus camaldulensis*, *Thymus vulgaris*, *Artemisia dracunculus* were evaluated against *Pseudomonas tolaasii*, under *in vitro* condition. Extraction of EOs from leaves were made with clevenger. Bioassays for inhibition activities of EOs in three concentrations of 10^{-1}, 10^{-2} and pure were performed against *P. tolaasii* at 10^5 cfu/ml on two agar media of NA and KB. After one day of incubation at 27°C, pure essential oil of Eucalyptus with 17 mm inhibition zone, and pure EOs of Mentha and Tarragon with 2 mm inhibition zone on KB medium exhibited the most and least antibacterial activities. No inhibition zone was observed for 10^{-2} concentration of Mentha, Thyme and Tarragon on NA medium. These were more pronounced when compared to least inhibitory effects of antibiotics erythromycin, penicillin and gentamicin in both concentrations of 0.1 and 0.01 mg/ml. Tetracycline was an exceptional with similar result obtained from pure Eucalyptus EO. On the other hand, gentamicin and tetracyclin were more effective when the concentrations were increased into 1, 5, 10 mg/ml.

Spray drift from aerial application on sugarcane in Brazil

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Phytopathology 101:S8

The aim of this study was to develop and evaluate a method to quantify drift from aerial application on a sugarcane field. A 120 ha sugarcane field was sprayed with a solution containing rhodamine tracer. The application was done with an EMB 202 aircraft at 30 L ha^{-1} with medium droplets, flight height from 2 to 3 m and 185 km h^{-1}. In order to evaluate the total drift by mass balance 20 horizontal glass collectors fixed on poles on the top of the canopy were randomly distributed on the field. Drift movement outside the field was evaluated by setting up a sampling line downwind were nylon string were placed at 10 m, 50 m, 200 m, 600 m, 1200 m and 2000 m from the field. The 2 m long strings were placed vertical on top of the canopy, with 8 replications for each distance. The spray application was done in 1:33 h, with meteorological averages of 19.6°C, 67.6% RH and 7.4 km h^{-1} of wind speed. After the application the tracer was washed from the collectors and analyzed by HPLC. Based on this data a model to calculate the drift potential was developed. The total losses (drift) calculated by mass balance was 19.6%. Drift data downwind showed that a sugarcane plant growing 2000 m apart from the field would receive up to 3.9% of the dose rate applied on the field.

Performance of aerial application for soybean rust control and drift under unsuitable meteorological conditions for spraying

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Phytopathology 101:S8

The aim of this study was to evaluate the drift potential and the performance of aerial application of fungicides for Asian Soybean Rust control under unsuitable meteorological conditions for spraying using a mixture of tebuconazol + picoxystrobin (0.5 + 0.25 L c.p ha^{-1}) at a volume rate of 15 L ha^{-1} and fine droplets. The five treatments were defined by the following adjuvants applied as tank mixtures with the fungicides: Nimbus (mineral oil) at 0.5% v v^{-1}, AgrexOil (vegetal oil) at 10% v v^{-1}, Li 700 (blend of acid, lecithin and surfactant) at 0.5% v v^{-1}, Nimbus + Li 700 at 0.25%+0.25% v v^{-1} and AgrosPred (organosilicon) at 0.2% v v^{-1}. The applications were arranged to be done under unsuitable meteorological conditions for aerial application (RH from 33.6 to 42.6%, temperature from 29.1 to 31.6°C and wind from 10 e 16.4 km h^{-1}). Data were collected related to soybean defoliation, Asian Soybean Rust control and spray deposits, in order to calculate the total losses (drift) by mass balance. The results showed that all the treatments failed to control the rust since the drift was very high (from 53.5 to 61.3% of total losses). The adjuvants were unable to control the drift under the unsuitable meteorological conditions for aerial application.

Influence of adjuvants and rain-free period on the application of haloxyfop-methyl

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Phytopathology 101:S8

The aim of this study was to characterize the performance of adjuvants on the application of haloxyfop-methyl (Verdict), looking at the effect of herbicide rainfastness on the control of *Digitaria* spp. The herbicide was applied at 60 g i.a. ha^{-1} with the following adjuvants, all of them at 0.5% v v^{-1}: Oppa (mineral oil 800 g L^{-1}), Assist (mineral oil 756 g L^{-1}), Joint Mineral Oil (mineral oil 761 g L^{-1}), Óleo Vegetal Nortox (vegetal oil 930 g L^{-1}) e Veget Oil (vegetal oil 930 g L^{-1}). The 24 treatments were defined by the interaction of the six spray solutions with four variations of a 15 mm simulated rain: rain-free (no rainfall after application), 0 min (rainfall immediately after the application), 30 min. (rainfall 30 minutes after the application) and 60 min. (rainfall 60 minutes after the application). The results showed that the highest control rates were obtained with adjuvants. There was advantage on the use of adjuvants when there was no rain or the rain-free period after the application was at least 60 minutes. When the rain was simulated immediately after the herbicide application none of the adjuvants presented significant difference to the herbicide alone, while the Oppa and the Joint Oil induced a reduction on the weed control rate and the Óleo Vegetal Nortox was the only treatment that provided more than 80% control. When the simulated rain was applied 30 minutes after the herbicide application, the Joint Oil showed the best performance.

Role of seaweeds occurring at Karachi coast in suppressing the root diseases of cotton and chili

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Phytopathology 101:S8

Considerable evidence has been accumulated in recent years to support and identify the benefits associated with the use of seaweed in crop production systems. In this study, Sargassum binderi, Rhizoclonium implexum, Stoechospermum marginatum, Spatoglossum variabile, Melanothamnus afaqhusainii, Stokeyia indica and Solieria robusta occurring at Karachi coast were applied as soil amendment two weeks before sowing of cotton (Gossypium hirsutum L.) seeds in clay pots. Application of some seaweeds and topsin-M (fungicide) and carbofuran (nematicide) showed more or less similar suppressive effect on root rotting fungi Macrophomina phaseolina, Rhizoctonia solani, Fusarium solani and root knot nematode Meloidogyne javanica on cotton. Seaweed also showed a positive effect on plant growth by enhancing fresh shoot weight and plant height. In field plot experiments, on cotton and chili (Capsicum annuumL.) seaweeds showed similar suppressive effect on soilborne pathogens and improved plant growth. Spatoglossum variabile and Melanothamnus afaqhusainii also increased numbers of ball per plant in cotton. Due to increasing concern over the chemical pesticides and fertilizers, seaweed resources could be used for the production of organic fruits and vegetables.

Canyon live oak (*Quercus chrysolepis*) is susceptible to bole infection by *Phytophthora ramorum*

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Phytopathology 101:S8

Canyon live oak (*Quercus chrysolepis*) was originally shown to be susceptible to leaf and twig infection by *Phytophthora ramorum* (cause of Sudden Oak Death, SOD). Recently, mortality of large *Q. chrysolepis* was observed in a SOD-affected forest along with trunk symptoms indicative of late-stage *P. ramorum* infection. Symptomatic trees showed spatial correlation with California bay (*Umbellularia californica*), the primary source of *P. ramorum* inoculum in oak-bay forests. However, the pathogen was not recovered from cankers sampled through 2009. To determine the susceptibility of *Q. chrysolepis* to *P. ramorum*, we inoculated 12–20 cm diameter, 120 cm long, logs of disease-free *Q. chrysolepis* and *Q. agrifolia* trees with mycelial plugs of 7 *P. ramorum* isolates. Inoculated logs were enclosed in plastic bags and maintained in 20°C growth chambers. At 8 weeks, 90% of the inoculations had resulted in visible cankers with a mean canker size of 57 cm^2 in *Q. chrysolepis* and 228 cm^2 in *Q. agrifolia*. *P. ramorum* was recovered from nearly all canker margins. Field inoculations were conducted on 18 *Q. chrysolepis* trees in July 2010. Over the next three months, only four inoculated trees showed minimal visible trunk symptoms (i.e., bleeding). In December 2010, four inoculated trees were destructively sampled. All inoculations resulted in cankers similar in size and appearance to those observed with the logs; *P. ramorum* was recovered from canker margins in all cases.

***Phytophthora ramorum*'s trophic nature suggests that it cannot utilize dead leaf litter in aquatic systems**

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Phytopathology 101:S8

Phytophthora ramorum, cause of Sudden Oak Death, is routinely isolated from waterways using leaf baits, but the nature of its presence in open waters

is unknown. It has often been recovered from waterways with no known terrestrial infestation nearby. To test *P. ramorum*'s capacity to utilize plant litter as a substrate in aquatic systems, we simultaneously exposed freshly picked rhododendron leaves with those killed by drying or freezing in two infested streams. Baits were deployed monthly during peak pathogen activity from January to June in each of two years. *P. ramorum* was recovered from 62% of fresh leaves, but only 6% and 2% of frozen and dried leaves, respectively. To further characterize *P. ramorum*'s trophic capacity, we incubated fresh, frozen and dried leaves separately in cups of water inoculated with *P. ramorum* or *P. gonapodyides* (an ubiquitous, presumed saprobic resident of open waters), or combined inoculum of both species for 7 days at 16°C. When incubated alone both species colonized all three bait types; however, *P. ramorum* was isolated more frequently from fresh and frozen leaves, while *P. gonapodyides* was isolated more frequently from frozen and dry leaves. When incubated together, *P. ramorum* was isolated more frequently from fresh leaves than *P. gonapodyides*, while the opposite occurred with frozen and dry leaves. These results indicate that *P. ramorum* may be limited biologically and ecologically from colonizing litter in aquatic environments.

Strategies for management of southern corn rust in Georgia

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Phytopathology 101:S9

Southern rust of corn caused by *Puccinia polysora* is a potentially devastating disease of sporadic importance in Georgia. Objectives here included initiation of sentinel plots for early detection of southern rust and assessment of fungicides for management. Sixteen sentinel plots planted to southern rust-susceptible Pioneer 33M57 and rust-resistant Pioneer 33M52 were assessed in 2010. Earliest detection of southern rust was on 2 July (P33M57 Turner Co.); latest detection was on 29 July (P33M57 Mitchell Co. and P33M52 Turner Co.). Fungicide studies were conducted in three locations. Plots in Decatur Co. were planted to P33M52 and P33M57, to P31D59 in Tift Co., and P31D59 and DKC69-71 in Mitchell Co. Treatments in Decatur and Tift Counties included pyraclostrobin, pyraclostrobin + metconazole, azoxystrobin + propiconazole, tebuconazole, and fluoxastrobin or trifloxystrobin + propiconazole. Fungicides were applied at tassel or silking. Fungicides at Mitchell Co. included pyraclostrobin and pyraclostrobin + metconazole applied at tassel or tassel and silking. Late season severity at Decatur and Tift Counties was less than 7% and 1% leaf area affected respectively; yields in plots treated with fungicides tended to be numerically but not significantly different than the untreated control. Late season severity at Mitchell Co. was less than 58% leaf area affected; yields in plots treated with fungicides were significantly greater than the untreated control.

Sensitive detection and discrimination of WSMV, TriMV and HPV using multiplex RT-PCR

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Phytopathology 101:S9

Wheat streak mosaic virus (WSMV), *High plains virus* (HPV) and *Triticum mosaic virus* (TriMV) all damage cereal crops in Oklahoma, Kansas and the High Plains, and it may be difficult to determine whether symptomatic plants are infected with one or multiple virus species. A multiple RT-PCR assay that facilitates early, accurate and sensitive detection of WSMV, TriMV and HPV from plant tissues is needed by plant health diagnosticians for quarantine applications, disease management, outbreak monitoring and host reservoir identification. Specific PCR primers and probes were designed for use in multiplex end point and real time RT-PCR, as well as in helicase dependant amplification targeting the CI protein gene of WSMV and TriMV, and the nucleoprotein gene of HPV. Primers were designed using the Web-interface applications and validated thermodynamic parameters. Due to high HPV strain diversity, five primer combinations were designed and tested. All primer pairs were validated *in silico* against published sequences and *in vitro* against infected plant samples. RT-PCR products were cloned and sequenced and artificial positive controls were developed. All primer sets allowed sensitive detection of all target species in infected plant samples. The RT-PCR assay is useful for multiplex detection as well as for other diagnostic applications. This assay can also be applied in breeding programs, and in biosecurity and microbial forensic investigations.

Detection and discrimination of *Pythium aphanidermatum* and *P. deliense* by single probe based real time PCR and multiplex end point PCR

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Phytopathology 101:S9

Pythium aphanidermatum and *P. deliense* are closely related species and plant pathogens causing seed disease, damping-off, and root rots on many relevant crops growing in warm regions and greenhouses. The discrimination between these two *Phytium* species is difficult if based only on morphological characteristics. To speed the specific detection and discrimination of these two *Pythium* spp. two systems are presented: 1) A single probe Taqman real time PCR in which the probe (PAD) was designed to anneal with a conserved region located between two sets of primers specific for *P. aphanidermatum* (PA4Fa /PA4R) and *P. delience* (PD1F/PD1R). These two sets of *Phytium* primers each amplify products of 143 bp. 2) A multiplex end point PCR that incorporates a new forward primer (PA4Fb) located upstream of the conserved rDNA-ITS sequence. In this assay primer PA4Fb and PA4R amplify a product of 340 bp that allows end point PCR discrimination of these two *Phytium* species. 5′ A/T rich sequence extensions were added to all primers to enhance multiplexing efficiency. None of the tested primers amplifies DNA from six other *Pythium* species. All PCR products were cloned and sequenced to confirm identity. The described PCR assays are useful for detection, quantification, microbial forensics discrimination, biosecurity monitoring, and study of Oomycete ecology and biology.

Multi-gene based detection and identification of *Phymatotrichopsis omnivora*

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Phytopathology 101:S9

The soilborne fungus *Phymatotrichopsis omnivora* is the cause of cotton root rot and of rots of several dicots in the southwestern U.S. and Mexico. Diagnosis relies on characteristic mycelial cords, which are not always obvious, on the roots of wilted plants. A method facilitating early, accurate and sensitive detection of *P. omnivora* in plant tissues is needed by plant health officials for inspection of products from quarantined locations, and by extension specialists to predict, identify and manage disease outbreaks and reservoir hosts. Specific PCR primers and probes were designed targeting *P. omnivora* genes for ITS-rDNA, beta-tubulin and the largest subunit of RNA polymerase II. Primer design was based on consensus sequences from multiple alignments. Three primer pairs and probes were validated *in silico* against published sequences and *in vivo* against infected plant samples. PCR products were cloned and sequenced to confirm identity. All primer sets allowed early detection of infected but non-wilted plants. Primer sets PO4 (116 bp product), PObt1 (126 bp product) and PORPB2-2 (135 bp product) detected as little as 1 fg of plasmid DNA carrying *P. omnivora* target sequences at cycle threshold (Ct) values of 31.24, 30.54 and 30.9, respectively. The described PCR assays are useful for pathogen detection and disease diagnosis, microbial quantification, breeding programs, and biosecurity and microbial forensics.

Implication of phenazine-1-carboxylic acid production by *Pseudomonas* sp. LBUM223 in the biocontrol of *S. scabies* causing common scab of potato

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Phytopathology 101:S9

Experiments performed in our laboratory have revealed that *Pseudomonas* sp. LBUM223, which produces the antibiotic phenazine-1-carboxilic acid (PCA), is able to significantly alter the growth of *Streptomyces scabies*, the development of common scab of potato, and the production of thaxtomin A by the pathogen, which is required for pathogenesis. A mutant of LBUM223, incapable of producing PCA, was incapable of triggering these responses. In order to better understand the impact of PCA production on the biocontrol of *S. scabies* under soil conditions, we characterized the population dynamics of LBUM223 (wild type and mutant) and the pathogen, as well the impact of PCA production on the expression of genes involved in thaxtomin A production in *S. scabies*. Potato plants were grown in pots and inoculated with different treatment combinations of S. scabies and LBUM223 (wild type and mutant) and harvested after 5, 10 and 15 weeks. Geocaulosphere, rhizosphere and bulk soil was collected and submitted to DNA and

RNA isolation. Nucleic acids were then analyzed by qPCR and qRT-PCR using specific primers and TaqMan probes to quantify population dynamics and gene expression leading to thaxtomin A production in the pathogen and PCA production in the biocontrol agent. Results suggest that alteration of gene expression leading to reduced thaxtomin A production in soil is a key mechanism for the biocontrol of *S. scabies* by PCA-producing LBUM223.

Current and future risk assessment of the spread of *Trioza erytreae* in citrus growing areas of North America
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Phytopathology 101:S10

Huanglongbing, caused by the phloem-limited bacteria *Candidatus* Liberibacter ssp., is a devastating disease of citrus vectored by two psyllids. Within North America, the Asian citrus psyllid *Diaphorina citri* Kuwayama is the only vector present. The threat of African citrus psyllid *Trioza erytreae* Del Guercio to North America remains a possibility. Previous work indicated that the Afican citrus psyllid is heat-intolerant, preferring temperatures less the 30 degrees Celsius, while the effects of precipitation on distribution have not been studied. To determine if the climatic variables of temperature and precipitation will serve as a barrier to the vector, current distribution data on citrus and T. erytreae were gathered. Specifically the two variables of annual mean temperature and annual precipitation were examined for their effects on the psyllid distribution. Fine scale ecological assessment was performed using the maximum entropy algorithm MaxEnt with Akaike information criteria to determine which climatic variables are influencing the distribution. Additionally, two atmospheric-climatic general circulation models, CCCM and CSIRO, were used to predict the changes in suitable habitat in 2050. Precipitation is more strongly correlated to the psyllid distribution than temperature placing a higher risk for southern California and western Mexico for possible psyllid introduction than Florida.

Novel broad spectrum highly potent fungicide: EV-050
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Phytopathology 101:S10

A novel proprietary molecule EV-050 exhibited high potency and broad spectrum activity against fungi belonging to Ascomycota, Basidiomycota and Oomycota. Fungi that cause devastating diseases on economically important food crops, horticultural crops, ornamental plants and trees were tested against EV-050. *In vitro* efficacy of EV-050 against resistant strains isolated from field such as *Botrytis cinerea*, *Alternaria solani*, *Coletotrichum gloeosporioides*, *Magnaporthe grisea* was assessed by radial mycelial growth inhibition and conidial germination inhibition. IC50 varied from 900ng/ml to 55 µg/ml on selective pathogens. Disease index on infected leaf disc and spore germination was assessed for biotrophic pathogens such as *Puccinia arachidis*, *P. tritici*, *Plasmopara viticola* and *Uncinula necator*. IC50 varied from 300ng/ml to 10 µg/ml. EV-050 was selective in inhibiting pathogens within and across the groups of fungi. Morphological and physiological observations revealed that EV-050 targets virulence factors by altering the hypal tip morphology and melanisation of hyphae. Also aborted and less melanised appressoria led to impaired conidial germination. These results imply that the compound has potential to be developed as an antifungal agent for plant protection against major fungal diseases. Development of formulations for field trials is under progress.

Rapid detection of *Ustilago nuda* on barley (*Hordeum vulgare*)
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Phytopathology 101:S10

Loose smut of barley (Ustilago nuda) is strictly a seed-borne fungus where the infection is located inside the embryo. By exchanging seeds, the transmission of this disease is highly likely in the field due to seed symptomless of this pathogen; therefore increasing seed movement necessitates specific solutions to guaranty smut free seeds and reduce unnecessary uses of seed treatment. ICARDA like most seed health and quarantine laboratories is detecting r Ustilago nuda using ISTA (7-013) method. This method is sensitive and reliable, but requires two days to complete the extraction. A new and fast detection method for barley loose smut was developed at ICARDA in collaboration with Aleppo university, which reduced the test's period significantly to five hours only, where about 100 g seed is soaked in NaOH for 2 h.; then heated for 30 sec at 70C0, the embryos are collected in 1 mesh; afterworlds embryos are separated using a solution of HCl, with mixture 1/1 of glycerol/water. Embryos are cleared for easier examination. The new method is fast, simple, reliable and very sensitive. This test can be used by seed health laboratories, regulatory and quarantine authorities to ensure that only loose smut seed free are introduced.

Analyses of nuclear and mitochondrial sequences reveal an ancient split in the evolutionary history of *Verticillium dahliae*
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Phytopathology 101:S10

Verticillium wilt, caused by the soilborne fungus *Verticillium dahliae*, is a ubiquitous disease of crops and ornamentals. In this study, the population structure of *V. dahliae* was examined using 16 microsatellite markers, eight nuclear exons and sequences of both nuclear and mitochondrial spacers. A total of 220 strains were collected from various hosts in four major agricultural valleys of California: Salinas, Santa Clara, San Joaquin and Sacramento. Migration analyses showed minimal gene flow among valleys. Interestingly, two major clades were consistently identified regardless of sequence targets. However, correlations of either clade with host and geographic origin, or virulence and vegetative compatibility phenotypes were undetectable. Using coalescent analyses on sequences of the eight nuclear exons, a molecular clock suggests that one cluster split soon after divergence from a common ancestor. Conversely, the larger of the two clusters appears to be more recent. Strains from Europe and South America were placed in both clusters. These two clusters reflect a historical population structure of *V. dahliae*, which predates the pathogen migration into modern day California agricultural system.

Characterization of a single chemosensory gene cluster in *Xylella fastidiosa* Pierce's disease pathogen of grape
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Phytopathology 101:S10

Virulence of *Xylella fastidiosa* Temecula1 involves the coordinate expression of a wide range of virulence factors including type IV pili which are associated with host colonization and twitching motility. Twitching motility in *X. fastidiosa* is regulated by a Pil-Chp chemosensory-like operon which is comprised of *pilG*, *pilI*, *pilJ*, *pilL*, *chpB*, and *chpC* genes and parallels the CheIV chemosensory cluster found in *Pseudomonas aeruginosa* that regulates twitching motility and pili biogenesis. Mutations in *pil-chp* genes resulted in twitching-negative phenotypes and significantly reduced disease severity on grape cv. Cabernet Sauvignon, except with *chpB* and *chpC*. The mutants *pilG*, *pilI*, *pilJ*, *pilL*, and *chpB* produced less biofilm than wild-type whereas the *chpC* mutant did not. Furthermore, *pilG*, *pilI*, and *chpB* mutants showed the least amount of aggregation followed by the *chpC* mutant. The *pilJ* and *pilL* mutants displayed the same level of aggregation as wild-type. Complemented mutants restored phenotypes to wild-type levels. These results indicate that this chemosensory system is also required for full virulence in *X. fastidiosa*.

Genetic diversity and population differentiation of *Sclerotinia sclerotiorum* collected from canola in China and in U.S.A.
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Phytopathology 101:S10

Sclerotinia sclerotiorum is an important pathogen of canola and many other crops worldwide. Genetic diversity and population differentiation of *S. sclerotiorum* collected from canola fields in Anhui Province, China (30 isolates) and in North Dakota, U.S.A. (29 isolates) were investigated in terms of genetic variation in 8 simple sequence repeat (SSR) marker loci, mycelial compatibility groups (MCGs) and three phenotypic traits: sensitivity to fungicides benomyl, iprodione and fluzinam, oxalic acid production, and pathogenicity. Significant genetic differences were observed; there were no shared SSR haplotypes and no shared MCGs between the two populations. Population differentiation was significant ($p = 0.000$) indicating lack of gene flow between the two populations. There were also significant differences between the two populations in oxalic acid production and in fungicide sensitivity. The Chinese population displayed high levels of insensitivity (faster growth rate) to benomyl and fluzinam and higher levels of oxalic acid production per unit dry weight of mycelium than did the U.S. population. However, there was no significant difference in pathogenicity between the two populations as measured by colonization of detached canola leaves. Data

suggest that despite geographic and genetic isolation the two populations of *S. sclerotiorum* were equally adapted to colonizing canola plants, and pathogenicity is under different selection pressure than the other genetic and phenotypic traits.

Role of soybean seed exudates in cultivar resistance to *Pythium aphanidermatum*

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Phytopathology 101:S11

Archer, a maturity group I cultivar with demonstrated flood tolerance and resistance to several Pythium spp., was compared to Hutcheson, a commercial group V cultivar, in response to the seed rot pathogen *Pythium aphanidermatum*. The purpose of this study was to identify and characterize seed exudates from two soybean cultivars presenting differential response to P. aphanidermatum seed rot. Dried seed composition was characterized by proximate analysis of moisture content, total phenolics, lipid content, and protein content. Isoflavones and carbohydrates identification and concentration were further determined for each cultivar. Hutcheson presented a higher concentration of carbohydrates, lipid content, and protein content, and a lower isoflavones concentration compared to Archer for both dried seed and seed exudates. An exchange exudates assay was conducted to determine the role of seed exudates to susceptibility to P. aphanidermatum. Hutcheson treated with Archer exudates presented a higher stand than Hutcheson control while Archer treated with Hutcheson exudates presented no differences in stand compared to Archer control. Likewise, a vegetative growth assay for P. aphanidermatum subjected to raw exudates showed differential diminution on radial growth with both cultivar exudates compared with the control. Overall, results suggest that seed exudates play a role in susceptibility to Pythium seed rot caused by *P. aphanidermatum* in soybean.

Development of a PCR-based assay for QoI resistance monitoring in the pecan scab pathogen, *Fusicladium effusum*

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Phytopathology 101:S11

Quinone outside inhibiting fungicides (QoIs) are an important component of integrated management programs for pecan scab, caused by *Fusicladium effusum*. The addition of salicylhydroxamic acid (SHAM) to fungicide-amended medium is toxic to *F. effusum*, rendering spore germination bioassays inadequate to quantify sensitivity of *F. effusum* to QoIs. An alternative molecular method was developed to assess QoI sensitivity in *F. effusum*, based on detection of amino acid substitutions G143A or F129L in the cytochrome bc1 protein (cyt bc1) that have been identified as the main mutations leading to QoI resistance in other fungi. A 530-bp cDNA fragment of the *cyt bc1* gene was amplified from a wild-type isolate of *F. effusum* using a primer pair set-1 designed based on highly conserved regions of *cyt bc1* gene sequences from several fungi, and sequenced. Based on the obtained sequence, a specific primer pair set-2 was designed to amplify the region containing the codons for G143 and F129 residues. DNA fragments from 77 *F. effusum* isolates collected from pecan orchards with a history of QoI use were amplified using this primer set and sequenced. Subsequent analysis revealed no differences in these *cyt bc1* sequences and all 77 isolates were predicted to be sensitive to QoIs. This qualitative procedure will enable us to detect QoI resistance in *F. effusum*, should it occur, and subsequently monitor the frequency of QoI resistance relative to the use patterns of these chemistries.

Effects of downy and powdery mildew on juice grapes in Michigan

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Phytopathology 101:S11

Viticulture in Michigan is limited by a cool and humid climate and foliar diseases, such as powdery mildew (PM) and downy mildew (DM) that require frequent fungicide applications. In juice grapes (*Vitis labrusca*), PM and DM often appear after fruit set and their impact on vine physiology is still under study. To evaluate their effects, we established two disease levels (low and high) by applying selective fungicides to control DM in 'Niagara' and PM in 'Concord' vines cropped at three levels (low, medium and high) by removing fruit clusters at 1200 growing-degree days (base 50 F, calculated from April 1). DM reached 53%, 57% and 73%, and PM <1%, 32% and 20% of the leaf area infected in 2008, 2009 and 2010, respectively. Fruit composition (Brix, pH and titratable acidity) was affected more by crop load than by disease, with the lowest Brix and pH values observed in diseased, high-cropped 'Niagara' vines. Cold hardiness of 'Niagara' canes was significantly reduced by DM in 2008 and 2010 and by high crop load in 2009. Cane cold hardiness was not significantly reduced in 'Concord'. Bud cold hardiness was unaffected in both cultivars. Starch content of the canes was reduced in both cultivars but only significantly in DM-infected 'Niagara' vines. At the levels of PM seen in 'Concord', fungicide applications did not appear to be cost-effective. However, 'Niagara' needs protection against DM to avoid a reduction in cold hardiness, particularly under high cropping levels.

Responses of *Rhizoctonia* spp. and *Sclerotium hydrophilum* to the plant extracts

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Phytopathology 101:S11

Soilborne phytopathogens affect rice production by inhabiting inoculum permanently in the soil. Adverse effects of chemical compounds in disease control strategies cause a serious problem to the natural environment. Pesticides of plant origin are preferred in order to reduce the risks involved in chemical control measure. Sixteen naturally available phytoextracts were tested in vitro for their potential to control phytopathogens of rice such as *Rhizoctonia* spp. and *Sclerotium* spp. Among the tested phytoextracts, clove, neem leaves, rosemary and pelargonium are potential phytoextracts to control of the tested soilborne phytopathogens. All of the tested fungal growths were suppressed 100% by using clove extract. Neem leaf extract, rosemary extract and pelargonium extract were found to give the second best suppression against the tested fungi. Neem leaf extract inhibited the growth of *R. solani* by 87.5%, *R. oryzae* by 92.5% and *R. oryzae-sativae* by 80.0%. However, the same extract inhibited *S. hydrophilum* by only 49.1%. Rosemary extract gave an inhibition of 67.7% for *R. solani*, 88.0% for *R. oryzae*, 86.0% for *R. oryzae-sativae* and 73.89% for *S. hydrophilum*. The inhibitory effect of pelargonium on the tested fungi showed 48.1% for *R. solani*, 90.8% for *R. oryzae*, 84.4% for *R. oryzae-sativae* and 83.3% for *S. hydrophilum*. The present finding provided the information on the sources of phytoextracts to control rice sheath pathogens.

Identifying *Macrophomina phaseolina* genes involved in phytotoxin phaseolinone production using cDNA-AFLP analysis

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Macrophomina phaseolina is a soil and seed-borne pathogen that causes charcoal rot in soybean. Charcoal rot is one of the most destructive diseases of soybean in the United States. Little is known about the mechanisms governing the interaction between soybeans and *M. phaseolina* that result in charcoal rot development. The ability of *M. phaseolina* to cause charcoal rot may be associated with the production of the phytotoxin phaseolinone by the fungus. A cDNA-AFLP (Amplified Fragment Length Polymorphism) approach was used as a qualitative and quantitative tool to identify *M. phaseolina* genes involved in the production of phaseolinone. *M. phaseolina* was grown in conditions conducive and non-conducive to the production of the phytotoxin. The cDNA AFLP screen was conducted using nine MseI/EcoRI AFLP primer combinations. Sixty four unique transcript-derived fragments (TDFs) were found to be differentially expressed across the different growing conditions. The expression patterns of the corresponding genes were confirmed by quantitative Real Time PCR. This is the first report of *M. phaseolina* genes potentially involved in the biosynthesis of phaseolinone. These findings can be used to develop new tools to study the interaction between soybean and *M. phaseolina*.

A qPCR assay to detect and quantify *Macrophomina phaseolina* in soybean roots

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Phytopathology 101:S11

Macrophomina phaseolina is a soil and seed-borne fungal pathogen that causes charcoal rot. Charcoal rot can result in significant losses in soybean yields in affected fields. The use of soybean varieties resistant to *M. phaseolina* is one of the main approaches recommended to combat the disease. Screening for resistance to charcoal rot relies on assessing disease severity by scoring for root and stem symptoms. Resistance is also assessed by estimating the extent of fungal colonization of the roots and stem of the infected plant. This is usually achieved by determining the number of fungal colony forming units (CFU) obtained from the infected plants. In this study, we have developed a new quantitative polymerase chain reaction (qPCR) assay to assess the extent of colonization of infected soybean plants by *M. phaseolina*. Two Taqman probes were designed. The specificity and sensitivity of the probes were assessed and the correlation between colonization estimates of *M. phaseolina* obtained by qPCR, CFU counts and

field symptoms rating were assessed. A specific and sensitive qPCR assay would enhance the ability to screen for resistance to *M. phaseolina* in soybean.

Bacterial spot (*Xanthomonas cucurbitae*): An emerging disease of pumpkin in Illinois

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Phytopathology 101:S12

Bacterial spot, caused by *Xanthomonas cucurbitae*, has become a serious threat to pumpkin production in Illinois. The pathogen infects foliage and fruit. Fruit infection by *X. cucurbitae*, results in fruit rot. A study in 2009 showed that the disease occurred in all 17 commercial fields surveyed in Illinois. Another survey of 50 commercial fields in 2010 showed that the disease occurred in 80% of the fields, overall 34% of fruits with the bacterial spot symptoms. Incidence of fruit infection by *X. cucurbitae* was greater than 50% in 18% of the fields, and the incidence of fruit infection exceeded 90% in 6% of the fields surveyed. During 2009–2010, 700 xanthomonad-like isolates were collected from infected pumpkin leaves and fruit using semi-selective medium [Kasugamycin-Cephalexin-Yeast extract- Peptone- Glucose-Agar (KC-Agar)]. Representative isolates (selected based on the colony morphology) were used in laboratory and greenhouse studies. Using genus-specific primers RST2 and RST3, xanthomonad isolates were selected. *X. cucurbitae* was identified based on the biochemical and physiological characteristics. Pathogenicity tests of 19 *X. cucurbitae* isolates were carried out on 3-week-old pumpkin (cultivar Howden) plants by spray-inoculation of 20 ml of the bacterial suspension (10^8 cells/ml water) on the leaves in a greenhouse. Leaf spots developed 2 weeks after inoculation and *X. cucurbitae* was isolated from inoculated leaves. Determining genetic variation among the isolates is underway.

A simple model for management of Fusarium crown rot in wheat

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Phytopathology 101:S12

Crown rot caused by *Fusarium pseudograminearum* remains a persistent problem in wheat production despite decades of research. One possible reason for this is the lack of an accessible framework for understanding the effects of rotation, resistance and other practices on populations of the pathogen in the medium to long term. A simple descriptive model was developed for the disease in bread wheat in the northern grains region of Australia. Inoculum potential was estimated from the square root of the product of crown rot incidence and yield (a surrogate for biomass) of the preceding crop. Incidence of disease was related to inoculum potential by an infection constant. If wheat was not sown in the next season, crown rot inoculum declined exponentially with time. These two relationships were combined to predict the behavior of the disease in different rotation systems. Over time, crown rot incidence converged to an equilibrium level that was determined by the infection constant, average yield, and the decomposition rate of inoculum. Modeled epidemics behaved in a way that was consistent with field data for continuous wheat, wheat-chickpea and wheat-sorghum rotations. The model is not intended to give an accurate prediction of crown rot incidence under all conditions, but rather to allow generalizations about how the disease behaves. These can then be used in extension or as a way of understanding how management and environment affect the disease.

Surfactin A isoforms characterizations in strains of *Bacillus mojavensis* for control of a maize pathogen, *Fusarium verticillioides*

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Phytopathology 101:S12

The endophytic bacterium *Bacillus mojavensis* has the potential for control of fungal diseases in maize and other plants. The bacterium and its cultural extracts have been shown to be antagonistic to the pathogenic and mycotoxic fungus, *Fusarium verticillioides*. An antifungal cyclic lipopeptide produced by 29 strains of B. mojavensis in culture was identified as isomers of surfactin A, a potent biosurfactant. HPLC-MS spectra analyses indicated that several stains of *B. mojavensis* produced Leu ^{7}-surfactin A as a surfactin, although other isoforms ranging from C-11 to C-15 were also identified. However, the data suggested that the in vitro antagonism observed on media is not correlated with total surfactin production, but may be related to amounts of specific isoforms of this biosurfactant. In this investigation, these *B. mojavensis* strains were screened on two media to determine the titer of isoforms produced and the antagonism to *F. verticillioides* induced by specific commercial surfactins A, B and C. Results indicated that there were significant levels of surfactin A produced by the surfactin producing strains during the first twenty-four hours. The results identified several strains as high producers of surfactin, as well as high producers of C-15 surfactin A, the most biologically active isoform for fungal toxicity, suggesting that it is these strains that should be used as biocontrol agents.

Analysis of the *Frankliniella occidentalis* proteome and differentially expressed proteins in response to *Tomato spotted wilt virus* infection

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Phytopathology 101:S12

The western flower thrips (WFT), *Frankliniella occidentalis*, is an insect pest of agricultural importance and worldwide distribution. It causes direct and indirect damage by feeding on plants and transmitting tospoviruses, respectively. WFT is the most efficient vector of *Tomato spotted wilt virus* (TSWV), which replicates in both the plant host and the insect vector. The overall goal of this work was to develop proteomic tools to study this insect and its interaction with TSWV. Larval thrips were collected and their proteins separated by two-dimensional gel electrophoresis. Following staining, 194 protein spots were excised, trypsinized, and subjected to liquid chromatography with tandem mass spectrometers (LC-MS/MS). The mass-to-charge ratio as a function of time was then used to generate peptide sequences that were analyzed against the NCBI Metazoan database and a WFT expressed sequence tag (EST) collection. We found that 52% and 30% of the protein spots had significant matches to the WFT EST collection and the Metazoan database, respectively. We also used a proteomic approach to identify differentially expressed proteins between TSWV exposed and non-exposed larval thrips. We found that there are indeed changes in the thrips protein profile due to virus infection. Our findings provide new insight into the molecular basis of the interaction of WFT and TSWV. Ultimately, this knowledge will enable the development of novel ways to control thrips and tospoviruses.

Genetic complementation between two viruses in an otherwise restrictive host

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Phytopathology 101:S12

Tospoviruses cause serious diseases in several important crop plants. The genome of tospoviruses consists of three RNAs, large (L), medium (M) and small (S). The L RNA is organized in negative sense orientation, whereas M and S RNAs are in ambisense. The S RNA codes for a non structural protein (NSs) in sense direction which was shown to function as viral suppressor of gene silencing in plants. We used datura (*Datura stramonium*) as a differential host for two distinct *tospovirus* species, Iris yellow spot virus (IYSV) and *Tomato spotted wilt virus* (TSWV). Following mechanical inoculation of datura, TSWV causes systemic infection, whereas IYSV infection of datura remains localized to inoculated leaves. We demonstrate that, in a mixed infection, the silencing suppressor NSs is expressed at a much higher level as compared to single infection in inoculated as well as systemic leaves. The systemic symptoms produced by TSWV in the presence of the IYSV silencing suppressor were more severe than those caused by TSWV infection alone. Even though the IYSV infection remained limited to the inoculated leaf, it was able to facilitate increased expression of TSWV NSs indicating complementation between two distinct tospovirus species.

Biological characterization of distinct strains of Iris yellow spot virus (genus *Tospovirus*)

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Phytopathology 101:S12

Thrips-transmitted Iris yellow spot virus (IYSV) is an important limiting factor to the production of bulb and seed crops. Several IYSV isolates were evaluated for their phenotype on two indicator hosts, *Datura stramonium* (a local lesion host) and *Nicotiana benthamiana* (a systemic host) following mechanical inoculation. Seedlings of both experimental hosts at four to six leaf-stages were mechanically inoculated. Host response was evaluated based on the following scorable phenotypic parameters: appearance of symptoms on inoculated leaves days post inoculation (DPI), DPI for the appearance of systemic symptoms on younger, uninoculated leaves, severity of symptoms, and effect on plant growth and vigor. Based on these parameters, two distinct strains of IYSV were identified. The duration in DPI that was necessary to

produce systemic symptoms and the subsequent death of inoculated plants varied between the mild and severe strains. In the case of the severe strain, systemic symptoms appeared 12 to 15 DPI and by 22 DPI, plants were severely infected and newly emerging leaves showed severe necrotic spots. By 50 DPI, inoculated plants died. The mild strain produced more benign symptoms as inoculated plants retained the vigor and optimal growth even after 60 DPI, with fewer new leaves showing systemic infection and the newly emerging leaves lacked symptoms.

Quantitative detection of *Verticillium longisporum* and *V. dahliae* in the soil of cabbage fields using nested real-time PCR

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Phytopathology 101:S13

Verticillium longisporum and *V. dahliae*, causal agents of Verticillium wilt, are spread in cabbage fields of Gunma Prefecture. Based on the *V. longisporum*-specific intron within the 18S rDNA and also difference of ITS 5.8S rDNA sequence between *V. longisporum* and *V. dahliae* in Japanese isolates, we developed 3 types of the quantitative nested real-time (QNRT) PCR assays; Vl-18S assay specific for *V. longisporum*, Vl-Va-5.8S assay specific for *V. longisporum* and *V. albo-atrum*, and Vd-5.8S assay specific for *V. dahliae*. Quantification of *V. longisporum* or *V. dahliae* in cabbage field soil using the QNRT-PCR assays was well consistent with the disease severity of Verticillium wilt in those fields. We also carried out the field trials to estimate the effect of the cultivation of a resistant cultivar on pathogen population in soil. When resistant cultivar YR Ranpo was planted for 3 seasons, both cabbage disease severity and pathogen density in the soil were significantly reduced in the field moderately contaminated by *V. dahliae*, but only slightly reduced in the highly contaminated field. In an additional field trial, initial pathogen density was correlated with Verticillium wilt disease severity. QNRT-PCR assays we developed here could be used to monitor the *Verticillium* pathogen population in field soils, and is a useful tool to assess the risk of Verticillium wilt prior to cabbage planting, to determine susceptibility to Verticillium wilt in cabbage cultivars.

How many species cause common and dwarf bunt of wheat?

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Phytopathology 101:S13

The wheat bunt pathogens are recognized as *Tilletia caries*, *T. laevis* (common bunt), and *T. contraversa* (dwarf bunt). Characters used to delimit these species, including host symptomology, teliospore morphology and germination, are continuous and accurate identification based on single isolates is not possible. Previous studies showing that the three species are reproductively compatible and possibly conspecific were based on a limited number of isolates that may not reflect the diversity present within this group of fungi. We tested the hypothesis that the wheat bunts represent recently diverged species, and that monophyletic lineages can be identified among a geographically diverse set of isolates. Multilocus phylogenetic analyses based on three anonymous loci and a portion of RPB2 using 60 isolates from North America, Eurasia and Australia revealed two major clades but neither corresponded to recognized species. However, there was evidence of incongruence among gene trees and network analyses revealed a reticulated pattern suggestive of recombination. Hybridization, incomplete lineage sorting, and limited genetic variation, all of which are expected among recently diverged groups, complicate our ability to delimit species within these fungi. Different approaches are required for estimating species trees including the application of stochastic models to accommodate lineage sorting and to identify species in a way that is robust to both sampling and method.

The major fungal diseases of ornamental plants in Kerman Province, Iran

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Phytopathology 101:S13

Ornamental plants play an important role in interior scapes of our homes, offices, and cities. Ornamental plants in Kerman Province are produced under a wide rang of environmental and cultural conditions and are affected by numerous pathogenic agents. Plant pathogenic fungi are the most important causal agents of ornamental plant diseases. To identify the fungal pathogens causing ornamental plants decline in Kerman Province, during 2010–2011 numerous samples were collected from ornamental plants (*Tagetes* spp., *Petunia* hybrida, *Rosmarinus officinalis*, *Poa pratensis*, *Mattiola* sp.) which were showing yellows, chlorosis and root and crown rot and decline symptoms. Several fungi were isolated from root, crown and stem of declining plants. The results showed that the prevalent species were *Phytophthora cactorum* on *Mattiola* sp., *Phytophthora palmivora* on *Petunia hybrida*, *Rhizoctonia solani* on *Rosmarinus officinalis* and *Tagetes* spp., *Fusarium solani* on *Poa pratensis*. The Kokh's postulation for each isolate were carried out and disease incidence were compared. Among fungal species recovered *Phytophthora cactorum* and *Rhizoctonia solani* were more pathogenic than the others and incidence of root and crown rot were observed more than the other disease symptoms.

Effect of immersion depth, dwell time and fruit-water temperature differences on water uptake by flumed tomatoes

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Phytopathology 101:S13

To prevent an internalization of decay and human pathogens into tomato fruit, packinghouses are instructed to limit the dwell time of fruit (≤2 min), to warm the water at least 5 C higher than incoming fruit temperatures, and to limit the immersion of fruit to 1 ft (31 cm). The impact of these requirements on water uptake by fruit and the potential for subsequent decay development is unclear. Fruit immersed to 30.2 cm are exposed to a hydrostatic pressure = 29.6 mbar. Fruit that cool from 35 to 25 C are exposed to a final pressure differential of 33.1 mbar. However, this temperature decrease requires a dwell time >5 min. In comparisons of immersion depth versus fruit-water temperature differences (fruit warmer than water) with ≤2 min dwell time, depth was much more important to water uptake than up to a 20-C difference in temperatures. This is consistent with the percentage change in pressures on a cooling fruit, where a change in degrees Kelvin (degrees Celsius + 273) affects the gas pressure inside versus outside the fruit. When suspensions of *Erwinia carotovora* were added to the water and the fruit dwell time was ≤2 min, the subsequent incidence and severity of bacterial soft rot were affected more by immersion depth than initial fruit temperature. Thus, with the current handling rules, water is likely to penetrate wounds on tomatoes exposed to a combination of the maximum depth of immersion and longest dwell time, whereas fruit-water temperature differences have little influence.

Relevance of the deposit structure for the biological efficacy of glyphosate as evaluated on four weed species

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Phytopathology 101:S13

The deposit pattern of foliar-applied agrochemicals, and its relation to the biological efficacy, has a big practical importance, but from the scientific point-of-view it is less understood. Thus, in our experiments we evaluated the relevance of the deposit properties for the bio-efficacy of hydrophilic a.i. - glyphosate. Deposition patterns were influenced by combining unformulated glyphosate with one selected ethoxylated rapeseed oil (RSO) surfactant having 5, 10, 30 or 60 ethylene oxide (EO) units. Treatment solutions were applied to the foliage of easy-to-wet *(Viola arvensis* and *Stellaria media*) and difficult-to-wet (*Setaria viridis* and *Chenopodium album*) weed species. Deposit structure was determined using scanning electron microscope with energy dispersive X-ray microanalysis. Adjuvant-aided bio-efficacy of glyphosate was observed on all weed species. Surfactants with higher EO units reduced both a.i. and droplet spread area, and enhanced glyphosate toxicity in difficult-to-wet species. In general, on these plants, increasing EO unit led to shrinking of a.i. and droplet spread area which could have increased the localized concentration, contributing for increased bio-efficacy. On easy-to-wet species, the number of EO units had only minor effect on deposition pattern and on the bio-efficacy of glyphosate. In several cases, a 'coffee-ring' like deposit was observed. Summarizing, a.i. deposition pattern does have impact on the bio-efficacy of glyphosate.

Characterization of *Tomato necrotic spot virus* (ToNSV), a new ilarvirus species infecting processing tomatoes in the Central Valley of California

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Phytopathology 101:S13

In 2008, virus-like symptoms including necrotic spots and streaks were observed in leaves, stems, and petioles of processing tomato plants in the Central Valley of California. Although these symptoms were similar to those induced by the ilarvirus *Tobacco streak virus*, tests for this and other known tomato-infecting viruses were negative. Results of sap- and graft-transmission experiments and sequence analyses of the capsid protein and replicase genes indicated that this disease was caused by a new ilarvirus species, provisionally named *Tomato necrotic spot virus* (ToNSV). ToNSV is most closely related to *Parietaria mottle virus*, another ilarvirus that infects tomato in Europe. Although ToNSV symptoms were common in processing tomatoes in the

Central Valley in 2008, the incidence was sporadic and relatively low (<%1) in most production areas in 2009. However, in 2010, ToNSV symptoms appeared early in the season and at higher incidences (up to 20% in some fields), and this was associated with high thrips populations. Additionally, the virus was detected in symptomatic field-collected peppers and onions. The results of ongoing studies on characterization and detection of ToNSV will be presented. This will include development of a rapid PCR-based detection method and studies on the mode of transmission, susceptibility of other crops and weeds, and the role of other hosts in the epidemiology of this new disease of processing tomatoes.

New species of the toxic fungal endophyte, *Undifilum*, from western United States locoweeds

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Phytopathology 101:S14

For over 100 years, locoweeds, from the genera *Oxytropis* and *Astragalus*, have been problematic for ranchers in the western United States due to their associated swainsonine toxin producing fungal endophytes. First described as *Undifilum oxytropis* from *Oxytropis* locoweeds, we present two new species of *Undifilum*. Taxonomic placement of fungi isolated from *A. mollissimus* and *A. lentiginosus* was based on growth rate, morphology, and molecular analyses. Isolates from *A. lentiginosus* grew more on alfalfa and potato carrot media than *U. oxytropis*. *Astragalus mollissimus* isolates grew only on potato dextrose media. *Undifilum oxytropis* were dark black with hardened outer surfaces, whereas isolates from *Astragalus* species did not exhibit the hard surface and were unique in coloring (*A. mollissimus* gray to black and *A. lentiginosus* tan to brown). Only fungi isolated from A. lentiginosus produced spores and these were similar to *U. oxytropis* with a slightly lower average number of septa. Maximum parsimony analyses of the ITS region and the gpd gene produced three clades (one for *Undifilum oxytropis* and one each for isolates from *A. mollissimus* and *A. lentiginosus*) and one distinct clade (*A. lentiginosus* isolates), resp. Neighbor-joining analyses of RAPD banding patterns showed one clade for *U. oxytropis* and one for isolates from *Astragalus* species. The results show fungi that are similar to *U. oxytropis* but have distinct features corresponding to new species within the genus.

Population genetics of *Eutypa lata* in the major grape-growing regions of the world and historical patterns of viticulture

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Phytopathology 101:S14

The causal agent of Eutypa dieback of grape, *Eutypa lata* (Ascomycota), is a destructive disease worldwide. The pathogen has a broad host range, but causes severe symptoms on only a few cultivated hosts (e.g., apricot & grape). To decipher its cosmopolitan distribution, we examined the population genetic structure of 19 geographic samples from grape in four continental regions (Australia, California, Europe, So. Africa), based on analyses of 287 isolates genotyped with nine microsatellite markers. High genotypic diversity in all regions (G_d = 0.9 to 1) and absence of multilocus linkage disequilibrium among loci supported the importance of sexual reproduction in all regions. The highest allelic richness (R' = 3.9) and gene diversity (H = 0.66 to 0.69) were in Europe, namely from coastal areas of the Mediterranean Sea. The lowest genetic diversity was in South Africa (R' = 1.6 to 2.9; H = 0.2 to 0.6). California, Australia and So. Africa, all of which had lower genetic diversity than Europe, were also characterized by demographic disequilibrium and, thus, may represent founding populations of *E. lata*. Low genetic differentiation among all samples (D_{EST} = 0.2, P = 0.001; F_{ST} = 0.03, P = 0.001) suggests that gene flow among continents prevents differentiation. Human-mediated spread of *E. lata*, possibly via infected plant material (from grape or another host), may have resulted in its current global distribution. High genetic diversity of *E. lata* in European samples near the Mediterranean Sea may reflect this region's more ancient history of viticulture.

Effect of fungicide seed treatments and cultivars on *Pythium* damping-off and root rot of edamame soybean

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Phytopathology 101:S14

Production of edamame soybeans is increasing in Ohio, but *Pythium* damping-off and root rot can reduce productivity. The efficacy of fungicide seed treatments and cultivars against *Pythium* spp. was tested in 2009 and 2010. Seedling emergence and severity of *Pythium* root rot were assessed 3 and 10 weeks after seeding, respectively. *Pythium* colonies were recovered from root samples on PIBNC medium. Plant biomass was determined based on the weight of plants from the center 5 ft of each row. Seed treatment with Apron XL+Maxim 4 FS+Cruiser (w or w/o *Rhizobium* inoculant) increased emergence and reduced root rot severity and the number of *Pythium* colonies compared to the untreated control in both years. Plant biomass was higher in plots treated with Apron XL+Maxim 4 FS+Cruiser than in the untreated control in 2010. Percent emergence was higher in the cultivar BeSweet 2015 than in BeSweet 2001 and BeSweet 292 in 2009. However, emergence was higher in BeSweet 292 than the other two cultivars in 2010. BeSweet 2015 had lower root rot severity and fewer *Pythium* colonies than BeSweet 2001 and BeSweet 292 in 2009 but there were no differences between cultivars in biomass. BeSweet 2015 had lower root rot severity than BeSweet 2001 and more *Pythium* colonies but higher biomass than the other two cultivars in 2010. Both cultivar and fungicide treatment should be considered to improve edamame emergence and root health in *Pythium*-prone soils.

Identification and evaluation of apple scab in Vf-resistant apple cultivars

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Phytopathology 101:S14

Apple scab, caused by the fungus *Venturia inaequalis* (Cke.) Wint., is one of the most important diseases of apples in the world. Up to 15 fungicide applications per year may be required in the eastern United States. An alternative approach for disease management is the use of *Vf*-resistant cultivars that derive their scab resistance from *Malus floribunda* 821. *Vf*-resistant cultivars were regarded as reliably resistant until 1993, when Parisi et al. reported the development of a new race of scab, capable of infecting the *Vf*-resistant cultivar, 'Prima', in Europe. Due to the process of introgressing the *Vf* gene into different genetic backgrounds to develop differing fruit qualities, cultivar resistances to scab were noted, and the need to identify which *Vf*-possessing cultivars are durably resistant to scab remains. In 2007, we identified scab on *M. floribunda* 821 in a breeding block in West Lafayette, IN. Field evaluations were performed in the following years in this breeding block, and has resulted in the identification of four *Vf*-resistant cultivars as being susceptible to a new race or races of *V. inaequalis*. These newly susceptible cultivars include 'Pristine', 'Enterprise', 'Jona Free' and both F2 progeny from Crandall's original crosses, but not 'Prima'. Laboratory evaluation of pathogenicity by leaf disk assay was consistent with field studies, and suggests that some cultivars may be escapes from this new population of *V. inaequalis* that can infect Vf-resistant cultivars.

Detection of *Colletotrichum cereale* specimens from modern and historical collections using culture-independent, real-time PCR methods

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Phytopathology 101:S14

Colletotrichum cereale, the causal agent of anthracnose, is commonly found on cereals, forage and prairie grass, and cool-season turfgrasses. Molecular analysis of *C. cereale* has revealed considerable diversity within the species, with isolates forming two primary lineages (clade A and clade B). Research of *C. cereale* is limited by the need to establish pure cultures, a time-consuming process that requires several sub-culturing steps and is often hindered by the presence of faster growing organisms. To test whether *C. cereale* could be detected directly from DNA extracted from infected plant tissue, two real-time PCR probes specific for clades A and B were developed using the single-copy Apn2 (DNA lyase) gene. Over 730 cultured isolates of *C. cereale* from turfgrass, prairie, and wheat hosts were screened, along with 23 field samples collected from diseased annual bluegrass putting green turf, 32 asymptomatic wheat plants, and 106 herbarium specimens on various host substrates. The probes were 100% accurate for *C. cereale* detection from cultured samples and for the discrimination of known clade A and clade B isolates (avg. cycle threshold (CT): A = 28.15; B = 26.82). *C. cereale* was also successfully detected in a clade-specific manner from diseased field samples and 68–103 year-old herbarium specimens (avg. CT = 24.88 and 34.75, respectively). These probes will be useful for culture-independent, high-throughput molecular analysis of *C. cereale* populations.

Effects of acute low temperature events on establishment of *Erysiphe necator* and susceptibility of *Vitis* species

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Phytopathology 101:S14

The obligate biotrophic pathogen *Erysiphe necator* causes grapevine powdery mildew, and coevolved with its hosts: Vitis species native to eastern North America. When the warm-climate species *Vitis vinifera* (the European winegrape) was exposed to acute cold events, there was a significant reduction in conidial germination, sporulation, and colony development. Our objective was to investigate the effects of similar acute cold treatments using the grape species *V. labrusca*, *V. rupestris*, *V. amurensis*, *V. riparia*, and *V. cinerea*, which represent a gradient of cold-tolerance and climatic range within Vitaceae. Leaves from each species were exposed to 2C for 8 h, incubated for an additional 24 h at 24C for 24 h, inoculated with conidia, and finally incubated at 24C for an additional 44–48 h. Control leaves did not undergo cold treatment. Commassie Blue stain was used in microscopic assessments of conidial development as (i) germtube only, (ii) primary hyphae or (iii) branched hyphae. Results showed that cooler climate Vitis species, *V. labrusca* and *V. rupestris*, did not demonstrate cold-induced resistance to powdery mildew while *V. vinifera* and *V. amurensis*, warm climate species, did demonstrate cold-induced resistance. *V. riparia*, and *V. cinerea* exhibited a higher basal level of resistance that was nonetheless enhanced by the cold shock treatment.

Achieving sustainable potato production through the use of new potato varieties with reduced fungicide requirements

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Phytopathology 101:S15

One of the primary goals of the potato industry is to enhance sustainability by optimizing the efficient use of agronomic inputs. One approach to achieving this goal is to exploit the higher levels of disease resistance in new potato varieties. The Northwest Potato Variety Development Program (NPVDP) has developed new varieties with higher disease resistance than the standard, Russet Burbank. The objective of this study was to determine the extent to which fungicide inputs for newly released NPVDP potato varieties can be reduced. The performances of five NPVDP varieties and Russet Burbank were evaluated in traditional and reduced-fungicide management programs. Plots were fumigated with metam sodium at zero, low, medium and high rates prior to planting to control Verticillium wilt. Fungicide treatments consisted of a seed treatment, in-furrow treatment and one foliar fungicide application, a seed treatment, in-furrow application and four foliar applications, and no fungicides. Foliar disease severity of early blight and white mold over the season was rated as the relative area under the disease progress curve. Levels of Verticillium wilt were estimated in the field by visually rating plant wilt and soil levels of the wilt pathogens *V. dahliae* and *Colletotrichum coccodes* were assessed by qPCR. Yield data were collected at harvest. Overall results showed that all NPVDP varieties performed well under reduced-fungicide management programs and significantly better than Russet Burbank.

Economic analysis of small plot and on-farm fungicide trials on soybean in Iowa

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Phytopathology 101:S15

Fungicide use on soybean has greatly increased in Iowa during the last decade and a growing number of producers each year are applying fungicides to the crop. With numerous fungicides available for soybean producers, it is important to be able to quickly compare and evaluate fungicide efficacies. In Iowa, fungicide trials have largely been conducted either as on-farm research studies using strip test comparisons or as small plot studies. On-farm research arguably has more real life application since larger test strips that span an entire field are used to compare one or two products with a nonsprayed control. In small plot research, the efficacy of many fungicide products is more easily compared by using a small area. The goal of this research is to compare yield responses of on-farm research and small plot research through economic analyses that are formulated to show profitability of spraying fungicides. Data obtained for these analyses were collected between 2006 through 2009 from on-farm research conducted by the Iowa Soybean Association and Iowa State University, and from small plot trials conducted by Iowa State University by multiple researchers. Mean yield responses in on-farm fungicide trials was 2.42 bushels per acre compared with 1.67 bushels per acre in small plot research. Our aim is to improve how fungicides are evaluated in Iowa and also improve soybean management recommendations for growers.

Quantification of *Cylindrocarpon* sp. in roots of almond and peach trees from orchards affected by Prunus replant disease

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Phytopathology 101:S15

Prunus replant disease (PRD) is a poorly understood soilborne complex that suppresses replanted almond and peach orchards in California. Using culture-dependent and culture-independent approaches, we found *Cylindrocarpon* (Cyl) *macrodidymum* among microorganisms associated with PRD. We developed a qPCR assay to further examine the Cyl-PRD association; a selective primer pair that amplified a 374-bp rDNA fragment from *C. macrodidymum* was coupled with a specific hydrolysis probe. The assay was optimized and validated using genomic DNA from the target and 70 non-target microorganisms and rootstocks. The lower detection limit was 100 fg Cyl DNA per 25 µl of PCR mix. The assay was used with root samples from replicate healthy and PRD-affected almond and peach trees (in fumigated and non-fumigated plots, respectively) in five California orchards. All orchards were planted in winter and expressed PRD symptoms the following summer. Root samples were collected on 1 to 5 dates per orchard from Apr.-Sept. of the year trees were planted. In orchards 1-3, Cyl levels were significantly higher in PRD-affected than in healthy roots on some dates (7 of 11 sampling dates), but in orchards 4 and 5 (1 date each) Cyl levels were near the lower detection limit and did not differ in relation to PRD incidence. We conclude that Cyl concentration in roots is positively associated with PRD in some orchards; the relationship may be seasonal, requiring systematic temporal sampling for quantification.

A novel endophytic biocontrol agent of oomycete pathogens with the activity of plant growth promotion, resistance induction and nitrogen fixation

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Phytopathology 101:S15

A novel antagonistic bacterial strain against oomycete plant pathogens (*Pythium ultimum* and *Phytophthora capsici*) was isolated from surface sterilized root of a halophyte, *Rosa rugosa*. On the basis of morphological, physiological, biochemical characteristics and phylogenetic analysis, the strain was found to be a new species of genus *Martelella* and proposed as *Martelella endophytica* YC6887 sp. nov. Strain YC6887 could inhibit mycelial growth of *Py. ultimum* and *P. capsici* on yeast casein soybean medium. The incidence of Phytophthora blight on pepper treated with the strain was significantly lower than that of non-treated control in pot tests under greenhouse condition. Antibiotic metabolite produced in culture media was extracted with ethyl acetate and purified by column chromatography. In pot bioassay using Arabidopsis and pepper as host plants to determine plant growth promotion and induced systemic resistance, strain YC6887 could promote the growth of both plants by drenching of cell suspension to the pot and induce systemic resistance of Arabidopsis against infection by the bacterial leaf pathogen *Pseudomonas syringae* pv. *tomato*. To examine whether the strain is able to colonize plants endophytically, the *gfp* gene tagged strain YC 6887 was introduced and observed by fluorescence stereomicroscopy. This *gfp* gene tagged bacteria colonized intracellular spaces in the leaf of tobacco. Additionally this strain formed nodules on the root of soybean and was found to have *nod* and *nif* genes.

Molecular detection of banana bacterial soft rot pathogen, *Dickeya* sp. (*Pectobacterium chrysanthemi*)

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Phytopathology 101:S15

Dickeya sp. (*Pectobacterium chrysanthemi*) is a plant-pathogenic enterobacterium responsible for soft rot disease in a wide range of species. The gram-negative bacterium first identified on banana in 2009 at Panyu, Guangzhou, China, was accountable for significantly economic losses to banana production. Polymerse chain reaction (PCR) techniques offer advantages over traditional methods of detection and diagnosis. At present, primers for detecting *Dickeya* sp. (*P. chrysanthemi*) are not available. In this study, based on differences in 16S-23S rDNA internal transcribed spacer (ITS) sequences of *Dickeya* sp. (*P. chrysanthemi*) and other bacteria, a pair of species-specific primers, LF/LR was synthesized. The specificity and sensitivity of the reaction were tested and the PCR protocols were used to detect diseased plant tissues, irrigation water, and diseased soil samples collected in the field. Specificity was tested against more than eight bacterial organisms associated with banana and the LF/LR primers amplified only a single PCR band of approximately 171 bp from *Dickeya* sp. (*P. chrysanthemi*). The detection sensitivity was determined to be 0.44 fg for pure

genomic DNA per 25 µl reaction volume. The results suggested that the assay detected the pathogen more rapidly and accurately than standard isolation methods. The PCR-based methods developed here could simplify both plant disease diagnosis and pathogen monitoring, as well as guiding plant disease management.

Fusarium head blight in southeastern Idaho

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Phytopathology 101:S16

Since Fusarium Head Blight (FHB) was identified in the late 1800s, cultural practices, crop rotations, and weather patterns have changed, allowing for the development of FHB in areas where it was previously rare to non-existent. The primary causal organism of FHB in Idaho is *F. culmorum*, whereas in other areas of the country, *F. graminearum* predominates, especially following corn. Every year, the University of Idaho Extension program plants variety trials in several locations throughout the region, including varieties representative of the barley and wheat varieties present in Southern and Eastern Idaho. During the summer of 2010, varieties in the Idaho Falls trial developed FHB symptomatic wheat heads. Observational analysis revealed the presence of FHB in several hard spring wheat varieties during late July 2010. This has been the only variety trial in Southern Idaho that reported visible symptoms and signs of infection during the 2010 season. Each head of hard spring wheat in this trial was inspected for visible symptoms of infection during late July and early August of 2010. Isolates were obtained from FHB infected samples and the primary species causing infection were identified. Initial identification determined the primary causal species to be *F. culmorum* with minimal contribution by other species, including *F. graminearum*. Species prevalence will influence recommended crop rotations and potential economic damage to the local grain industry.

Volatile-mediated plant growth promotion by *Fusarium oxysporum*

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Phytopathology 101:S16

Fusarium oxysporum is a cosmopolitan soil-borne fungus that is known to cause wilt diseases in a large number of crop plants. However, not all *F. oxysporum* isolates are pathogenic, and some isolates have been used as biocontrol agents protecting plants from other pathogens. We have discovered, that certain *F. oxysporum* strains produce volatile compounds that promote growth of the model plant *Arabidopsis thaliana*. 47 *F. oxysporum* isolates belonging to 22 *formae speciales* were co-cultivated with *A. thaliana* seedlings in Petri dishes containing a center partition, allowing only for the exchange of gasses between the two sides of the dish. After 14 days, plant fresh weight measurements revealed seven *F. oxysporum* isolates that strongly promote plant growth through the emission of volatile compounds. In order to determine the mode of action and perception mechanism by which these volatiles affect plant growth, we studied how *A. thaliana* mutants, defective in several hormonal regulatory pathways controlling plant growth, respond to these volatiles. We are also studying the cellular and developmental changes of *A. thaliana* plants in response to *F. oxysporum* volatiles and identifying those compounds through the use of gas chromatography and mass spectrometry. Identification of fungal volatiles underpinning plant growth promotion and the elucidation of their synthesis and mechanisms of action will help us better understand chemical-mediated plant-microbe interactions in the soil.

Switchgrass rust epidemics (*Puccinia emaculata*) in agronomic fields in Tennessee

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Phytopathology 101:S16

Recently, agronomic switchgrass production has increased due to its usage as a crop for production of cellulosic ethanol. In July and August 2007, *Puccinia emaculata* was reported to cause rust disease on switchgrass in Tennessee. In this study, four agronomic fields of switchgrass in eastern Tennessee were evaluated to determine the epidemiological characteristics of rust on switchgrass. Individual leaves on twenty-five plants were rated for disease severity in each of the four fields once per week over a period of fifteen weeks. Disease progress curves were then developed from disease severity data in order to make comparisons between plots as well as between fields. Rust was first detected in late May. Disease severity progressed exponentially, leveling off in late August to early September. Log phase of disease progression occurred between mid-June to mid-August. Final disease ratings were taken immediately before harvest, revealing an average of greater than five percent of leaf surfaces were infected. Leaves began dying in mid-to-late June, but it is not apparent if death was caused by disease or other factors.

Establishment of a foundational Federal-academic partnership for the enhancement of forensic plant pathology

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Phytopathology 101:S16

The U.S. National Biosecurity Defense Plan recognizes the need for robust capability in agricultural biosecurity and microbial forensics. A collaborative partnership between the National Institute for Microbial Forensics & Food and Agricultural Biosecurity (NIMFFAB) and the National Bioforensics Analysis Center (NBFAC) builds strengths in forensic plant pathology. NBFAC's forensic range is expanded by collaborative research with NIMFFAB to develop and validate real time PCR assays for forensic applications to plant pathogens. As an NBFAC Spoke Laboratory, NIMFFAB provides a link with the plant pathology community through the American Phytopathological Society's Microbial Forensics Interest Group, in which academic, industry and Federal phytopathologists and security personnel interact and provide input to programs and priorities. As a part of Oklahoma State University, NIMFFAB prepares graduate students through coursework, research and internships to meet future staffing needs of security agencies. Finally, NIMFFAB conducts focused outreach and training to increase awareness and appreciation among the plant pathology and security communities for their respective roles in preventing and responding to potentially criminal plant health emergencies. The NIMFFAB-NBFAC relationship enhances the security of our nation's plant resources and agricultural enterprise, and demonstrates the potential for multi-disciplinary collaborative efforts between academia and government.

Gene trees versus species trees for resolving the *Phytophthora* Clade 1C phylogeny

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Phytopathology 101:S16

Phytophthora Clade 1C contains foliar blight pathogens, including the causal agent of potato and tomato late blight, *P. infestans*. Four of the five species within this subclade are found sympatrically in the central highlands of Mexico; a fifth species, *P. andina*, has recently been described from the Andean highlands of Ecuador. These species are found on specific plant family hosts, although *P. infestans* and *P. andina* infect diverse sections of the Solanaceae. Due to their rapid and most likely recent diversification, resolving the branching order among the Clade 1C species is challenging. Here we have used sequence data of seven nuclear and six mitochondrial loci, including intron and spacer regions, from 58 isolates to reconstruct a robust species tree. We have also used computational and experimental methods to identify haplotypes within the heterothallic members of this group. Although phylogenetic signal is low, we have used maximum likelihood methods to test several hypotheses for the temporal diversification of this important group.

Comparison of nine PCR primer sets designed to detect *Pantoea stewartii* subsp. *stewartii* in maize

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Phytopathology 101:S16

Pantoea stewartii subsp. *stewartii*, the causal agent of Stewart's bacterial wilt of maize, is a major quarantine pest in maize seed. Verifying freedom from *P. stewartii* remains a significant hurdle in exporting corn seed from the U.S. Several PCR primer sets have been developed and suggested as being potentially useful for routine seed health testing. Nine published PCR primer sets were evaluated for their ability to specifically detect *P. stewartii* and for potential cross reactivity (false positives) with other *Pantoea* species. Six conventional PCR primer sets and three real-time TaqMan primer sets were compared using *Pantoea* isolates that included *P. stewartii*, *P. agglomerans*, *P. ananatis* from multiple hosts, and non-*P. stewartii* isolates from maize. The primer sets targeted sequences within the *cps* gene cluster, *hrpS* gene, 16s rRNA ITS region, and the *pstS-glmS* region. None of the primer sets was 100% specific for *P. stewartii* exclusively. Each primer set amplified DNA from additional isolates that included *P. ananatis* and/or *Pantoea* (non-*P. stewartii*) isolates from maize seed. These results suggest that these primer sets may not be suitable for use in routine testing for *P. stewartii* in maize seed due to the potential for false positive reactions.

Pestalotiopsis and _Colletotrichum_ species causing latent infection on persimmon fruits in Brazil

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Phytopathology 101:S17

Persimmons grown in Brazil have suffered an anthracnose disease (*Colletotrichum gloeosporioides*) causing early defoliation and twig cankers and, recently, a *Pestalotiopsis* sp. was also identified causing twig and branch cankers. Fruits appear damage at harvest and postharvest storage. In addition, unripe, putatively infected fruits drop before harvest. The objective of this study was to evaluate the incidence of latent infection of both pathogens in unripe persimmon fruits. The level of latent infection was determined using the overnight freezing incubation technique. Samples of 50 unripe fruits of the cultivars 'Fuyu' and 'Kakimel' were collected in two orchards, first was under an organic and second under a conventional production system. Fruit were collected 120 and 150 days after full bloom. The fruits were surface-sterilized with 92% ethanol, sodium hypochloride at 400 µg/ml+0.5 mL of Tween 80, and water solution for 5 min. After placing the fruit in a freezer at –15°C for 20 h, the fruits were incubated in a moist chamber at 25 ± 2°C, and evaluated daily for 7 days. The first symptoms of *C. gloeosporioides* appeared after 3 days while 80% of the Fuyu fruits showed infections after 7 days (both collections). Furthermore, 66 and 87% of the fruit of the first and second collection, respectively, had infections by a Pestalotiopsis sp. However, in Kakimel fruits, 61 and 36% were indected by *C. gloeosporioides* and 6.7 and 19% by a *Pestalotipsis* sp., respectively, for the first and second fruit collections.

Typhula ishikariensis and _Typhula incarnata_ vary in sensitivity to fludioxonil, propiconazole and chlorothalonil

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Phytopathology 101:S17

High elevation golf courses in Colorado typically treat their putting greens and fairways with preventive fungicides in late October each year to reduce damage caused by gray and speckled snow molds (*Typhula incarnata* and *T. ishikariensis* respectively). Over the years, we have observed that applications of chlorothalonil (10–100 ug/g) and fludioxonil (1–5 ug/g), either alone or in combination, fail to provide adequate control of snow molds even though their concentrations in the verdure under snow cover do not diminish significantly during winter. We tested the *in vitro* sensitivity of 200 *T. incarnata* and *T. ishikariensis* isolates to various concentrations of fludioxonil, propiconazole and chlorothalonil. Fludioxonil at 1 ug/g completely inhibited the growth of 90% of the isolates and the rest were inhibited at 10 ug/g. Chlorothalonil at 1–10 ug/g inhibited or significantly reduced the growth of the majority of *T. ishikariensis* but not *T. incarnata* isolates. Growth of all but three isolates was inhibited by more than 80% at 10 ug/g propiconazole, but growth of many isolates was unaffected at 1 ug/g. These results indicate intra- and interspecific variability in sensitivity to these fungicides, but do not explain why they are not more effective in the field.

Onion cultivar resistance to _Iris yellow spot virus_ and onion thrips in Colorado

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Phytopathology 101:S17

Onion, Allium cepa, is the most important monocot outside of the grasses. The crop suffers significant physical damage from onion thrips, Thrips tabaci, which also vectors an important tospovirus, iris yellow spot virus (IYSV). Synthetic pyrethroid, organophosphate and carbamate insecticides are intensively used for onion thrips control. Due to high risk of insecticide resistance development, and environmental and user safety issues with insecticide use, research is underway to ensure U.S. onion sustainability through breeding and genomics, with the goal to identify, validate and deliver resistances to IYSV and its vector. Field evaluations were conducted in 2009 and 2010 in northern Colorado and southern New Mexico to identify onion cultivars with resistance to IYSV and/or onion thrips. Eighty two and 109 germplasms, respectively, were evaluated in 2009 and 2010 for thrips populations and IYSV incidence and severity. Agronomic characteristics including number of leaves per plant, leaf color, days to bulbing, cropping and maturity, as well as final bulb size and weight, were determined. In 2009 and 2010, 16 and 18 entries respectively, were resistant to the virus and thrips in Colorado. Of these, PIs 258956, 264320, 546140, 546188 and 546192 were consistently resistant, had acceptable agronomic traits, and were selected as candidates for the translational genomics study coordinated by our colleagues involved with the USDA-SCRI Project 2008-04804.

Effects of _Iris yellow spot virus_ and onion thrips on onion physiology, growth and productivity

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Phytopathology 101:S17

The tospovirus *Iris yellow spot virus* (IYSV) and its vector, the onion thrips (*Thrips tabaci*) are yield-limiting pests of onions in all onion production regions where these pests are prevalent. The polyphagous vector damages plants by disrupting the leaf surface and removing mesophyll cell contents. This feeding habit adversely affects the photosynthetic capacity of plants. In 2009 and 2010, greenhouse experiments were conducted to investigate the effects on onion physiology, growth and productivity of 4 treatments: thrips only (T), IYSV only (V), Thrips+IYSV (TV) and a healthy control (HC) for a field resistant variety, Colorado 6 and a susceptible variety, Talon. Net photosynthesis (P_N), stomatal conductance and intercellular CO_2 content showed a decreasing pattern for all treatments throughout the season but the rates were generally higher in Colorado 6 than in Talon. In both varieties, P_N taken at different PAR at 127 DAS showed a similar trend for all treatments but was significantly lower for V. There was no variety difference for both bulb weight ($P = 0.9073$, $F = 0.01$) and bulb size ($P = 0.0734$, $F = 3.30$). However, there was significant treatment difference for bulb weight ($P < 0.0001$, $F = 49.84$) of 57.94, 36.77, 20.12 and 13.99 g/bulb, respectively, for HC, V, T and TV. There also was a significant bulb size difference between the treatments ($P < 0.001$, $F = 22.92$) with HC having the largest bulb size of 4.50, followed by V, then T and finally by TV with sizes 4.11, 3.48 and 3.07 cm diameter, respectively.

Activity of citrus canker lesions on leaves, shoots and fruit of grapefruit in a Florida orchard from June 2010 to January 2011

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Phytopathology 101:S17

Lesions of citrus canker, caused by Xanthomonas citri subsp. citri (Xcc), on citrus fruit preclude export to certain markets. Characterizing the population dynamics of bacteria in canker lesions in commercial orchards can help gauge risk associated with diseased fruit entering fresh markets. The aim of this study was to quantify and compare lesion activity in citrus tissues up to harvest in an east central Florida grapefruit orchard. Each month from June 2010 to January 2011, twenty, fifty and eighty lesions were sampled from shoots, leaves and fruit, respectively. Lesion activity was quantified by dilution plating on nutrient agar and bioassay by injection infiltration into leaves of cv Duncan grapefruit. From June 2010 to January 2011, linear regression analysis indicated a decline in the proportion of active lesions for fruit from 98% to 6% ($R^2 = 0.80$), for leaves from 100% to 66% ($R^2 = 0.44$) and for stems from 45% to 10% ($R^2 = 0.41$). Lesion activity was most erratic for stems. The maximum bacteria flux density (BFD, mm^{-2} min^{-1}), a measure of inoculum production was 2.7 × 105, 2.4 × 105 and, 1.4 × 104, bacteria mm^{-2} min^{-1} on fruit, leaves, and stems, respectively. Although the greatest reduction in BFD occurred for fruit lesions, considering lesions are active up to the point of harvest there is a role for postharvest disinfection treatments to mitigate Xcc on fresh fruit.

Evaluation of phosphite to control scab on pecan in the southeastern U.S.A.

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Phytopathology 101:S17

Pecan scab (caused by *Fusicladium effusum*) infects pecan and severe disease can cause yield loss. The efficacy of phosphite, an elicitor of systemic acquired resistance (SAR) was evaluated in field experiments in 2009 and 2010. Biweekly applications of phosphite (Prophyt at 2.64 L 1000 L^{-1} ha^{-1}) were compared to an industry standard fungicide, triphenyltin hydroxide (TPTH, Super-tin at 0.90 L 1000 L^{-1} ha^{-1}). Both phosphite and TPTH reduced scab provided equally good control of leaf scab, with the exception of one of the TPTH treatments in 2010. Phosphite and TPTH also gave equally good control of scab early in fruit development (Jul/Aug); however, by the final assessment (Sep/Oct), fruit scab severity on phosphite treated trees was greater than those receiving TPTH and in 2010, scab severity was equivalent to the untreated control. There was no difference in fruit volume between phosphite and TPTH-treated plots in 2009, and no difference in nut volume between treatments in 2010, although there were treatment differences in kernel weight and fruit weight in 2010. Results indicate that phosphite provides useful control of pecan scab on both foliage and fruit early in the growing season, but does not provide as good prolonged late-season protection compared to an industry standard, TPTH.

Continued deployment of moderate resistance to Cephalosporium stripe in Kansas winter wheat cultivars

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Phytopathology 101:S18

Cephalosporium stripe was a significant problem in Kansas during the 1970s because of the widespread planting of highly-susceptible cultivars. However, beginning with the release of Arkan in 1982, Kansas has had a history of releasing cultivars with moderate levels of resistance. As a result, stripe has declined to trace levels. Because of the virtual disappearance of stripe, little conscious effort is currently placed on developing cultivars with resistance. Due to the lack of emphasis, it is important to periodically test the reaction of cultivars to stripe. Therefore, six winter wheat cultivars were tested in the field for their reaction to stripe. Sturdy was the susceptible check and Plainsman V the moderately-resistant check. Karl 92 was released in 1992, Jagger in 1994, Fuller in 2006, and Everest in 2009. Karl 92, Jagger, and Fuller have been very popular and risen to be the number one cultivar in Kansas. In the field, the susceptible cultivar Sturdy showed 77.6% whiteheads and 51.7% yield loss while moderately-resistant Plainsman V had only 25.4% and 24.1%, respectively. All of the popular Kansas cultivars had disease severity and yield loss values which were equal to or less than Plainsman V. Thus, moderate levels of resistance to stripe appear to have been maintained in cultivars popular in Kansas during the past 20 years including the two most recent releases from Kansas State University (Fuller and Everest).

Multiplex detection of *Phytophthora*: Padlock probe based Universal detection Multiplex Array (PUMA)

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Phytopathology 101:S18

Phytophthora spp. is responsible for many diseases worldwide, can occur on a wide range of different crops and the number of species is increasing. To detect and identify those species several molecular methods have been developed for single species only. A uniform method for detection of all *Phytophthora* species would be very useful for research and regulatory communities. Therefore we developed a diagnostic method to detect a range of *Phytophthora* species, including *P. ramorum*. The method includes a generic TaqMan PCR amplification method for all *Phytophthora* species combined with species specific padlock probe (PLP) detection on a dedicated universal micro-array. Twentythree padlock probes for 22 *Phytophthora* species relevant for the Netherlands were developed based on sequence differences in the ITS-1 region. After point mutation specific ligation of a mixture of the 23 PLPs on the generic amplicon, exonuclease treatment to degrade the unreacted probes, amplification of the ligated probes and hybridization on a micro-array, a unique signature on the micro-array can be obtained for each *Phytophthora* species included in the test. In this paper the specificity and sensitivity of a padlock based diagnostic tool is combined with a cost effective microtiter plate array detection device and has been evaluated using reference *Phytophthora* cultures as well as mixed infected material collected from field surveys, including air-, root-, water- and plant tissue samples.

Influence of defective RNAs of *Tomato black ring virus* on symptoms expression

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Phytopathology 101:S18

Tomato black ring virus (TBRV) infects a wide host range, including vegetables, grapevine, soft fruits (raspberry, strawberry), fruit trees and non-cultivated species. TBRV genome consists of two positive-sense, single-strandedRNAs of about 7500 and 4500 nucleotides for RNA1 and RNA2, respectively. A small additional satellite RNA occurs in some isolates. The existence of defective RNA molecules (DIs) associated with TBRV has also been shown. D-RNAs frequently from virus genome during prolonged incubation in a host species and affect on symptoms. We collected several isolates of TBRV from different plants species. The goal of our work was to confirm whether defective particular act as silencing factors during viral infection. For each isolate originally devoid of defective RNA serial passages in *Chenopidum quinoa* have been performed. After each passage cycle virions purification and RNA extraction was performed. The RNA was isolated from purified viral particles of all isolates after 6, 10, 15 and 20 passages. Analysis of viral RNA revealed the presence of an additional, small RNA segments in the three Polish isolates collected from *Robinia pseudoacacia* and tomato after 15 passages. No additional bands appeared from isolates collected from zucchini and potato. Those isolates in which small genomic RNA segments were generated induced milder symptoms in comparison to TBRV without D-RNAs. This findings can be used in developing new strategies for plant protection.

Fusarium ear rot pathogens and their mycotoxins associated with South African maize

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Phytopathology 101:S18

Maize is the most important agricultural crop produced in Southern Africa, and is consumed daily by millions of Africans as a staple food. In South Africa, the crop is often affected by ear rot pathogens belonging to the genus *Fusarium* and their mycotoxins. To determine the prevalence of *Fusarium* species and their toxins, samples were collected from two susceptible maize cultivars at 14 localities in South Africa during 2008 and 2009. *Fusarium* species was quantified by real-time PCR and their mycotoxins by multi-toxin analysis using HPLC-MS. In 2008, *F. graminearum* was the predominant species in the eastern Free State, Mpumalanga and KwaZulu-Natal provinces, while *F. verticillioides* was predominant in the Northwest, the western Free State and the Northern Cape provinces. In 2009, maize ear rot infection was higher and *F. graminearum* became the predominant species found in the Northwest Province. *Fusarium subglutinans* was associated with maize ear rot in both years at most of the localities, while *F. proliferatum* was not detected from any of the localities. Deoxynivalenol and zearalenone correlated well with the amount of *F. graminearum* found in maize grain, fumonisins with *F. verticillioides*, and moniliformin and beauvericin with *F. subglutinans*. Our findings suggest a shift in the occurrence of *Fusarium* species and their mycotoxins in South African maize, which could be contributed to changing agricultural practices and climatic changes in production areas.

Meta-analysis of *Solanum* resistance gene analogs—towards a comprehensive catalog of R-gene alleles for research and crop improvement

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Phytopathology 101:S18

Wild *Solanum* species are rich sources of disease resistance (R) genes for improvement of potato, tomato, and eggplant. Comprehensive surveys of *Solanum* R-gene alleles will enable research on R-gene evolution and provide informed criteria for the use of wild germplasm. Most R-genes encode a nucleotide binding site (NBS). PCR-based approaches targeting NBS-encoding DNA sequences have been developed, yielding fragments termed "resistance gene analogs" (RGAs) that originate from R-genes. We generated 91 RGAs from a disease-resistant relative of potato, *S. bulbocastanum*, complementing smaller existing RGA collections from six *Solanum* species. By combining 236 RGA and 42 cloned R-gene sequences into a single meta-analysis, we generated SolaR80, a comparative sequence-based framework for assigning gene fragments to R-gene lineages that reflect both evolutionary relationships (and probable orthology) and DNA cross-hybridization results. The SolaR80 framework provides *Solanum* researchers with a common terminology for cross-species analyses. Our research shows that most R-gene lineages are present in most *Solanum* species. One R-gene lineage has undergone expansion and diversification in *S. bulbocastanum*. We hypothesize that this gene lineage is of significance to the health of the species. Our ongoing efforts include a bioinformatics survey of whole genome sequence of tomato and potato and next generation sequencing of R-gene alleles from a broad array of *Solanum* species.

FRET probe genotyping of *Xylella fastidiosa* strains

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Phytopathology 101:S18

Epidemiological studies of Pierce's disease (PD) can be confounded by a lack of genetic information on the bacterial causative agent, *Xylella fastidiosa* (*Xf*). PD in grape is caused by genetically distinct strains of *Xylella fastidiosa* subsp. *fastidiosa* (*Xff*), but is not caused by numerous other strains or subspecies of *Xf* that typically colonize plants other than grape. Detection assays such as ELISA and qPCR are effective at detecting and quantifying *Xf* presence or absence, but offer no information on *Xf* subspecies or strain identity. Surveying insects or host plants for *Xf* by current ELISA or qPCR methods provides only presence/absence and quantity information for any and

all *Xf* subspecies, potentially leading to false assessments of disease threat. This study provides a series of adjacent-binding fluorescence resonance energy transfer (FRET) DNA melt analysis probes that are capable of efficiently discriminating *Xf* subspecies and strain relationships in rapid real-time PCR reactions. These dual hybridization probes are used on *Xf* positive insect and grape DNA extractions to provide *Xf* genotype information, and constitute a Multilocus Melt Typing (MLMT) assay for *Xf*.

Disease severity and microsclerotium properties of the sorghum sooty stripe pathogen, *Ramulispora sorghi*

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Phytopathology 101:S19

Ramulispora sorghi causes sooty stripe of sorghum. Disease severity in irrigated and dryland plots was measured for 25 susceptible genotypes during the 2007 and 2008 growing seasons using a rating scale based upon percent leaf infected. Disease severity ratings were approximately 1.4 points higher ($P < 0.0001$) on the rating scale in the irrigated plots than dryland plots for 2007 and 2008. Sooty stripe lesions were collected from each sorghum genotype in irrigated plots and assessed for mean microsclerotium production within lesions, microsclerotium size, and sporogenic germination, with significant differences apparent between for microsclerotium size ($P = 0.01$) and sporogenic germination ($P = 0.01$). There was no relationship between disease severity and microsclerotium production within leaf lesions, microsclerotium size, or sporogenic germination; however, there was a positive and significant correlation between microsclerotia production within a lesion and microsclerotium size ($R^2 = 0.19$, $P < 0.0001$). Although microsclerotia from sorghum lesions varied in structural characteristics and their ability to produce spore masses, these qualities were dependent upon the sorghum genotype from which the microsclerotia were derived, since the *R. sorghi* population was genetically uniform as determined by ITS sequences and RAPD PCR.

Comparative genomics of *Salmonella enterica* serovar Weltevreden plant and animal isolates

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Phytopathology 101:S19

Salmonella enterica serovars are a prevalent cause of human gastroenteritis linked to fresh plant produce. *Salmonella* Weltevreden has been a predominant food-safety serovar in South-East Asia associated with animal products. Recently, it has emerged as a global problem also associated with vegetables. We sequenced the complete genome of a *S.* Weltevreden isolate from a Scandinavian outbreak traced to alfalfa sprouts. Illumina and 454 sequencing delivered 18.7/46.4x coverage, with read assembling into 91 contigs. The 4.9 Mb genome has a 52.1% G+C content, 4858 coding sequences (CDS), and a novel plasmid pSW82 (81.9 bp, 93 CDS). Comparative analysis of the plant isolate against a resequenced genome of a scallop isolate revealed intriguing differences despite high overall homology. Analysis of clustered interspaced short palindromic repeat regions (CRISPRs) that record extrachromosomal infections indicate recent divergent evolution of plant and animal isolates. Comparative analysis with other *S. enterica* serovars showed high synteny in gene content, but revealed notable differences. *S.* Weltevreden is one of the few serovars to have a type VI secretion system (T6SS) with two functional gene clusters. Differential presence/absence of several phosphotransferase systems (PTS) was revealed, which may enhance survival of *S.* Weltevreden on a broader range of hosts. Differential transcriptomics analysis in planta is underway and will be discussed.

Aggressiveness of *Rhizoctonia solani* AG 2-2 ISGs IV and IIIB on sugar beet and rotation crops

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Phytopathology 101:S19

Rhizoctonia crown and root rot (RCRR) of sugar beet is caused by Rhizoctonia solani AG 2-2 intraspecific groups (ISGs) IV and IIIB. Isolates from plants with RCRR (24/ISG) were tested for aggressiveness on seedlings and adult plants of sugar beet, pinto bean, soybean, corn, and hard red spring wheat. A commercial greenhouse soil was infested with each ISG isolate before sowing. Two weeks after planting (WAP), seedlings were evaluated with a root rot index (RRI) for sugar beet (0–100 scale); beans and corn (1–5 scale); and wheat (0–3 scale). Adult sugar beet roots were inoculated 8 WAP; bean crops at/near flowering; corn at V5/V6; and wheat at 6 WAP. Two weeks after inoculation, adult crops were rated for disease with the same RRI used for seedlings (except for sugar beet, 0–7 scale). There was significant variability among isolates within ISGs and ranges overlapped. On seedlings, IIIB was more aggressive than IV and average RRIs on sugar beet were 78 and 51, on pinto bean were 4.4 and 2.7, on soybean were 4.1 and 3.2, on corn were 3.1 and 2.1, and on wheat were 1.0 and 0.7, respectively. Aggressiveness was similar for ISGs on adult sugar beet (5.0 for IV, 4.9 for IIIB), pinto bean (2.9 for IV, 3.1 for IIIB) and soybean (3.5 for both ISGs). On corn, RRI averaged 1.7 for IV and 2.3 for IIIB and on wheat were 0.1 for IV and 0.3 for IIIB. Thus, RCRR of sugar beet is less likely to occur with hard red spring wheat in the rotation compared to beans and corn.

***Pantoea agglomerans* fire blight biocontrol strain- and species-specific real-time PCR tools to monitor environmental impact and behavior in orchards**

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Phytopathology 101:S19

Pantoea agglomerans E325 (Northwest Agricultural Products, Bloomtime) is a promising biocontrol alternative for fire blight management. To facilitate European registration, we developed quantitative real-time PCR (qPCR) tools for environmental impact assessment. Strain-specific ID based on unique sequences of a 3-kb genomic island discriminated E325 from other *Pantoea* products (Blight-Ban C9-1, BlossomBless P10c). Impact assessment on indigenous *Pantoea* was enabled by species-specific ID (conserved pagRI autoinducer genes) and mechanism-of-action genes (pantocin A *paaA*; novel E325 antibacterial metabolite). Monitoring in apple orchard trials (2009–2010; 3 locations) validated sensitivity/specificity of qPCR assays (LOD 3 cells/reaction, 700 CFU/flower). E325 established on 80–100% of flowers, with secondary colonization restricted to treated plots and no escape outside orchards. Population dynamics followed expected models for seasonal low temperatures (stable log 4-5 CFU/flower after 5-6 d). There was no significant impact on (or risk to) native *Pantoea* flower communities. E325 did not persist on foliage or soil after 120 d. No residues were detected on harvested fruit averting consumer exposure issues. Biocontrol features were rare in native *Pantoea* (eg, pantocin A genes <7.4% frequency). Monitoring results indicate no adverse impact/risk of E325 applied to control fire blight.

***Erwinia amylovora* early detection in orchards using lateral-flow immunostrips Ea AgriStrip and quantitative PCR for flower monitoring**

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Phytopathology 101:S19

Fire blight forecasting models predicting infection risk are routinely used to decide need/timing of control product application, but they assume pathogen presence. We developed tools to monitor *Erwinia amylovora* flower populations and optimize grower decision making. We developed a lateral-flow immunostrip (Ea AgriStrip, Bioreba) and quantitative PCR tools and evaluated sensitivity/specificity in Swiss/U.S.A. orchards. Both methods have the advantage of rapid detection without interference from non-target microflora. Quantitative PCR delivered superior sensitivity (LOD 3.6 log cfu/flower vs. Ea AgriStrip LOD ≥ 5 log cfu/flower). However, Ea AgriStrip was simple to use and suitable for on-site monitoring giving results within 15 min. Depending upon flower sampling scheme, qPCR may be overly sensitive, leading to excessive application of control products, while the sensitivity of Ea AgriStrip may better reflect infectious populations warranting intervention.

Early emergence applications of prothioconazole for management of Cylindrocladium black rot of peanut

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Phytopathology 101:S19

Prothioconazole (199 g ai/ha) is applied to peanut as an in furrow (IF) spray to control Cylindrocladium black rot (CBR), a disease of roots and pods caused by *C. parasiticum*. Early emergence (EE) applications (2–3 weeks after planting) in an 8 cm band in a high volume (373 L/ha) spray were evaluated in 2006. The EE application followed by four sprays of Provost (0.58 L/ha, prothioconazole + tebuconazole) reduced CBR incidence 18% and increased yield 1042 kg/ha versus the Provost alone. In 2007, both IF and EE applications followed by Provost significantly increased yield in two tests by an average of 672 and 837 kg/ha, respectively, and the final CBR incidence was reduced 46 and 42%, respectively. In 2008, CBR levels were lower, and

only the IF treatment plus Provost had lower disease incidence than the untreated plots. Delaying application of EE sprays by 2–4 weeks lowered the level of CBR control. This method of application, combined with later season Provost sprays, can help reduce losses to this damaging disease.

Localization of *Banana bunchy top virus* within the aphid vector, *Pentalonia nigronervosa* as revealed by Immunofluorescence, TEM, and PCR assays
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Phytopathology 101:S20

We have used Immunofluorescence, Transmission Electron Microscopy (TEM), and PCR assays to specifically localize *Banana bunchy top virus* (BBTV; family *Nanoviridae*, genus *Babuvirus*) within its aphid vector, *Pentalonia nigronervosa* (Hemiptera, Aphididae). Aphids were exposed to BBTV-infected plants for an acquisition access period of 7–10 days; thereafter, guts and salivary glands were dissected and processed for the localization of the virus. BBTV was localized in Immunofluorescence using either monoclonal or polyclonal antibodies into the anterior midgut. TEM observations revealed the presence of vesicles of 0.5-1 µm as diameter containing paracrystalline structures in the cytoplasm of cells composing the anterior midgut. These structures were absent in aphids raised from healthy banana plants. BBTV was localized in immunofluorescence within the principal salivary glands with no evidence for labelling into the accessory salivary glands. The virus genomic DNA was detected by using a diagnostic PCR from the guts, salivary glands, and hemolymph. A likely path of translocation of BBTV may therefore include the penetration of ingested viral particles through the anterior midgut, hemolymph infection, and the invasion of the principal salivary glands. An alternative virus translocation path, which may involve the direct penetration of the salivary glands from the anterior midgut, is suggested because cells forming the principal salivary glands lay in direct contact to the stomach wall.

Mixed modes of reproduction and spatial aggregation of genotypes of the grape powdery mildew fungus, *Erysiphe necator*, within vineyards
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Phytopathology 101:S20

Random mating leads to high genotypic diversity, 1:1 mating-type ratios, and random associations of alleles at different loci, *i.e.*, linkage equilibrium. To test for random mating in populations of the grape powdery mildew fungus, *Erysiphe necator*, we sampled isolates from two vineyards of *Vitis vinifera* in October 2009 (Watkins Glen, NY: N = 62; Winchester, VA: N = 78). The location of each isolate within a vineyard was recorded. We also sampled isolates from the VA vineyard in June 2010 (N = 69). Isolates were genotyped for mating-type and 11 SSR markers. Genotypic diversity was high in all populations. The October 2009 populations contained a large proportion of clones (NY: 35%; VA: 56%) dominated by few genotypes; however, only two clones of the same genotype were detected in the June 2010 population. After clone correction, mating-type ratios in the three populations did not deviate from 1:1. Yet, significant linkage disequilibrium was detected in all three populations even after clone correction. Mantel tests resulted in positive correlations between genetic and geographic distances within vineyards demonstrating that similar genotypes were spatially aggregated and that inoculum disperses short distances. These results suggest that clonal selection and spatial aggregation contribute to linkage disequilibrium even though the population undergoes an annual sexual cycle.

Pest interceptions on live plants at U.S. ports of entry: A system overwhelmed
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Phytopathology 101:S20

More than half of the non-native forest pathogens established in the U.S. today arrived via the plants for planting pathway. often with disastrous ecosystem effects. Currently U.S. regulations rely on 1) phytosanitary certificates issued by the exporting country's national plant protection organization, and 2) inspections at certain ports of entry with plant inspection stations. Plant imports increased 500% since 1967, with 2.5 billion plants imported in 2010. About 56 inspectors are employed nationwide to inspect live plants. We examined two data sources, both maintained by the USDA Animal and Plant Health Inspection Service, to estimate the approach rate of plant pests (insects and pathogens) in shipments of live plants for propagation entering the U.S., and determined the inspection efficiency of port inspections. Under normal port operations, only 2.3% of plant shipments were identified as containing actionable pests. In contrast, the Agricultural Quarantine Inspection Monitoring system dataset, a random subset of much more intensively inspected shipments, identified 9.9% of incoming shipments as infested with actionable pests. This discrepancy suggests that at least 76.5% of incoming infested plant shipments pass the ports undetected. The International Plant Protection Convention is developing a standard to harmonize regulations of plants for planting. These data suggest it would be wise to limit reliance on inspections at ports of entry.

Population structure of *Ophiognomonia clavigignenti-juglandacearum* reveals multiple introductions of the butternut canker fungus into North America
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Phytopathology 101:S20

Butternut canker caused by the fungal pathogen *Ophiognomonia clavigignenti-juglandacearum* (*Oc-j*) remains the primary cause for range-wide mortality of butternut. The disease was first reported in Wisconsin in 1967, however the fungus may have been present for several years prior. Therefore, our objectives were to evaluate the genetic diversity of isolates of *Oc-j* recovered from butternut, black walnut, and heartnut in North America, and to correlate these results with selected assessments of host range and virulence to identify possible associations among these variables. A total of 48 isolates of *Oc-j* from across North America including isolates from black walnut and heartnut were analyzed. Clustering analyses based on 16 SNP markers reveal that *Oc-j* populations are made of four differentiated genetic clusters. This result suggests multiple introductions of the pathgen through successive or simultaneous introductions of isolates having differentiated genetic backgrounds. Isolates recovered from heartnut and black walnut caused larger lesions on all three Juglans species compared to isolates originally recovered from butternut. Eight of the nine isolates, which caused the largest lesions on butternut belonged to a single genetic cluster. The pathogenicity data in combination with geographic and population structure data indicate this fungus was introduced into North America on multiple occasions, and genetic clusters differ in their level of virulence.

Effect of post-inoculation relative humidity on peanut infection by Sclerotinia minor
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Phytopathology 101:S20

Stems of six-week-old plants of the cv Okrun (susceptible to Sclerotinia blight) were inoculated with S. minor. Two post-inoculation humidity regimes of 100% RH were used. In the first RH regime, one inoculation chamber was kept open for the duration of experiment (DOE), and five were closed for durations of 1, 2, 3, 4 or 7 days post-inoculation (PI). In the second RH regime, one chamber was kept open for the DOE, and five were closed for durations of 12, 24, 36, 48 and 60 hour PI. No infection occurred in chambers opened for the DOE or closed for 12 hr. Closure for 24 hr resulted in 50–75% infection, and closure for 48 hr or more resulted in 88–100% infection. Lesions on infected stems were measured up to 7 days after inoculation to calculate area under lesion expansion curve (AULEC). Closure for 24 hr produced AULEC of 8.2–9.7 cm2, whereas significantly (P = 0.05) higher AULEC of 18.0–16.0 cm2 were obtained with closure of >48 hr. These findings indicate the importance of providing 100% RH for at least 48 hr post-inoculation to effectively quantify lesion expansion.

Characterization of two newly described Curtovirus isolated from spinach in south-central Arizona
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Phytopathology 101:S20

A commercial spinach field in south-central Arizona developed geminivirus-like disease symptoms. Total DNA extracts was used as template for rolling circle amplification (RCA) of the viral genomes. RCA products were digested and cloned using PstI to obtain full length curtoviral genomes. A first curtovirus consisted on 3065-bp in length and it was tentatively named Spinach severe curly top virus-[AZ] (SSCTV-[AZ]){Genbank No. GU734126}. An ClustalV alignment with sequences of all curtovirus species available in GenBank indicated that this spinach isolate shared the highest nt sequence identity at 59% with Horseradish curly top virus (HrCTV). The genome consists of six open reading frames and lacks the AC3 gene, an arrangement most similar to HrCTV. A second curtovirus consisted on 2860-bp in length and it was provisionally named Spinach curly top Arizona virus

(SCTAzV). This isolate shared the highest nt sequence identity at 66% with Beet curly top Iran virus (BCTIV). The genome of this isolate comprises three virion-sense and two complementary sense ORFs, however, C3 and C4 were not found in the SCTAzV genome. This genome arrangement is similar to that of Beet curly top Iran virus (BCTIV). Recombination analysis indicated that a fragment comprising part of the intergenic region and Rep gene of the SSCTV genome arose from interspecific recombination. SSCTV and SCTAzV infectious dimeric clones were constructed and agroinoculated alone and in mixture to Nicotiana benthamiana, seedlings to test infectivity.

Development of an efficient system for assessing gene function in the cotton plant using virus-induced gene silencing (VIGS)
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Phytopathology 101:S21

A vector for virus induced gene silencing (VIGS) based on the Cotton leaf crumple virus (CLCrV) DNA-A, was used to demonstrate the use of VIGS for transient gene silencing of different selected cotton genes: 1) fatty acid desaturase (FAD2-4); 2) a steroid 5α-reductase (GhDET2); and 3) chitinase like genes (GhCTL1 and GhCTL2). Total RNA was isolated from cotton (leaves, roots, cotton ball) and used as template to amplify (by RT-PCR) different size length fragments using specific primers for each gene of interest. These fragments were cloned into the VIGS CLCrV vector. VIGS CLCrV vectors carrying FAD-2-4, DET2, CTL1 and CTL2 were used to inoculate cotton seedlings (Gossypium hirsutum cv Deltapine 5415) with 1.1-µm diameter tungsten microprojectiles (BioRad) coated with a mixture of 1 µg each of the following plasmids. Inoculated plants were kept in the growth room at 28°C, 16/8 h photoperiod. Healthy cotton plants were maintained as controls. Results showed that cotton plants inoculated with the CLCrV – FAD2-4, CTL1 and CTL2 VIGS vectors and the CLCrV DNA B dimer developed mild CLCrV symptoms at four weeks post inoculation. All vectors were detected successfully replicating in cotton, analysis of FAD2-4, CTL and DET gene expression confirmed the silencing of these genes in cotton.

Screening Taro (*Colocasia esculenta*) for resistance to Taro Leaf Blight (TLB) using a detached-leaf disc bioassay and marker-assisted selection
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Phytopathology 101:S21

Taro (*Colocasia esculenta*) is a tropical root crop cultivated primarily for its starchy corm. A major disease that threatens the sustainability of taro is Taro Leaf Blight (TLB) caused by the oomycete pathogen *Phytophthora colocasiae*. While Hawaiian varieties of taro are susceptible to *P. colocasiae*, tolerance to TLB has been found within taro germplasm from Palau, Thailand, and Guam. Hybrid lines can be tested for tolerance to TLB using a detached-leaf disc bioassay. Four 36 mm discs are cut from the first fully developed leaf of each hybrid. Discs are inoculated with approximately 50 zoospores of a local isolation of *P. colocasiae* and incubated at 27°C for four days. Mean lesion size is measured on day three and four for each hybrid. Preliminary analysis showed approximately 25 hybrids that were highly tolerant to TLB. The majority of the tolerant hybrids were a cross between Dirratengadik/Moi and (Red Moi/PH15)/Sawahn Kurasae. When the hybrid screening is complete, 150 of the most tolerant and 150 of the most susceptible hybrids will be transferred into the field for further analysis. Additionally, genetic markers are needed for taro germplasm characterization and to accelerate the resistance screening for early selection of desirable hybrids. Both micro-satellites and SNPs are being evaluated for use in marker-assisted selection.

Proteins associated with aflatoxin-resistance are identified and characterized towards candidacy for breeding markers
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Phytopathology 101:S21

Aflatoxins, the toxic and carcinogenic secondary metabolites of *Aspergillus flavus* and *A. parasiticus* are produced during infection of maize, causing serious preharvest and postharvest problems. Host resistance has become a viable approach to controlling aflatoxin contamination since the discovery of maize lines with aflatoxin-resistance. However, to transfer this resistance into lines with commercially-useful genetic traits, gene markers need to be identified. To accomplish this, kernel resistance-associated proteins (RAPs) have been identified through the employment of comparative proteomics to investigate maize lines including closely-related lines varying in aflatoxin accumulation. RAPs also have been further characterized through physiological and biochemical studies to determine their causal role in resistance. Three RAPs, a 14 kDa trypsin inhibitor, pathogenesis-related protein 10 and glyoxalase I have been investigated using RNAi gene silencing and plant transformation. Several resistant lines have been subjected to QTL mapping to identify loci associated with the aflatoxin-resistance phenotype, while gene expression studies have been performed to provide a more thorough picture of the maize resistance response to infection by aflatoxigenic fungi. Results of proteome, RAP characterization, RNAi silencing and gene microarray studies highlight the potential of several RAPs as gene markers.

The *Rpg5* NBS-LRR-STPK gene and a second NBS-LRR gene are required together for *rpg4* mediated wheat stem rust resistance in barley
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Phytopathology 101:S21

Preliminary data indicated that the recessive and temperature sensitive *rpg4* gene and the dominant *Rpg5* gene are required together for resistance to *Puccinia graminis* f. sp. *tritici* races MCCF, QCCJ and TTKSK. We have cloned the *Rpg5* gene but validation of the *rpg4* gene is hindered by the complex nature of the locus. Recombinant analysis of the Q21861 (resistant) X Steptoe (susceptible) high resolution mapping population determined that *rpg4* was distinct from but tightly linked to *Rpg5*. The *rpg4* gene is required for full resistance to the wheat stem rust races but resistance is only expressed in the presence of *Rpg5*; however, *Rpg5* alone confers full resistance against the rye stem rust isolate 92-MN-90. Using virus-induced gene silencing (VIGS), we silenced each gene within the *Rpg5* genetic interval (*Rpg5*, *Hv*RGA1, *Hv*Adf2 and *Hv*Adf3) followed by inoculation with *Pgt* race QCCJ. Preliminary data determined that *Rpg5* was required for *Pgt* race QCCJ and TTKSK resistance, but a second NBS-LRR gene, *Hv*RGA1, is also required for resistance against QCCJ. Thus, the *Rpg5* resistance mechanism may follow the emerging theme that pairs of unrelated genetically linked NBS-LRR genes are required for pathogen recognition and resistance. We will report on the post-transcriptional gene silencing and recombinant analysis data indicating that the complex stem rust resistance locus contains three genes required for wheat stem rust resistance against several races including TTKSK.

Characterization of endophytic microflora colonizing wood tissues of healthy and Esca-diseased vines
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Phytopathology 101:S21

Microbial communities colonizing Cabernet-Sauvignon vine plants infected or not by the trunk-wood disease Esca are studied. Both cultivation and molecular techniques were used to investigate the endophytic microflora colonizing the various plant tissues. High fungal, bacterial and yeast diversity of communities that colonize healthy wood tissues of healthy and Esca-diseased plants were pointed out. Bark and internal healthy wood tissues communities were different for fungal, bacterial and yeast communities. The asymptomatic wood of healthy and Esca-plants had a similar microflora. Analyses evidenced that there is a seasonal evolution (from winter to autumn) of fungal communities in the woods, but these communities were similar for summer to autumn. For all samples, the cultivation techniques indicated that the same pathogenic-, saprophytic- and antagonistic-fungal species were isolated from healthy wood tissues of grapevine affected or not by Esca. These fungi were the fastest to grow on media-culture in laboratory conditions. As for pathogenic fungi, some species usually associated with grapevine diseases were frequently isolated from healthy wood of stock and trunk, e.g. Botryosphaeriaceae spp. The comparison between young and 15–25 years old vine plants evidenced that Trichoderma spp. colonized more young plants than older ones. The grapevine fungal microflora evolved with seasons. Abiotic or biotic factors have likely some influence on microbial communities' changes in the plant.

Characterization of biocontrol strains of Pythium oligandrum and control of an Esca pathogenic fungus attack
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Phytopathology 101:S21

The Oomycota Pythium oligandrum is a biocontrol agent which is known for its ability to induce plant resistance and to control pathogens in the rhizosphere. For instance, this antagonistic oomycete produces 3 elicitin-like proteins (oligandrin POD1, POD2) which induce resistance when they are applied on plants. In the present experiment, P. oligandrum strains were isolated from various areas of the Bordeaux vineyard. Subsequently, the strains were characterized and tested for vine protection against an, Phaeomoniella chlamydospora. Roots were sampled from vine plants grown in various soils (silt-clay, sandy-clay, stony) from the Bordeaux vineyard region. Whatever the conditions, P. oligandrum strains were isolated from nearly all the samples. It seems therefore ecologically adapted to vineyard soils. Forty strains were collected, purified and identified by sequencing of the rDNA ITS. P. oligandrum elicitor detections were carried out by amplification of oligandrin and POD1 genes. These genes were detected and sequenced in most of the strains, Inter Simple Sequence Repeat were used to assess genetic diversity too. A greenhouse experiment pointed out that Cabernet Sauvignon cuttings infected by P. chlamydospora were protected up to 50% when the rhizosphere of vines was colonized with P. oligandrum. Taken together these results provide useful information for screening and developing of P. oligandrum biocontrol strategy.

The role of mycotoxins produced by *Fusarium verticillioides* and *Fusarium graminearum* in maize seedling infection

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Phytopathology 101:S22

Fusarium verticillioides and *Fusarium graminearum* are fungi that produce mycotoxins (fumonisins and deoxynivalenol, respectively) and affect plant health by causing disease. While it is well known that these mycotoxins reduce the value of contaminated crops as food or feed, their impacts on seedling health have not been fully described. In order to characterize these effects on seedling infection and disease, wild-type and mycotoxin-nonproducing mutant strains of each fungus were grown on washed and sterilized rice hulls and mixed with sterile vermiculite. Maize seeds were planted into the infested vermiculite and harvested after 8, 14 and 21 days. Root and shoot weights and shoot length were measured and roots were scanned for image analysis of root structure and symptoms. Crown tissue was excised, surface sterilized and used for fungal isolation and quantitative PCR to determine levels of fungal DNA present in the tissue. For *Fusarium verticillioides* there were significant differences between the fumonisin and non-fumonisin producers for all the measured variables by 14 days after planting. Plants exposed to the fumonisin-knockout strain had longer shoot lengths ($p < 0.0001$) and higher root ($p = 0.0026$) and shoot weights ($p = 0.0004$) and less fungal DNA ($p < 0.0001$) present in the tissue than plants exposed to the wild-type. For *F. graminearum*, seedling disease symptoms and infection were similar for the wild-type and trichothecene knockout strains.

First report of the yeast *Eremothecium coryli* associated with brown marmorated stink bug feeding injury on tomato and apple

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Phytopathology 101:S22

An outbreak of brown marmorated stink bug (BMSB) (*Halyomorpha halys*), an exotic species first found in the U.S. in 2001, occurred in several mid-Atlantic states in 2010, causing damage to tree fruit and vegetables. Feeding damage to apple fruit consisted of slightly sunken areas approximately 0.3–0.6 cm in diameter. In tomatoes, the feeding sites appeared as irregular yellowish spots on ripe fruit. Damaged apple and tomato fruit were surface disinfested with 95% ethanol, the outer epidermis removed aseptically, and the discolored internal tissue plated on agar. A yeast was recovered from all tissue pieces after 4 days. The isolates were identified by morphology and 28S rRNA analysis as *Eremothecium coryli*, a plant pathogenic yeast associated with native stink bug species. To confirm transmission of *E. coryli* by BMSB, fifty adults were captured and enclosed in a screen cage in the greenhouse. Unblemished apples and tomatoes were placed in the cage and after 7 days examined for symptoms. Feeding injury similar to that seen in the field occurred on all fruit and *E. coryli* was recovered from all lesions tested. To our knowledge, this is the first report of this yeast associated with *H. halys*. Studies are underway to determine the role of this yeast in fruit damage and how to best manage its transmission.

Managing daylily rust with fungicide dips, drenches and foliar spray applications

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Phytopathology 101:S22

Daylily rust, caused by *Puccinia hemerocallidis*, was introduced into the U.S. in 2000 and continues to be problematic to growers in southern production areas. Experiments were conducted to assess the efficacy of various fungicides applied as root dips, drenches, or foliar sprays to manage daylily rust. Root dip treatments included six fungicides and sodium hypochlorite. Single drench applications of four fungicides (azoxystrobin, fluoxastrobin, myclobutanil and tebuconazole) at three rates (0.06, 0.13, and 0.25 g a.i./plant) were evaluated in greenhouse trials and two fungicides (azoxystrobin and tebuconazole) at one rate (0.15 g a.i/plant) were evaluated in the field. Eight foliar treatments were applied at 14-day intervals in the field. Dip treatments with azoxystrobin significantly reduced disease development. All drench treatments, except myclobutanil at 0.06 g a.i./plant, significantly reduced rust development in potted daylily when plants were challenged inoculated 3 weeks post-treatment. Drench or foliar applications of azoxystrobin provided the greatest suppression of rust development on daylily in the field. One drench application of azoxystrobin protected plants from rust for over four months. Fungicides dips and drenches could minimize the threat of accidentally introducing daylily rust on propagative materials to new locations and reduce disease development in established field plantings.

Managing resistance of *Cercospora beticola* Sacc for integrated disease management in sugar beet

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Phytopathology 101:S22

In 2010, a field trial with artificial inoculations was conducted in order to evaluate the efficacy of flutriafol (DMIs) and carbendazim (MBCs) based fungicides for the control of Cercospora leaf spot. Individual plots were inoculated with isolates: a) sensitive to flutriafol and carbendazim, b) resistant to flutriafol and sensitive to carbendazim, c) resistant to flutriafol and carbendazim and d) sensitive to flutriafol and resistant to carbendazim. A total of four fungicide applications were made and three disease evaluations were performed during the growing season. Additionally, at harvest, yield and sugar content were calculated. The highest disease intensity was detected in inoculated plots without fungicide applications and in treatments inoculated with resistant isolates plus fungicide applications. Root yield and sugar content were significantly higher in plots inoculated with sensitive isolates and subsequently treated with fungicides. Detection of fungicide resistance and its management is an important tool in disease control.

Blue-green and chlorophyll fluorescence-based differentiation between simultaneously occurring N-deficiency and pathogen infection in winter wheat

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Phytopathology 101:S22

In recent years several sensor-based approaches have been established to early detect non-destructively single stress factors, but the challenge to discriminate simultaneously occurring stressors still remains. Earlier studies on wheat plants strongly affected by pathogens and nitrogen deficiency indicate that the chlorophyll fluorescence might be suited to distinguish between both types of stressors. Nevertheless, there is lack of information on the pre-symptomatic detection of synchronized occurrence of slight N-deficiency and the early stages of pathogen development. The usefulness of the blue, green, and yellow fluorescence signals in this context has not yet been exploited. Based on this, we hypothesised that a differentiation between physiological reaction of wheat plants due to N-deficiency and leaf rust (*Puccinia triticina*) as well as N-deficiency and powdery mildew (*Blumeria graminis* f. sp. *tritici*) might be accomplished by means of UV laser-induced fluorescence spectral measurements. Results reveal that both fluorescence amplitude ratios R/FR and B/G enable a reliable and robust discrimination between the experimental groups. Furthermore, the discrimination was done as early as one and two days after inoculation for powdery mildew and leaf rust infection, respectively. Moreover, several additional amplitude ratios and half-bandwidth ratios were suited to early detect the pathogen infection, irrespective of the nitrogen status of the plants.

The use of field bioassay to facilitate the deregulation of fields formerly infested with *Globodera rostochiensis* in New York

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Phytopathology 101:S22

Globodera rostochiensis (Golden Nematode or GN) is a potato cyst nematode, causing much crop damage worldwide and is a quarantine pest in the U.S. due to its exotic nature and its potential for crop damage and trade impacts. First detected on Long Island in 1941, GN has been found on fields in portions of 8 New York Counties with a total of 1.277 million acres under regulation and 6000 acres known to be infested with GN. The PPQ/NYDAM GN Program has been in place since 1948. Until recently there have been few mechanisms to release infested potato fields from regulation without removing them completely from host production. A bilateral agreement between the USDA APHIS PPQ and the Canadian Food Inspection Agency was recently adopted that allows for the release of fields formerly infested with potato cyst nematodes. A combination of repeated field surveys, cyst population suppression, time and finally challenging the remaining cysts in the field to the repeated presence of host plant material (a bioassay) are utilized to determine if any of the remaining cysts in the field are viable. The first fields for bioassay have been out of host crop production for nearly 30 years and have not had viable GN cysts detected for a minimum of 10 years. The bioassay includes planting susceptible host crops in field foci for 3 years followed by soil sampling to a depth of 25cm obtaining 9 2000cc samples per acre. Fields may be partially released based on negative bioassay results.

The role of an oxidative stress sensor in the oxidative stress response, virulence and host colonization of *Pantoea stewartii* subsp. *stewartii*
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Phytopathology 101:S23

Pantoea stewartii subsp. *stewartii*, the etiological agent of Stewart's wilt, is a serious pathogen of sweet corn. An important aspect of bacterial plant colonization is the ability to withstand exposure to reactive oxygen species (ROS) arising from the host defense response or normal plant developmental processes. The transcriptional regulator, OxyR, modulates the oxidative stress response in many bacteria through production of ROS detoxifying enzymes and other important bacterial survival mechanisms such as biofilm formation. A *P. stewartii* ΔoxyR mutant showed increased sensitivity to ROS and changes in OxyR dependent catalase expression. Moreover, the ΔoxyR mutant displays a marked decrease in the production of stewartan exopolysaccharide, a key component of the mature biofilm matrix, which is essential to the infection process. The ΔoxyR mutant was less virulent in sweet corn, but was capable of colonizing plants at levels twice as high as wild type. Following treatment with ROS, ΔoxyR showed a striking increase in expression of soxS, part of a second oxidative stress response pathway (SoxR/S). We hypothesize that there is partial overlap of the OxyR and SoxR/S regulons and that *P. stewartii* compensates for the lack of OxyR by upregulating the SoxR/S pathway, causing the increase in host colonization. Further characterization of the OxyR regulon of *P. stewartii* will provide insight into the vulnerabilities of xylem dwelling bacteria during plant colonization.

Influence of *Pythium aphanidermatum*, *P. irregulare*, and *P. cryptoirregulare* on the bacterial community in recycled irrigated water
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Phytopathology 101:S23

Pythium species are among the most damaging pathogens in horticulture causing damping-off, root and stem rots in ornamental plants. They have been recovered from recycled irrigated water in commercial greenhouses in PA. An understanding of the interaction among these oomycetes and the microbial community present in this ecosystem is vital for establishing a long term management strategy. Little is known about the impact that *Pythium* spp. have on the bacteria communities in recycled water reservoirs. Responses of bacteria communities changes may be associated to the presence of *Pythium* species in the water. A study showed that *Pythium ultimum* favors specific genera such as Actinobacteria, Proteobacteria, Chytridiomycota and Sordariomycetes in compost whereas compost without *P. ultimum* was dominated by Homobasiodiomycetes. Culture-independent techniques such as automated ribosomal intergenic spacer analysis (ARISA), have expanded our understanding of the diversity of bacteria populations in various ecosystems including soil and water. This study is examining the impact the presence of *P. aphanidermatum*, *P. irregulare*, or *P. cryptoirregulare* has on bacteria diversity and community composition in water in order to develop a better understanding of this ecosystem. Profiles obtained using ARISA will help us assess the community-specific profile for three *Pythium* species. Preliminary results suggest that *Pythium* presence might play a role in structuring bacterial community composition.

Induction of grape tissue necrosis and tobacco leaf HR by *Agrobacterium vitis* requires a polyketide synthase and a nonribosomal peptide synthase
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Phytopathology 101:S23

Mutation of an Spf-type of phosphopantetheinyl tansferase (PPTase) gene (Avi5813) in chromosome of *Agrobacterium vitis* strain F2/5 leads to modifications of multiple phenotypes including the hypersensitive response (HR) in tobacco and necrosis in grape. Complementation with the full length of PPTase gene fully restored the HR and necrosis responses. Screening of the *A. vitis* strain F25 genome revealed a single copy of Spf-type PPTase gene that must be involved in polyketide and non-ribosomal peptide biosynthesis. Mutagenesis of the genes encoding proteins that require post-translational modification by PPTase resulted in identification of a type-1 polyketide synthase (PKS) gene (Avi4330) and a non-ribisomal peptide synthase (NRPS) gene (Avi3342) that are both required for induction of HR and necrosis. Knockout of either Avi4330 or Avi3342 caused a necrosis and HR minus phenotype regardless of cell concentration or culture age. This result suggests that an effector molecule required for induction of HR and grape necrosis is likely a hybrid peptide-polyketide product.

Precence and levels of aflatoxins in common bean (*Phaseolus vulgaris* L.) samples from Uganda
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Phytopathology 101:S23

Aflatoxins are produced by *Aspergillus flavus* which grows on a variety of grains and in humans are associated with aflatoxicosis. Occurrence and extent of contamination is influenced by environmental factors and vary with geographic location, agronomic practices, and conditions during pre-storage, and/or processing periods. This study was meant to assay the presence and levels of aflatoxins on common bean grain sampled from different growing and storage conditions in Uganda. Grain samples were collected from markets and farmers' stores in three districts of Uganda. More than 51% of the collected seeds had been stored between 2 to 5 months. Each of the samples was subjected to standard seed health tests and also screened using a one-step lateral flow immunochromatographic assay with a cut-off of 4 ppb using AgraStrip® Aflatoxin Test kit. Positive samples with aflatoxin greater or equal to 4ppb were subjected to an ELISA-based quantification procedure. Seed heath tests revealed 15 species of fungal contaminants on the seed samples with Aspergillus flavus occurring on more than 96% of the samples. Qualitative aflatoxin tests detected over 10% positive cases (1.9 to 24.8 ppb). Most of the positive cases and highest levels of contamination were from the warm areas while samples from the cooler areas had levels below the detection level. Further extensive assessment is planned to establish the extent and significance of aflatoxins on the safety of bean grain used for human consumption.

Arbuscular mycorrhizal fungi diversity associated with coexisting cheatgrass and big sagebrush communities
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Phytopathology 101:S23

Arbuscular mycorrhizal fungi (AMF) are important plant symbionts, and have been implicated in successful plant invasions through multiple mechanisms. Very little is known about the role of AMF on cheatgrass (Bromus tectorum) invasion and persistence. Cheatgrass has been shown to reduce the AMF diversity of neighboring vegetation (Hawkes et al. 2006), and soils from cheatgrass dominated areas have lower mycorrhizal inoculum than uninvaded soils (Al-Qawari 2003). However, the identities of AMF species associated with cheatgrass, as well as the diversity of cheatgrass-AMF associations are largely unknown. On the other hand, big sagebrush (Artemisia tridentata) has been shown to have high diversity of associated AMF (Allen et al. 1995). An important question in understanding replacement of big sagebrush dominance by cheatgrass is how this shift alters the diversity of AMF across the sagebrush steppe. The research presented here compares diversity of AMF associating with coexisting cheatgrass and big sagebrush. A MF species were identified from three distinct locations in Colorado, Utah and Wyoming using trap cultures containing field-collected soil and root material grown for one year in a greenhouse. Diversity was also measured using DNA extracted from the same soil and root samples. DNA was amplified using AMF-specific primers, cloned and sequenced. The understanding of key plant-microbe interactions like AMF will improve effective management of invasive plants.

Preliminary results of the distribution and genetic diversity of *Potato virus Y* (PVY) in the main Turkish pepper growing areas

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Phytopathology 101:S24

Potato virus Y (PVY) is the type member of the genus*Potyvirus* (family Potyviridae), the largest group of RNA plant viruses. PVY isolates were collected according to hierarchical (nested) sampling design. Collections were made in 100 fields in seven localities in Hatay, thirteen localities in Kahramanmaraş (Eastern Mediterranean) and seven localities in Gaziantep (Southeast Anatolia). DAS-ELISA revealed the infection of 167 samples with PVY from a total of 1098 pepper (*Capsicum annuum*) plants showing symptoms of viral infection. Genetic diversity among these 167 samples was examined by RT-PCR-RFLP analysis of coat protein (CP) cistron. The entire CP cistron was amplified using polyvalent primers for all PVY groups and amplified a 1159 nucleotide fragment. Two restriction endonucleases (*Hae*III and *Mse*I) used for RFLP analyses of RT-PCR products were chosen based on genomic sequences available for PVY group C, which includes almost all pepper isolates. RT-PCR-RFLP analyses revealed between two and four DNA profiles. Conseuently, only 85 of the 167 PVY positive samples from Hatay were analysed for CP genome segment and they all had the diversity. None of the samples from all other localities had the diversity.

Co-inoculation of wheat with *Triticum mosaic virus* and *Wheat streak mosaic virus* exacerbates loss of fresh and dry matter

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Phytopathology 101:S24

Triticum mosaic virus (TriMV) is a recently discovered virus of wheat in the Great Plains of the United States. Information on TriMV's effect on yield when it infects wheat alone or in combination with *Wheat streak mosaic virus* (WSMV) is scant. In a greenhouse experiment, winter wheat cultivars Millennium (WSMV-susceptible) and Mace (WSMV-resistant) were mechanically inoculated with TriMV, WSMV, TriMV+WSMV, or sterile water at the 2-leaf growth stage. At 28 days post inoculation, the number of tillers per plant (TPP), shoot fresh weight (SFW), shoot dry weight (SDW), root fresh weight (RFW), and root dry weight (RDW) were determined. TPP was significantly (LSD, P = 0.05) lower only in TriMV+WSMV-inoculated plants in Millennium. In TriMV-inoculated plants, SDW was significantly reduced only in Millennium. SFW and SDW were similar in TriMV- and WSMV-inoculated plants in both cultivars. RFW and RDW did not differ between TriMV- and WSMV-inoculated plants in Millennium or Mace, but were significantly lower in inoculated than in non-inoculated Millennium plants. In TriMV + WSMV-inoculated plants, SFW was reduced by 64.1% in Millennium and 11.3% in Mace; SDW was reduced by 47.3% in Millennium and 3.5% in Mace; RFW was reduced by 74.5% in Millennium and 15.5% in Mace; and RDW was reduced by 79.3% in Millennium and 29.5% in Mace. These results imply a high risk for yield loss when susceptible wheat is co-infected with TriMV and WSMV.

Dissecting the mode of transmission of *Maize chlorotic mottle virus* by the corn thrips, *Frankliniella williamsi*

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Phytopathology 101:S24

Maize chlorotic mottle virus (MCMV, Machlomovirus, Tombusviridae) has been recorded in Hawaii since the early 1990's and has since become one of the most widespread viruses affecting corn production on the Islands of Kauai and Oahu. MCMV is transmitted semi-persistently by several Chrysomelid beetles including the western corn rootworm, *Diabrotica virgifera* (Coleoptera, Chrysomelidae). However, beetle species vectors of MCMV are not present in Hawaii where the main vector has been reported as the corn thrips, *Frankliniella williamsi* (Thysanoptera, Thripidae). We have examined the mode of transmission of MCMV by the corn thrips by using leaf disk assays. Adults of the corn thrips transmitted the virus right after acquisition, with no evidence for latent periods. Thrips were able to transmit the virus for up to 6 days after acquisition, with decreasing efficiency as time progressed. Transmission efficiency increased with the time thrips were allowed to feed on infected plant leaves and to healthy leaf disks. Transmission rate peaked to 82% of inoculated leaf disks after an Acquisition Access Period of 48 hours and an Inoculation Access Period of 96 hours; 3 hours were estimated as the minimum time for virus acquisition and inoculation. MCMV was transmissible by both larvae and adults; however, adults were more efficient vectors than larvae. Our data suggests that corn thrips transmits MCMV in a semi-persistent manner.

The impact of plant pathogens on post-weed biocontrol restoration

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Phytopathology 101:S24

Successful biological control of the deep-rooted perennial invasive plant *Euphorbia esula/virgata*, has dramatically reduced stand density at several locations within Theodore Roosevelt National Park in North Dakota, U. S. A. The synergistic interaction of root-feeding larvae of *Aphthona* spp. with soilborne plant pathogens has been shown to be the essential mechanism through which infestations of *E. esula/virgata* were controlled. We investigated the hypothesis that the biotic legacy from biocontrol would be significant soilborne disease on native species transplanted into restoration plots at sites where *E. esula/virgata* is under successful biocontrol. Seedlings of several native forbs and grasses were transplanted into plots at five different locations within the park where *E. esula/virgata* had been controlled. Surveys of the plots were conducted. Transplanted seedlings had a low a disease incidence based on isolations from roots and crowns of sampled plants. Native forbs showed the most apparent disease; among these, the native species *Ratibida columnifera*, *Aster ericoides*, and *Helianthus pauciflorus* had the highest incidence of root and crown disease from which *Rhizoctonia*, *Fusarium* and *Pythium* spp. were isolated. Insect/pathogen interaction-driven stimulation of soilborne pathogen inoculum potential may affect the success of restoration following biological control, particularly for forbs. The results of pathogenicity tests of fungal isolates on native forbs will be reported.

Enzyme-linked immunosorbent assay for *Pyrenophora teres* in soil

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Phytopathology 101:S24

Net blotch foliar disease, caused by the necrotrophic fungal pathogen *Pyrenophora teres* Drechsl, is a serious disease of barley (*Hordeum vulgare* L.) in eastern Montana, U.S.A., which results in premature leaf death and poor grain development and can reduce both yield and quality of the crop. The fungus survives between seasons primarily on barley residue and volunteer barley plants, but also on some grasses, and seed. An enzyme-linked immunosorbent assay (ELISA) was developed in an attempt to quantify *P. teres* mycelial and spore biomass in soil. Amounts as small as 0.5 ng of freeze-dried *P. teres* mycelia and spores dispersed in carbonate buffer were detected. Common field fungi found in eastern Montana such as Sordariomycetes and Dothidiomycetes of Ascomycota and Agaricomycetes of Basidiomycota showed negligible cross reactivity with the antibodies. Experiments performed on soils spiked with *P. teres* mycelia and spores and on naturally infested soils indicated that antigens are not degraded in soil and a proportion of immobilized antigens can be extracted from soil and detected by ELISA. *Pyrenophora teres* in naturally infested field soil detected by ELISA was also detected by PCR thus confirming the ELISA by itself as a viable technique for detection of *P. teres* in soil.

A Decision Support System for management of organic vineyards against downy mildew

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Phytopathology 101:S24

A DSS (Decision Support System) called *Vitebio.net* is described for guiding decision about tactical management of downy mildew in organic vineyards. The DSS is available for registered users via the internet in an interactive way. It is based on: (i) a network of weather stations that measure environmental variables; (ii) a 72-h weather forecast system; (iii) a file repository that stores weather data; (iv) a set of mathematical models that use weather data and vineyard-specific information to predict the biological events relevant for decision making; (v) a user interface that makes it possible to readily input vineyard-specific information and obtain supports for decision making; and (vi) a register of the vine-management options already applied that could influence future decisions (e.g., fungicides used, rates, etc.). Output provides information on: (i) current weather conditions and 3-days forecasts; (ii) model output for primary and secondary infections of *Plasmopara viticola*; (iii) optimum dose of copper to be applied depending on disease risk, residual coverage of the previous fungicide application, and grapevine growth and development. Different users can access information at different levels of complexity, depending on their role (i.e., providers, advisors, or growers). Each year, experimental sites are managed so as to compare advantages arising from using the DSS in comparison with the usual grower's practice.

Genome sequencing and analysis of *Anisogramma anomala*, the causal agent of eastern filbert blight

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Phytopathology 101:S25

Eastern filbert blight (EFB), caused by the obligate filamentous ascomycete *Anisogramma anomala* (Peck) E. Müller, is a devastating disease of European hazelnut, *Corylus avellana*. The causal fungus is native to a wide area east of the Rocky Mountains, where it is found associated with its natural host, *C. americana*. Despite quarantine efforts, EFB was discovered in Washington in the late 1960s and now threatens the U.S. hazelnut industry, located primarily in the Willamette Valley of Oregon. Commercial cultivars carrying a single dominant resistance gene from 'Gasaway' have shown complete resistance to *A. anomala* infection over several decades of exposure in the west coast where, due probably to a single point introduction, the fungus has limited diversity. However, isolates from the east coast have been shown to overcome the 'Gasaway' gene, suggesting greater diversity across its natural range. To explore the pathogen genome for mechanisms of pathogenicity and look for molecular markers to trace pathogen diversity and movement, we have sequenced the genome of *A. anomala* using the Illumina GA IIX platform. Approximately 24M 151x2 paired-end reads were generated from an insert library of ~400 bp. Initial *de novo* genome assembly achieved contig N_{50} of 1.6 kb and N_{max} of 20 kb. We are using a small number of high-quality Sanger sequences to validate and guide the improvement of the assembly, which will be followed by scaffolding. Genome assembly, annotation and analysis will be presented.

Genome-wide identification and characterization of microsatellite markers in *Anisogramma anomala*

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Phytopathology 101:S25

Anisogramma anomala (Peck) E. Müller is an ascomycete that causes eastern filbert blight (EFB) of European hazelnut, *Corylus avellana*. In the Willamette Valley of Oregon, where 99% of the U.S. crop is produced, EFB is controlled by fungicide applications and more recently through resistant cultivars carrying a single dominant resistance gene from 'Gasaway'. The pathogen appeared in the Willamette Valley only after the 1960s. Harbored by its natural host, *C. americana*, it is believed to have a more diverse population east of the Rocky Mountains than in Oregon. Supporting this premise, some isolates from the east have been shown to overcome the 'Gasaway' resistance gene in field and greenhouse studies. To develop molecular markers for investigation of its genetic diversity and movement, we screened for perfect and compound microsatellite sequences in a draft genome assembly of *A. anomala*. A total of 44,530 microsatellites were identified, with 134 identified per Megabase of genome. Compound microsatellites were found in 2157 loci. PCR primers were designed to amplify each microsatellite. Clustering analysis is being conducted to identify redundant loci. We assembled a collection of *A. anomala* isolates from diverse locales spanning its native range, including those expressing differences in virulence on 'Gasaway'. Isolates were cultured, DNA was extracted, and we are now using them to screen non-redundant microsatellite markers with motifs of two or more nucleotides.

Co-infection of a single *Phytophthora infestans* isolate by two distinct viruses

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Phytopathology 101:S25

Phytophthora infestans is the highly destructive pathogen that causes potato and tomato late blight. It has fungal-like morphology and habitat, but belongs to a distinct group, the oomycetes. Screening of *P. infestans* isolates revealed two double-stranded (ds) RNAs, 8.2 kb and 3.0 kb, in a single isolate from Florida. The dsRNAs represent genomes of two distinct viruses, as evidenced from their complete sequences and biological properties. The 8.2 kb dsRNA, named *Phytophthora infestans* RNA virus 3 (PiRV-3), has two open reading frames (ORFs) showing closest similarity to the respective ORFs in *Phlebiopsis gigantea* mycovirus dsRNA2 and *Fusarium graminearum* dsRNA mycovirus-3, two viruses with similarity to members of the family *Totiviridae*. ORF1 and ORF2 are in different frames on the same strand with a small overlap, but no translational frameshifing signal was detected. The 3.0 kb dsRNA, named *Phytophthora infestans* RNA virus 4 (PiRV-4), contains a single ORF predicted to encode a protein with greatest similarity to the RNA-dependent RNA polymerases of *Saccharomyces cerevisiae* 20s and 23s viruses, two members of the family *Narnaviridae*. PiRV-4 was also detected in isolates without PiRV-3. A combination of chemotherapy and hyphal-tipping cured PiRV-3 from the host strain, resulting in a PiRV-3-free strain with denser mycelium in culture.

Identification of a soybean G-protein coupled receptor and its role in plant defense responses

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Phytopathology 101:S25

G-protein coupled receptors (GPCR) comprise large a family of transmembrane receptors. After binding a ligand, the GPCR activates a cognate G-protein which, in turn will trigger a myriad of second messengers that regulate numerous cell physiological responses. Recent studies in plants have demonstrated direct roles for G-proteins in plant defense responses including the production of reactive oxygen species (ROS), the activation of NADPH oxidases, ion channels and phospholipases. A heterotrimeric G-protein in *Arabidopsis thaliana* was demonstrated to play a role in the jasmonate-mediated signaling response to the necrotrophic pathogen *Alternaria brassicola*). Based on our previous microarray studies we have identified several components of the G-protein signaling cascade that consistently change in expression during pathogenesis. To further assess if there is an actual GPCR involved directly or indirectly in the interaction between the necrotrophic fungal pathogen *Sclerotinia sclerotiourum* and soybean, we used the Arabidopsis GCR1 sequence to screen the soybean genome for candidate GPCR coding genes. Two putative GPCR genes were identified. An RNAi silencing construct was generated with the sequence and was introduced into soybean. We are currently in the process of obtaining viable seeds from the silenced plants. Additionally, Arabidopsis gcr1 mutants are being evaluated for their response to *Sclerotinia sclerotiorum* and other pathogens.

Novel rust resistance in wheat (*Triticum aestivum* L.)

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Phytopathology 101:S25

The *Puccinia* fungi that cause wheat rust diseases are among the most globally destructive agricultural pathogens. The most effective and utilized defense against rust is genetic resistance. The vast majority of rust resistance is race-specific conferred by single genes rapidly overcome by the pathogens. From EMS mutagenesis using a soft white spring wheat cultivar 'Alpowa', we have identified a mutant, $MNs220^{Alp}$, expressing broad spectrum, slow-rusting, all plant stage resistance. The mutation is likely to be associated with the change of negative regulation of resistance that is known to suppress either active or passive defense responses. In wheat, negative regulators have been observed to inhibit rust resistance genes. $MNs220^{Alp}$ has enhanced resistance to leaf, stem (including Ug99 and its derivative races), and stripe rusts, as well as powdery mildew. Genetic analysis in several backgrounds demonstrated that the resistance found in $MNs220^{Alp}$ is conferred by a single dominant gene. Gene expression profiling of several pathogenesis-related (PR) genes indicates that $MNs220^{Alp}$ has a rapid and elevated pathogen induced response. The mutant has an indistinguishable phenotype from the wild type in the absence of pathogens allowing for the immediate deployment into breeding programs. Beyond the immediate benefit, continued analysis of the locus will lead to a better understanding of the regulation of defense response network in wheat.

Effects of plant growth regulators on a DMI insensitive *Sclerotinia homoeocarpa* population

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Phytopathology 101:S25

Dollar spot (caused by *Sclerotinia homoeocarpa*) is primarily controlled by fungicide applications on golf courses. Demethylation inhibitor fungicides (DMIs) are the most widely used of the three fungicide classes with confirmed resistance in dollar spot. Plant growth regulators (PGRs; flurprimidol and paclobutrazol), which inhibit gibberellic acid, have a correlation *in vitro* sensitivity to DMIs and exhibit a fungistatic effect on dollar spot in the field. There is no report of PGRs selecting DMI insensitive isolates in the field. The objective of this study was to evaluate the effect of two PGRs on a golf course population of *S. homoeocarpa* consisting of both DMI sensitive and insensitive isolates. The effects of the PGRs in rotation and tank-mixed with the DMI, propiconazole were studied to determine changes in efficacy of dollar spot and *in vitro* sensitivity of *S. homoeocarpa*. Results indicate that the tank-mix of propiconazole (1 oz/1,000 ft2) with either flurprimidol and paclobutrazol at 0.28 and 0.18 oz/1,000 ft2, respectively reduced dollar spot infection centers similar to propiconazole alone at 2 oz/1,000 ft2. These treatments also selected for DMI insensitive isolates 7 days after application.

PGRs applied consecutively increased the number of insensitive isolates, but rotational and tank-mixed applications that included propiconazole provided the most selection pressure for insensitive isolates.

Xanthomonas leaf blight of *Ficus elastica*
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Phytopathology 101:S26

Ficus elastica is popular for its lush foliage and most often used as an interior container plant. During late summer 2010, a severe outbreak of Xanthomonas leaf blight affecting several cultivars of *F. elastica* was observed in South Florida. On young plants symptoms appear as small, water soaked lesions with irregular borders near the leaf margin. Leaf spots on older plants turn brownish-black with a chlorotic halo and leaf tissue appears water soaked on the abaxial side. A PCR was performed on the 16S rRNA gene and subsequent DNA sequencing and GenBank search showed the isolated strain is 99% identical to that of *Xanthomonas campestris*. Pathogenicity was confirmed by spraying a bacterial cell suspension of 1×10^8 CFU/ml onto 12 potted *F. elastica* 'Burgandy'. Twelve plants were inoculated with water as controls. Plants were placed in a greenhouse and shade house where temperature ranged from 23–32 C and 60–95% relative humidity. Symptoms started to develop within 10 days and *X. campestris* was re-isolated and identified using the above methods. *Xanthomonas campestris* has been previously reported on other species of Ficus in Florida. Further characterization of the pathogen, host range studies, and the effect of temperature and light on disease development are underway.

A sensitive molecular method for detecting virus in orchids
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Phytopathology 101:S26

Cymbidium mosaic virus (CymMV) and *Odontoglossum ringspot virus* (ORSV) are the most prevalent orchid viruses in nursery production worldwide. These two economically important viruses are a threat to the industry as they cause size reduction and decrease foliar and flower quality in numerous orchid species. Virus symptoms in orchids are typically not distinct and often asymptomatic, making visual detection unreliable. Common virus detection methods include immunological (i.e. double antibody sandwich (DAS-ELISA) and Immunostrips) nucleic acid hybridization (i.e. dot-blot) and molecular assays (i.e. PCR and real time PCR). In this study, high-fidelity and standard RT-PCRs were used to detect CymMV and ORSV from infected orchid leaf tissue. The high-fidelity RT-PCR detected 0.001 ng/µl of CymMV and ORSV in a total plant RNA extraction compared to 1 ng/µl of CymMV and 0.1 ng/µl of ORSV respectively. These results indicate a 10^3 increase in sensitivity for detecting CymMV and ORSV using the high-fidelity RT-PCR. We present a highly sensitive method that can be used to ensure virus free orchids that meet phytosanitary requirements. For this purpose a diagnostic manual with detailed methodologies is in progress.

Increases in snap bean and soybean seedling diseases associated with a chloride salt and changes in the micro-partitioning of tap root calcium
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Phytopathology 101:S26

In a series of field experiments from 1995 through 2010, the incidence of seedling diseases of snap bean and soybean caused by *Rhizoctonia solani*, *Macrophomina phaseolina*, *Pythium* spp., and *Fusarium* spp. were greater with an application of KCl than with K_2SO_4 applied at 93 kg K/ha. To determine if the observed increases could be due to a change in root calcium associated with chloride salts, soybeans were grown in pasteurized silt loam field soil and treated with KCl, K_2SO_4, $MgCl_2$, and NaCl at 250 or 400 µg Cl/g soil (276 or 441 µg K/g soil). After 10 to 13 da, the soybean roots were freed from the soil. Root segments were gently wiped clean of adhering soil with damp cotton swabs, dried, and subjected to elemental analysis using a Philips XL30 ESEM equipped with an energy dispersive x-ray analyzer. Calcium levels in the outer cell layers of tap roots treated with KCl, $MgCl_2$, and NaCl were lower than with the untreated control. Compared to the untreated control, there was no change in root calcium with K_2SO_4. The chloride salt, KCl, may have predisposed the seedling roots to pathogen infection in the earlier field studies by reducing the amount of root calcium available for plant defense mechanisms.

Detection of wheat powdery mildew by using hyperspectral remote sensing
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Phytopathology 101:S26

Wheat powdery mildew is an important disease of wheat in China. The field experiments were conducted at the experimental station of CAAS in Hebei Province in 2007, 2008 and 2009. Two wheat varieties were used in the experiments and different epidemic patterns of powdery mildew were obtained by fungicide spraying. The canopy reflectance of wheat with different disease intensity was measured by using spectroradiometer. The results indicated that as disease index increasing, reflectance in the near infrared region decreased significantly, and there were highly significant negative correlations between them in both varieties at the anthesise and milk-filling stages. Meanwhile the first derivative reflectance in visible region and in near infrared region were positive and negative correlations with disease index, respectively. Red edge position moved to short wavelength when wheat infected with powdery mildew, and a significant correlation was existed between red edge slope and disease index. Several vegetation indices such as DVI, RVI, NDVI and SAVI had high the absolute values of correlation coefficients with disease index. Based on above results, the models of relationships between disease index and hyperspectral parameters were constructed for both varieties. These results demonstrated that hyperspectral remote sensing has the potential to monitor wheat powdery mildew in fields. This study was supported by Special Fund for Agro-scientific Research in the Public Interest (No.3-15).

Evaluation of wheat varieties for resistance to stripe rust in south Gansu in China during 2006–2010
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Phytopathology 101:S26

Wheat stripe rust is one of the most important and destructive disease in southern Gansu, China. Growing resistant wheat cultivars is the most effective, economical, and environmentally friendly method of disease control. Evaluation of the resistance to stripe rust on wheat cultivars was the basic work before using it. By artificial inoculation with different races of *Puccinia striiformis* f. sp. *tritici.*, 2449 wheat varieties (1ines) were identified for resistance in Gansu during 2006–2010. The results showed that 722 varieties (1ines) are resistant to testing races including CYR33, CYR32 and mixed races in whole stage such as Lantian 25. 619 wheat varieties (1ines) are resistant in seedling stage. The resistance of Gansu's wheat varieties (1ines) was better than other province's in China. The results in the fields indicated that about 95% wheat varieties (1ines) which released in the huanghuaihai region are susceptible. Some of resistant sources, such as C591 is susceptible after 2007. It is notable that other progeny lines which possess Yr26 become middle susceptible in South Gansu from 2009. Now, the wheat varieties (1ines) which possessing Yr26 were grown over a large area in Gansu and Sichuan provinces. Using resistant diversity, growing different wheat varieties (1ines) which possessed different genes in some distinct area, has become a great concern to reduced the speed of losing resistance to stripe rust in south Gansu, China.

Plasmid content of *Erwinia amylovora* isolates from orchards in Washington and Oregon
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Phytopathology 101:S26

We examined the plasmid content of a collection of 305 isolates of *Erwinia amylovora* from Washington and Oregon in the Pacific Northwest of the U.S.A. with PCR assays and RFLP. Nearly all isolates of *E. amylovora* carried plasmid pEA29, which is not found in other species of bacteria, but 4% of the isolates from this region lacked pEA29. The plasmid pEU30, previously reported in pathogen strains from western states in the U.S.A., was detected in 28% of isolates. The RFLP patterns of plasmid preparations from a third of isolates from an epidemic in Washington in 1988 had altered RFLP patterns, possibly due to the presence of plasmid(s) in addition to pEA29 or pEU30. Considering all samples, the majority of isolates in this region were typical of *E. amylovora* and harbored only pEA29. Nonetheless, many of the pathogen isolates had altered plasmid content, indicating that plasmid acquisition and propagation in populations of *E. amylovora* in orchards in the Pacific Northwest is more common than previously assumed.

Epidemiology of grape anthracnose: Identification of factors associated with defoliation of grape leaves infected by *Elsinoe ampelina*
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Phytopathology 101:S26

Anthracnose is a serious disease on several winter hardy grape cultivars. Infected leaves drop prematurely and severe epidemics may result in poor or

no yield. Hence, management program should prevent early infection and defoliation in order to protect berry from being infected and maintain plant vigour. There is a scarcity of information on anthracnose epidemiology. Factors associated with grape defoliation due to *E. ampelina* infections were not known; hence the objective of this study was to investigate host, pathogen and weather factors that influence leaf survival. Leaf emergence and anthracnose severity was monitored in non sprayed experimental vineyard (cv. Vandal Cliche) from 2006 to 2008. The number of leaves on two shoots on 10 randomly selected vines was monitored 3 times per week and the proportion of leaf area infected by *E. ampelina* monitored weekly from leaf emergence to natural defoliation. Initial proportion leaf area diseased (IPLAD) and mean IPLAD per shoot ($IPLAD_{shoot}$) were the most significant variables influencing leaf survival. An increase of 1% in both variables reduced leaf survival by 0.65 and 0.95%, respectively. In addition, new leaves that emerged when the disease is well established have shorter life duration. The information gained from this study improved our understanding of grape anthracnose epidemiology and suggests that early control measures may be necessary to avoid premature leaf drop.

Management of strawberry anthracnose fruit rot in North Carolina with reduced fungicide spray schedules

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Phytopathology 101:S27

Strawberry anthracnose fruit rot (AFR), caused by *Colletotrichum acutatum* is an economically important disease in the Southeastern United States. Dispersal of inoculum requires splashing rain while conidial germination and host infection is enhanced by prolonged wetness hours at optimum temperature. These weather variables can be monitored and incorporated into disease forecasting models (courtesy N. Peres, U of FL). Conventional management of AFR includes weekly application of fungicide sprays during the fruiting season. This practice is expensive and poses a risk for selecting fungicide resistant populations. Results from our study indicated that both adjustment in timing of spray applications and disease prediction based spray programs can significantly reduce the number of sprays without compromising the level of disease control. Reduced spray schedules in 2008 and 2009 starting at 10% bloom (4 applications) had 29.87% and 9.5% AFR compared to 21.6% and 9.2%, respectively in a season long schedule (8 applications) and 46.72% and 37.6% in non-sprayed plots. A disease forecast based spray program in 2010 indicated further reduction in the number of sprays would be possible with similar levels of disease control compared to a season-long schedule. In 2010 the non-sprayed controls had 8.45% AFR and the forecast-based spray schedule and season-long schedule had 3.2% and 4.4% AFR, respectively.

Evaluation of pruning techniques and bactericides for managing bacterial canker of sweet cherry

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Phytopathology 101:S27

Bacterial canker of sweet cherry occurs worldwide, causing bud mortality, twig cankers, leaf spots, flower and fruit lesions, and severe collapse and death of trees. In New York, *Pseudomonas syringae* pv. *syringae* (Pss) is most commonly isolated from infected tissues. Given that the pathogen may enter through pruning wounds, our objectives were to determine if, by leaving pruning stubs, trunk and scaffold cankers could be reduced, and if pruning time or bactericides reduce stub infection severity. Pruning techniques and bactericides (copper and phosphorous acid, applied at March and April pruning times) were evaluated in replicate orchard blocks in Geneva and Highland, NY. Stub pruning (avg 20-cm-long × 3.5 cm diam) and inoculation were done in March, April, May and post-harvest. Cut surfaces were inoculated with Cu-sensitive Pss (10^8 cfu/ml). Canker progression down stubs (severity) was assessed during the growing season. Stub infections rarely progressed into scaffolds or trunks. Cankers progressed furthest in stubs pruned in March and least when pruning was done post-harvest. Bactericide treatments failed to prevent infections and provided less than 19% reduction in canker severity. Our results indicate the ineffectiveness of bactericides at pruning and the effectiveness of post-harvest, stub pruning to manage cankers. Reducing copper applications in orchards will slow the emergence of Cu-resistant bacterial strains and reduce copper build-up in soils.

Efficacy of seed treatments on *Thielaviopsis basicola* in soybean

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Phytopathology 101:S27

To determine the affects of *Thielaviopsis basicola* on soybean, a greenhouse disease screening trial was conducted to evaluate the susceptibility of popular varieties grown in Arkansas and determine the efficacy of seed treatments. Since, *Thielaviopsis basicola* is considered a cool wet weather pathogen; a temperature controlled soil bed was constructed using a chiller that circulates water through copper coil tubing in the soil to maintain soil temperatures in the mid-50 o F. The soil was autoclaved and artificially inoculated with T. basicola at ~100 propogules/gram of soil. Four Delta Grow soybean varieties (5700, 4970, 4880, 4975, and 5160) were treated with four different seed treatments (Untreated, Experimental Treatment 1, Apron Max, Apron Max + Experimental Treatment 1) and planted in the inoculated soil in a randomized complete block design. Growing conditions for the soybeans were 14 hour days at 86°F air temperature for 35 consecutive days. On the 35th day, the roots were washed for 20 minutes. They were then surface sterilized in 10% bleach solution for 1 minute and 30 seconds. The roots were platted on a TBCEN selective media. Initial results indicated all soybean varieties tested were susceptible to *Thielaviopsis basicola*. After only two week after planting, untreated plant roots were discolored and found to have T. basicola chlamydospores present on damaged tissue.

Alternative control of citrus black fly *Aleurocanthus woglumi* Ashby, 1915 in the northeast of Brazil

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Phytopathology 101:S27

Citrus black fly *Aleurocanthus woglumi* is an invasive pest in Brazil and is restricted to a few states. Its detection in the northeastern states in 2010 caused an interruption of exportation of fruits from the attacked area, originating huge losses. Local government has urged the producers to spray their crops with imidacloprid which is the only product registered in Brazil for its control. This action has displeased the organic producers who were left without options. So, this study had the objective of testing alternative products on the control of citrus black fly. Mineral oil, vegetal oil, neem oil, orange peel oil, detergent and soaps, at concentrations of 1.0% and 0.5%, were sprayed weekly over adults, eggs and larvae of citrus black fly, in the field, in a completely randomized design with 14 treatments and 10 replicates. Infested leaves were checked under stereo microscope. The efficiency of alternative products varied according to each phase of development. No alternative product was effective against hatching of eggs. Detergent, orange peel oil, mineral oil and powdered soap killed 100% of larvae of 2nd and 3rd instars with just one application. Only powdered soap and orange peel oil killed 100% of pupas. Both detergent and orange peel oil killed 100% of adults instantly. Thus, these products can be used as an alternative approach on the control of citrus black fly.

Effect of alternative products on mortality of adults of citrus black fly *Aleurocanthus woglumi* Ashby, 1915 on leaves of orange trees

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Phytopathology 101:S27

Citrus black fly is an invasive species in Brazil and it is restricted to a few states. The only product registered in Brazil for its control is imidacloprid, but its utilization has been avoided by ecological farmers. This study had the objective of testing the effect of alternative products sprayed over infested leaves of orange trees. Highly infested newly developed branches were carefully cut, turned upside dow and sprayed with a 0.5 L manual garden sprayer containing water based solutions of neem oil, orange peel oil, mineral oil, vegetal oil, detergent and powdered soap at 1.0% concentration in a completely randomized design with 7 treatments and 10 replicates. Each replicate consisted of one infested leaf. Ten infested leaves were also sprayed with water only as the control treatment. Leaves were immediately collected after been sprayed and were taken to the lab. The mortality of adults of citrus black fly was checked under stereo microscope. Except for the treatment control, all the other treatments left dead adults of citrus black fly on the leaves. Thus, detergent, powdered soap, and (mineral, vegetal, neem and orange peel) oils can be used on the alternative control of adults of citrus black fly.

Effect of concentrations of detergent on mortality of adults of citrus black fly *Aleurocanthus woglumi* Ashby, 1915 on leaves of orange trees

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Phytopathology 101:S27

Citrus black fly is an invasive species in Brazil and is restricted to a few states. The only product registered in Brazil for its control is imidacloprid, but its utilization has been avoided by ecological farmers. Among other alternative products, detergent was found to kill the adults of citrus black fly at 1.0% concentration. This study had the objective of testing the effect of other concentrations of detergent sprayed over infested leaves of orange trees. Highly infested newly developed branches were carefully cut, turned upside

down and sprayed with 0.5 L garden sprayer containing water based solutions of detergent at the following concentrations: 5.0%, 4.0%, 3.0%, 2.0%, 1.0%, 0.5%, 0.4%, 0.3%, 0.2%, 0.1% and 0.0% (water only - control) in a completely randomized design with 11 treatments and 10 replicates. Each replicate consisted of one infested leaf. From each sprayed branch, 10 leaves were collected and checked under stereo microscope. No live flies were found on leaves sprayed at concentrations ranging from 0.5% down to 0.3%. Both dead and agonizing flies were found at 0.2% and 0.1% concentrations. No dead flies were found on the water only control treatment. Thus, since it is effective until 0.3% concentration, detergent can be used on the alternative control of adults of citrus black fly.

Efficiency of concentrations of detergent on mortality of adults of citrus black fly *Aleurocanthus woglumi* on leaves of tangerine trees
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Phytopathology 101:S28

Citrus black fly is an invasive species in Brazil and is restricted to a few states. The only product registered in Brazil for its control is imidacloprid, but its utilization has been avoided by ecological farmers. Detergent has been found to kill the adults of citrus black fly at concentrations as low as 0.3%. However it was unknown if a spray would kill all adult insects found on a leaf or if some would escape. This study had the objective of testing the efficiency of concentrations of detergent sprayed over slightly infested leaves of tangerine trees. Infested leaves were carefully cut, turned upside down and sprayed from a distance of 10 cm with a 20 L manual costal sprayer containing solutions of detergent at 0.5%, 0.4%, 0.3% and 0.2% in a completely randomized design with 4 treatments and 10 replicates. Each replicate consisted of one infested leaf. Flies were counted before and after each spraying. A cloth was put behind each leaf to capture the flies forced out by spraying. Leaves and cloth were checked under stereo microscope. The concentrations of 0.5%, 0.4% and 0.3% killed 100% of flies. At 0.2% concentration, the number of dead flies did not match the number of live flies found before the spray, indicating that some had managed to escape. Thus detergent is 100% efficient at concentrations as low as 0.3% on the control of adults of citrus black fly.

Efficiency of alternative products on mortality of adults of citrus black fly *Aleurocanthus woglumi* on leaves of tangerine trees
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Phytopathology 101:S28

Citrus black fly is an invasive species in Brazil and is restricted to a few states. The only product registered in Brazil for its control is imidacloprid, but its utilization has been avoided by ecological farmers. Alternative products were found to kill adults of citrus black fly a 1.0% concentration. However, it was unknown if a spray would kill all adult insects found on a leaf or if some would escape. This study had the objective of testing the efficiency of alternative products sprayed at a concentration of 1.0% over slightly infested leaves of tangerine trees. Infested leaves were carefully cut, turned upside down and sprayed from a distance of 10 cm with a 20 L manual costal sprayer containing solutions of mineral oil, vegetal oil, neem oil, orange peel oil, detergent and powdered soap in a completely randomized design with 6 treatments and 10 replicates. Each replicate consisted of one infested leaf. Flies were counted before and after each spraying. A cloth was put behing each leaf to capture the flies forced out by spraying. Leaves and cloth were checked under stereo microscope. Detergent, powdered soap and orange peel oil killed 100% of flies. Mineral oil, vegetal oil, and neem oil left dead flies on leaves and cloth but their final numbers were inferior to the ones found before spraying, indicating that some had managed to escape. Thus, detergent, powdered soap and orange peel oil are 100% efficient at 1.0% concentration on the control of adults of citrus black fly.

Efficiency of concentrations of orange peel oil on mortality of adults of citrus black fly *Aleurocanthus woglumi* on leaves of tangerine trees
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Phytopathology 101:S28

Citrus black fly is an invasive species in Brazil and is restricted to a few states. The only product registered in Brazil for its control is imidacloprid, but its utilization has been avoided by ecological farmers. Orange peel oil has been found to kill the adults of citrus black fly at concentrations as low as 0.2%. However, it was unknown if a spray would kill all adult insects found on a leaf or if some would escape. This study had the objective of testing the efficiency of concentrations of orange peel oil sprayed over infested leaves of tangerine trees. Slightly infested leaves were carefully cut, turned upside down and sprayed from a distance of 10 cm with a 20 L manual costal sprayer containing solutions of orange peel oil at 0.5%, 0.4%, 0.3%, 0.2%, and 0.1% concentrations in a completely randomized design with 5 treatments and 10 replicates. Each replicate consisted of one infested leaf. Flies were counted before and after each spraying. A cloth was put behind each sprayed leaf to capture the flies forced out by the spraying. Leaves and cloth were checked under stereo microscope. The concentrations of 0.5%, 0.4%, 0.3% and 0.2% killed 100% of flies. At 0.1% concentration, the number of dead flies did not match the number of live flies, indicating that some have managed to escape. Thus, orange peel oil is 100% efficient at concentrations as low as 0.2% on the control of adults of citrus black fly.

Effect of concentrations of orange peel oil on mortality of adults of citrus black fly *Aleurocanthus woglumi* Ashby, 1915 on leaves of orange trees
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Phytopathology 101:S28

Citrus black fly is an invasive species in Brazil and is restricted to a few states. The only product registered in Brazil for its control is imidacloprid, but its utilization has been avoided by ecological farmers. Among other alternative products, orange peel oil was found to kill the adults of citrus black fly at 1.0% concentration. This study had the objective of testing the effect of other concentrations of orange peel oil sprayed over infested leaves of orange trees. Highly infested newly developed branches were carefully cut, turned upside down and sprayed with a 0.5 L garden sprayer containing water based solutions of orange peel oil at the following concentrations: 2.0%, 1.0%, 0.8%, 0.6%, 0.4%, 0.2%, 0.1%, 0.08%, 0.06%, 0.04%, 0.02% and 0.0% (water only - control) in a completely randomized design with 12 treatments and 10 replicates. Each replicate consisted of one infested leaf. From each sprayed branch, 10 leaves were collected and checked under stereo microscope. No live flies were found on leaves sprayed at concentrations ranging from 2.0% down to 0.2%. From 0.1% down to 0.04% both dead and agonizing flies were found. No dead flies were found at 0.02% concentration nor on the control treatment. Thus, since it is effective at even very low concentrations, orange peel oil can be safely used on the alternative control of adults of citrus black fly.

Effect of alternative products on mortality of 2nd and 3rd instar larvae of citrus black fly *Aleurocanthus woglumi* on leaves of orange trees
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Phytopathology 101:S28

Citrus black fly is an invasive species in Brazil and is restricted to a few states. The only product registered in Brazil for its control is imidacloprid, but its utilization has been avoided by ecological farmers. Some alternative products have been found to be effective against adults of citrus black fly. This study had the objective of testing the effect of alternative products on larvae of 2nd and 3rd instar. Powdered soap, mineral oil, vegetal oil, and orange peel oil were tested at 1.0% and 0.5% concentrations. Detergent was tested at 5.0% and 2.5% concentrations. Larvae infested leaves were sprayed with a 0.5 L garden sprayer from a distance of 10 cm. Two weekly applications were done. One weak after each application 4 leaves were collected and checked under stereo microscope. Mineral oil, vegetal oil, orange peel oil, detergent and powdered soap left no live 2nd and 3rd instar larvae of citrus black fly on treated leaves after both 1 and 2 applications, being effective alternatives for controlling this pest at these phases.

Effect of concentrations of orange oil on mortality of 2nd and 3rd instar larvae of citrus black fly *Aleurocanthus woglumi* on leaves of lemon trees
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Phytopathology 101:S28

Citrus black fly is an invasive species in Brazil and is restricted to a few states. The only product registered in Brazil for its control is imidacloprid, but its utilization has been avoided by ecological farmers. Orange peel oil has been found to control 2nd and 3rd instar larvae of citrus black fly on leaves of orange trees at 1.0% and 0.5% concentrations. This study had the objective of testing the effect of lower concentrations of orange peel oil on mortality of 2nd and 3rd instar larvae of citrus black fly on leaves of lemon trees. Infested leaves were sprayed from a distance of 10 cm with a 0.5 L garden sprayer containing solutions of orange peel oil at 0.5%, 0.4%, 0.3%, 0.2% and 0.1% concentrations in a completely randomized design with 6 treatments and 10 replicates. Each replicate consisted of one infested leaf. One week after spraying, 10 treated leaves were collected from each treatment and checked under stereo microscope. In each leaf an area of 1 cm^2 was used for evaluation. The orange peel oil concentrations of 0.5%, 0.4% and 0.3% left no survivors on the leaves. Both dead and live larvae were found on leaves treated at 0.2% and 0.1% concentrations. Only live larvae were found on leaves of the untreated control. Thus, orange peel oil can be used effectively on the control of 2nd and 3rd instar larvae of citrus black fly at concentrations as low as 0.3%.

Effect of alternative products on mortality of 4th instar larvae (pupas) of citrus black fly *Aleurocanthus woglumi* on leaves of orange trees

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Phytopathology 101:S29

Citrus black fly is an invasive species in Brazil and is restricted to a few states. The only product registered in Brazil for its control is imidacloprid, but its utilization has been avoided by ecological farmers. Some alternative products have been found to be effective against adults of citrus black fly. This study had the objective of testing the effect of alternative products on mortality of the 4th instar larvae (pupas). Powdered soap, mineral oil, vegetal oil, and orange peel oil were tested at 1.0% and 0.5% concentrations. Detergent was tested at 5.0% and 2.5% concentrations. Highly infested leaves received three weekly applications with a 0.5 L garden sprayer from a distance of 10 cm. Treated leaves were protected with a cloth in the field. From each treatment, 4 leaves were collected 7 days after the first application, 10 leaves were collected 7 days after the second application and more 10 leaves were collected 15 days after the third weekly application. Treated leaves were checked under stereo microscope. A needle was used to perforate each pupa in search for organic fluids. Powdered soap killed 100% of pupas only at 1.0% concentration since the first application. Orange peel oil killed 100% of pupas at both 1.0% and 0.5% concentrations. None of t he other alternative products tested in this study killed 100% of pupas of citrus black fly.

Multilocus analysis of *Phoma sclerotioides* isolates from Minnesota

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Phytopathology 101:S29

Phoma sclerotioides causes brown root rot in alfalfa, which reduces winter survival. Regional genotypes occur but local population structures have not been characterized. The population genetic structure and gene flow within alfalfa fields in Minnesota was inferred using multilocus analysis. Portions of chitin synthase 1 (CHS; 299 bp), glyceraldehyde-3-phosphate dehydrogenase (G3PD; 578 bp), the rDNA internal transcribed spacers (ITS; 498 bp), and their concatenated sequences (CS) (1,375 bp), were analyzed for 102 isolates from four sites. Diversity of the CS was similar for two collection years with regard to nucleotide (?) and haplotype (Hd) diversity, as well as Theta (?) values, although a different number of haplotypes were found each year. Sequence of G3PD was the most diverse for ? and ? values among other estimators, while CHS had the highest Hd in both years. In 2007, no population differentiation was found between isolates from two sites with the CS, and abundant gene flow (Nm = 31.50) was detected. In 2008, highly significant population differentiation between isolates from four sites, including the two sampled in 2007, and more restricted gene flow (Nm = 1.86) was detected. Neutrality tests on the individual or joint sequences were not statistically significant. The level of nucleotide and haplotype diversity indicates that the Minnesota population is not recently introduced and nucleotide variation is mostly driven by random genetic drift.

Assembling and exploring the *Cochliobolus miyabeanus* genome of a strain pathogenic on wildrice (*Zizania palustris*)

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Phytopathology 101:S29

The genome of a strain of *C. miyabeanus* was shotgun sequenced by paired-end reads with Illumina HiSeq 2000 technology. The genome was assembled with AbySS software yielding a total size of 34.96 Mb (114X), with N50 = 99.43 kb contained in the largest 105 scaffolds, and with a maximum scaffold length of 408.932 kb. The G + C content of the genome was 51%. GeneMark-ES v2.3a detected 12,344 protein-coding genes, with a mean size of 1,537 bp and an average of 2.55 exons per gene. The average size of encoded proteins was 437 aa. Annotation of fungal proteins was done using BLASTP against the protein database of NCBI and protein functional classification was done with Blast2GO. Complete genome blast searches to the pathogen-host interaction database identified 15% of the genes mostly related to virulence, and pathogenicity, and a few effector molecules. Additionally, several genes were associated with transport, resistance and sensitivity to chemicals. The most abundant repetitive elements identified belong to the LTR/Gypsy retrotransposons. An independent RNA sequencing experiment is being used to validate the genome assembly. DNA genomic sequences from *C. miyabeanus* were mapped onto a reference genome (*C. heterostrophus*). Understanding the organization of the *C. miyabeanus* genome in parallel with the fungal transcriptome can help in developing wildrice varieties that will resist the specific pathogenicity/virulence factors of the fungus.

Evaluation of *Bacillus firmus* strain GB-126 seed treatment for the biocontrol of the reniform nematode on cotton plants

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Phytopathology 101:S29

Previously we have demonstrated the biocontrol potential of *Bacillus firmus* strain GB-126 in a non competitive autoclaved soil environment. *Bacillus firmus* strain GB-126 reduced numbers of *Rotylenchulus reniformis* vermiform stages, females, and eggs per gram of cotton root at a rate of 0.71 mg of spores per seed. The objective of this study was to evaluate the seed formulation rates of 0.10, 0.71, and 1.40 mg of spores per seed of *B. firmus* strain GB-126 on cotton compared to aldicarb and an untreated control in a silt loam field soil under greenhouse conditions. Variables measured every 5 days were plant height, shoot and root weight, root architecture, females and eggs per gram of root, and vermiforms per 480 grams of soil. The highest seed treatment rate of 1.40 mg of spores of *B. firmus* strain GB-126 per seed provided protection to the cotton root from 20 DAP until 30 DAP where the number of females per gram of root were lower than the untreated seed control ($P \leq 0.027$), and resulted in lower numbers of eggs per gram of root 30 DAP ($P \leq 0.001$). The three seed treatment rates of *B. firmus* strain GB-126 reduced vermiform populations in soil 15 DAP ($P \leq 0.001$) with the highest rate suppressing the vermiform population through 30 DAP ($P \leq 0.005$). The 1.40 mg of spores per seed rate reduced numbers of the females, eggs, and vermiform life stages of *R. reniformis* 30 DAP, similarly to aldicarb ($P \leq 0.05$).

Occurrence of a soft-rot disease on *Oncidium* orchids caused by a *Dickeya* sp. in Florida

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Phytopathology 101:S29

Oncidium orchids have been subjected to extensive cultivation in the pot-plant and cut flower industries. In August 2008, approximately 50 *Oncidium* 'Gower Ramsey' orchids were discovered at a commercial orchid nursery in South Florida with brown, macerated leaves and pseudobulbs typical of soft-rot disease reported in other orchids. Ten plants were selected and sections were removed from the edge of symptomatic tissue. All isolates were Gram negative, anaerobic, degraded pectate, grew at 37°C, produced blue to brown pigment on NGM medium, were sensitive to erythromycin, were oxidase negative, and were positive for phosphatase and indole production. MIDI analysis (Sherlock version TSBA 4.10; Microbial Identification, Newark, DE) identified the strains as *Erwinia chrysanthemi* (SIM 0.880 to 0.929). PCRs were performed using the 16S primers 27f and 1495r and 1,423 bp of the 16S rDNA gene showed 98 to 99% similarity to *Pectobacterium chrysanthemi*. Pathogenicity tests were performed by injecting 10 *Oncidium* 'Gower Ramsey' orchids with 100 µl of a 1×10^8 CFU/ml of a bacterial suspension. A *Dickeya* sp. was re-isolated and identified according to the method described above. Although an *Erwinia* sp. has been reported to cause soft-rot symptoms on *Oncidium aureum*, to our knowledge this is the first report of a *Dickeya* sp. (=*E. chrysanthemi*) causing soft-rot symptoms on an economically important *Oncidium* orchid in large-scale production in the Florida.

Expression of the cloned IS53 transposase promoter from *Pseudomonas savastanoi* under stress conditions

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Phytopathology 101:S29

Pseudomonas savastanoi causes tumors on olive and oleander trees, in part by the synthesis of indole-3-acetic acid (IAA) and cytokinins (CK). Oleander isolates carry the IAA and CK genes on virulence plasmids which also contain IS elements that have been associated with loss of virulence due to deletion of the IAA genes. The role of IS elements in bacterial mutation is well-established, but it is not known if mutation rates, at least those mediated by IS transposition events, can change in response to environmental stresses. The transposase (*tnp*) gene of the *P. savastanoi* IS53 is homologous to heat-shock promoter sequences from *E. coli*. Thus, the *tnp* gene of IS53 may be upregulated by heat-shock or other environmental stresses. To test this hypothesis, IS53 was cloned into a TOPO TA cloning vector, and the putative tnp promoter (*ptnp*) was subcloned into pVO155 upstream of a promotorless GUS (*uidA*) reporter gene. If *ptnp* is a heat-shock promoter, then under different temperature conditions, there should be different levels of expression of the *ptnp/uidA* fusion. The purpose of this masters' research is to measure *tnp* activity under stress conditions, and to identify and quantify the movement of transposable genetic elements (TGEs) in *P. savastanoi* under different stress conditions as a potential mechanism for environmentally-regulated mutation.

Diversity in *Cotton leaf curl virus* (CLCuV) isolates prevalent in northwestern India in light of the breakdown of CLCuV resistance in cotton

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Phytopathology 101:S30

Following the reports of breakdown of *Cotton leaf curl virus* (ClCuV) resistance in popular cotton hybrids in northwestern India during the 2010 season, six strains of CLCuV, including Sri Ganganagar strain isolated from a severely infected CLCuV-resistant hybrid, were characterized and nucleotide sequences of DNA-A and βDNA components were determined. Sequence comparisons revealed 81–99% and 88.3–92% sequence identity of DNA-A and βDNA, respectively, with known CLCuV sequences. Recombination analysis revealed significant recombination in these six virulent Indian strains showing 25 recombination sites in DNA-A and 11 recombination sites in βDNA. The observed recombination in several regions of DNA-A and βDNA in the potential resistance breaking Sri Ganganagar strain of CLCuV was mapped to the highly virulent Burewala strain and several other strains.

Characterization of the ICRISAT mini-core peanut germplasm collection regarding *Sclerotinia* blight resistance and oleic acid composition

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Phytopathology 101:S30

Peanut production is consistently threatened by various diseases and pests. *Sclerotinia minor* Jagger (*S. minor*), the causal agent of Sclerotinia blight, is a major threat to peanut production in the Southwestern U.S., Virginia, and North Carolina and can reduce yield by up to 50% in severely infested fields. Although host plant resistance would provide the most effective solution to managing Sclerotinia blight, limited sources of resistance to the disease are available for use in breeding programs. Peanut germplasm collections are available for exploration and identification of new sources of resistance, but traditionally the process is lengthy, requiring years of field testing before those potential sources can be identified. Molecular markers associated with phenotypic traits can speed up the screening of germplasm accessions. This study objective of this study was to characterize the ICRISAT mini-core collection with regards to oleic acid composition and a molecular marker associated with Sclerotinia blight resistance. One hundred twenty-four (124) accessions from the collection were available and genotyped using the SSR marker and 67 were identified as potential new sources of resistance and targeted for further evaluation in field tests for Sclerotinia blight resistance. Capillary electrophoresis profiles of oil extracted from each accession determined that none were high oleic in composition.

The effect of biological control practices on inducible defense genes and metabolic genes in field-cultivated potato plants

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Phytopathology 101:S30

Because major plant signalling pathways and the resulting cellular responses often include similar or identical genes, competing physiological processes may lead to a reduction of the effectiveness of induced resistance or have detrimental effects on plant growth and yields. Therefore, it is important to develop an understanding of the costs and benefits of biological control treatments and compost amendments. Field experiments were conducted to assess the effect of biological control agents 1) hypovirulent *Rhizoctonia solani* (Rhs1A1), 2) *Trichoderma virens* strain GI-21 (SoilGard™), and 3) *Bacillus subtilis* (Kodiak™) applied in-furrow with or without compost on changes in the gene expression levels of *StSUT4* (sucrose transporter), *ci21A/Asr1* (glucose metabolism in tuber), *PR*1 (Systemic Acquired Resistance pathway, SAR) and *PinII* (Induced Systemic Resistance pathway, ISR) in 'Yukon Gold' potato plants using quantitative Real-Time Polymerase Chain Reaction. RNA was extracted from potato leaf samples collected early and late in the growing season. Expression level of *PR1* increased almost 3-fold in plants treated with *T. virens* and the increase in expression of *PR1* was generally greatest later in the growing season. Expression level of *StSUT4* increased later in the growing season in year 2.

Identification of the critical factors for mechanical transmissibility of *Tomato leaf curl New Delhi virus*

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Phytopathology 101:S30

Tomato leaf curl New Delhi virus (ToLCNDV) belonging to *Begomovirus* was identified from many plants in tropical and subtropical countries. ToLCNDV contains bipartite genome, designated as DNA-A and DNA-B and each is approximately 2.7 kb in size. In April 2007, a new isolate ToLCNDV-OM was isolated from oriental melon (*Cucumis melo*). This isolate could infect some important gourd crops like cucumber, luffa and zucchini. Interestingly, most geminiviruses cannot be mechanically transmitted to their original hosts. However, ToLCNDV-OM could be transmitted to its original host by mechanical inoculation. In order to investigate the factors affect the mechanical transmissibility of ToLCNDV, the DNA-A and DNA-B of ToLCNDV-OM were recombined with the genome of a mechanically non-transmissible ToLCNDV cucumber isolate (ToLCNDV-CB). The virus inocula were prepared from infected *Nicotiana benthamiana* by agro-infectious clones and then were mechanically introduced into oriental melon and tomato plants to evaluate the mechanical transmissibility of the virus. Results indicated that the combination of DNA-A of ToLCNDV-CB with DNA-B of ToLCNDV-OM became mechanically transmissible whereas that of DNA-A of ToLCNDV-OM with DNA-B of ToLCNDV-CB did not. It clearly suggested that DNA-B of ToLCNDV-OM contain critical factors for mechanical transmissibility. Study for identification of specific gene that is responsible for mechanical transmissibility of ToLCNDV-OM is ongoing and will be presented.

Functional characterization of two genes involved in cercosporin biosynthesis in *Cercospora kikuchii*

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Cercospora kikuchii is the causal agent of soybean leaf blight and purple seed stain diseases. Currently, leaf blight is a major concern in Louisiana and other southern states, and it has the potential of spreading into other major soybean producing regions, such as the Midwest. The pathogen produces a toxin, cercosporin, which was shown to play a crucial role in pathogenicity and virulence on soybeans. We utilized two-dimensional protein gel electrophoresis (2DGE) to identify proteins that may be involved in cercosporin biosynthesis by comparing protein profiles of *C. kikuchii* grown under cercosporin-favoring (light) and cercosporin-suppressive (dark) conditions. Several proteins were up-regulated in *C. kikuchii* grown under light, and these were sequenced and identified as hydroxynaphthalene reducatse (HNR) and adenosylhomocysteinase (AHC). The corresponding genes were cloned from *C. kikuchii* through genome walking. HNR gene is predicted to be 862 bp long with one intron, whereas AHC gene is predicted to be 1562 bp long with two introns. HNR and AHC gene disruption mutants were produced through a hygromycin split marker approach, and the mutants showed drastic reduction in cercosporin production *in vitro*. The resulting HNR and AHC mutants also are being tested for changes in pathogenicity or virulence on soybean.

Fungicide seed treatments to manage seedling blight of faba bean in Alberta, Canada, 2010

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Faba bean (*Vicia faba* L.) is a source of high energy and protein for livestock and humans. Root rot of faba bean caused by *Fusarium avenaceum* and *Rhizoctonia solani* is widespread on the Canadian prairies. To examine the efficacy of four fungicides Apron Maxx (metalaxyl +fludioxonil), Vitaflo 280 (carboxin + thiram), SP1020 and Trilex (trifloxystrobin + metalaxyl) against these pathogens, two inoculated field trials, each treated with one pathogen, were conducted at Lacombe, Alberta in 2010. Each trial consisted of the faba bean cultivars Earlibird and Snowbird, inoculum at 15, 30 or 35 mL/6-m row, the fungicides, in a randomized split plot design with cultivar as main plots and inoculum concentration as sub-plots, along with inoculated and non-inoculated controls. Seedling emergence and seed yield declined and root rot severity increased with increasing inoculum concentration for both pathogens. Diseased plants were shorter and had thinner stems than healthy plants and the lower foliage turned yellow. The cv. Snowbird showed more susceptibility compared to Earlibird. Seed from diseased plants was often shrunken. Each of the fungicides improved emergence and yield compared to the inoculated control. This indicates that seed-treatment fungicides may be useful in reducing the impact of these ubiquitous pathogens on seedling establishment and yield.

Pathological and molecular race determinations of *Fusarium oxysporum* f. sp. *lactucae* from Taiwan

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Phytopathology 101:S31

Fusarium wilt disease of lettuce (*Lactuca sativa* L.), caused by the vascular wilt pathogen *Fusarium oxysporum* f. sp. *lactucae* (Fola), is one of the major factors restricting stable production of lettuce in Taiwan. Identification of pathological races of Fola is a necessity for lettuce breeders to develop Fola-resistant cultivars and for growers to choose appropriate cultivars for production. For race determination, Japanese Fola reference strains and Fola isolates from Taiwan were subjected to pathogenicity test against three differential lettuce cultivars, Patriot, Costa Rica No. 4, and Banchu Red Fire. Results showed that most of the Fola isolates from Taiwan were race 1, with the exception of two isolates (Fola-10 and Fola-40) collected from Taoyuan County were identified as race 3. This is the first report revealing that the Fola race 3 was found outside of Japan. Based on random amplification of polymorphic DNA (RAPD) fingerprinting, genetic diversity in Fola races was observed. Several RAPD markers were selectively used as schematics to differentiate Fola races in Taiwan. (Supported by 95AS-13.3.1-BQ-B1(6), 96AS-14.3.1-BQ-B1(6), 97AS-14.3.1-BQ-B1(11), 99AS-9.3.1- BQ-B2(6); the ATU plan, Ministry of Education, Taiwan, R.O.C., and National Chung Hsing University, Taiwan, R.O.C.)

Phylogenetic relationship of *Xylella fastidiosa* between pear leaf scorch strains and strains of other host origins

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Phytopathology 101:S31

Pear leaf scorch, the only *Xylella fastidiosa*-induced disease reported from Taiwan, was found in area where the variety Hengshan *(Pyrus pyrifolia*) was grown. Strains of pear leaf scorch *X. fastidiosa* (XF-PLS) shared similarities to strains of other host origins in requirement of complex medium and rippled cell walls, however, recent serological and molecular biology studies showed difference among them. Four strains of XF-PLS were compared with fifteen strains isolated from oleander, pecan, plum, peach, mulberry, grapes, citrus, coffee, and sycamore by sequence analyses of 16S rRNA and 16S-23S rRNA spacer region. When sequence analysis of 16S rRNA based on fragment size of 1537-1540 bp was compared, the similarity index among 4 XF-PLS strains was 99.8–99.9%, whereas that was 98.1–98.7% between XF-PLS strains and strains from other hosts. When sequence analysis of 16S-23S rRNA spacer region based on fragment size of 510-540 bp was compared, the similarity index among 4 XF-PLS strains was 100%, whereas that was 87.6–88.4% between XF-PLS strains and strains from other hosts. The phylogenetic trees revealed that XF-PLS strains were separated from strains of other hosts. Strains of other hosts were divided into four subgroups: strains from 1) oleander, 2) grape and mulberry, 3) citrus and coffee, and 4) pecan, peach, plum and sycamore. Results indicate that XF-PLS strains were not closely related to the above-mentioned strains and could belong to a new subspecies of *X. fastidiosa*.

Monitoring *Cercospora zeae-maydis* sensitivity levels to quinone outside inhibitor fungicides across multiple years

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Gray leaf spot (GLS) of corn caused by *Cercospora zeae-maydis*, can cause serious losses to producers in the U.S. One GLS managment practice used by some producers is the application of quinone outside inhibitor (QoI) fungicides. This practice has increased in the North Central U.S., which also means the risk of QoI sensitivity shifts may be higher in corn pathogens like *C. zeae-maydis*. The EC_{50} sensitivity levels of C. zeae-maydis to QoI fungicides (azoxystrobin, pyraclostrobin, and trifloxystrobin) in isolates collected from different North Central states from 2008 to 2009 were compared with the sensitivity levels of baseline isolates. The mean azoxystrobin EC_{50} level of isolates collected in 2008 and 2009 was similar to the baseline EC_{50} level, but isolates collected in 2010 had a significantly greater ($P \leq 0.05$) azoxystrobin EC_{50} level compared to the means of the baseline isolates. The mean pyraclostrobin EC_{50} level of isolates collected in 2008 and 2010 was similar to the baseline EC_{50} level, but isolates collected in 2009 had a significantly greater pyraclostrobin EC_{50} level compared to the mean of the baseline isolates. The mean trifloxystrobin EC_{50} level of isolates collected in 2008, 2009, and 2010 were not significantly greater than the mean of the baseline isolates. These results indicate that sensitivity levels of *C. zeae-maydis* isolates to QoI fungicides can vary year to year, and that fungicide sensitivities should continue to be monitored.

Production of healthy seed potatoes on organic farms

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Phytopathology 101:S31

Organic growers face limited access to certified seed potatoes for specialty varieties. Our research will determine the feasibility of seed potato production on Wisconsin organic farms and aims to increase the number of varieties available. In 2010, trials on organic farms of potatoes grown from minitubers on raised beds covered with plastic mulch had yields of 40 lb/10 ft of row, which is approaching yields on conventional farms. Trials from 2007–2010 showed that Potato virus Y (PVY) incidence on organic farms was comparable to conventional seed potato farms. Winter wheat borders, live mulches, and mineral oil sprays were trialed as PVY control measures, but only mineral oil sprays provided virus control. Aphid landing data has not supported the hypothesis that aphids land on field edges, which could explain why winter wheat borders have been ineffective at controlling PVY. A disease-free tissue culture bank of 110 high priority varieties is being developed. In 2010, we conducted trials of 4 varieties each of red, yellow, russet and fingerling varieties on 4 organic farms. There were significant yield and size profile differences between varieties and locations. Varieties were tested for disease and leaf hopper resistance, nutritional attributes, and taste. Disease and leaf hopper resistance varied significantly across varieties. Economic analysis of selected varieties will include minituber production costs and potential profits for organic growers.

Occurrence of Northern stem canker in first soybean plantings following Conservation Reserve in South Dakota

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Northern stem canker, caused by *Diaporthe phaseolorum* var. *caulivora* (DPC), is a sporadic and unpredictable disease of soybeans in the North Central region. In 2009, a high level of disease (ca. 90% incidence) with severe yield loss (60–65%) was documented in a commercial field near Castlewood, South Dakota. Isolates from cankered soybean tissue showed typical DPC cultural characteristics and perithecia and are being confirmed by RFLP analysis of DNA amplified from the ITS region of rDNA. The remarkable feature of this outbreak is that the field had been maintained in the Conservation Reserve Program for the previous eleven years as a mixture of intermediate wheat grass and alfalfa. Perithecia formed abundantly on soybean residues in spring 2010, but fruiting was delayed in surface exposed residues that did not remain continuously moist. Epidemic levels of Northern stem canker occurred in no-till and conventional tillage plots established in 2010. Infection was documented by tissue isolations as early as 28 days following planting, but plants remained symptomless till late August. Foliar symptoms and basal petiole necrosis preceded canker formation.

Yearly variation in the development of current season needle necrosis on noble, Nordmann and Turkish fir Christmas trees in the U.S. Pacific Northwest

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Phytopathology 101:S31

Current season needle necrosis (CSNN) is a poorly understood disease that affects a number of *Abies* spp. that are grown as Christmas trees. CSNN has been reported on noble (*A. procera*), Nordmann (*A. nordmanniana*), and grand fir (*A. grandis*) in Europe and these species, plus Turkish fir (*A. bornmuelleriana*), in the U.S. Pacific Northwest. In 2002 and 2004, a series of replicated genetic field trials were established at the Washington State University Research Center in Puyallup, WA. This is a low elevation site that is very conducive to the development of CSNN. These trials contain 91 sources of noble fir, 15 sources of Nordmann fir and 4 sources of Turkish fir. CSNN data were collected annually for six years on the noble fir trees and five years on the Nordmann and Turkish fir trees. Disease severity was rated on a scale of 0 to 10 during late summer/early fall. Data analysis indicated that there was significant yearly variation in the severity of CSNN and differences

in the susceptibility of the different sources of trees in these trials to CSNN. Spearman rank order correlation analysis indicated that there was a highly significant correlation between the yearly susceptibility rankings of the sources during the years data were collected on the noble, Nordmann, and Turkish firs. These results indicate that the relative susceptibility of different sources of trees to CSNN can be determined after one or two years at conducive sites.

Spread of *Phytophthora ramorum* to water, soil, and vegetation outside a nursery in Pierce County, Washington

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Phytopathology 101:S32

Since its initial detection in a nursery in 2003, *Phytophthora ramorum* has been found in 48 nurseries, four streams, and three drainage ditches in Washington. In 2004, *P. ramorum*-positive plants were confirmed at a Pierce County nursery. For four years the nursery was inspected and no additional *P. ramorum* was detected, however in 2009 symptomatic plants again tested positive and genotype analysis indicated that both the EU1 and NA2 lineages were present on multiple plants, sometimes in the same lesion. In the spring of 2009, infested ditch water as well as infected salal (*Gaultheria shallon*) was confirmed along the perimeter of the nursery. The furthest infected plants were down the ditch, about 400 feet from the nursery. Genotype analysis of twelve salal samples and one water sample detected only the NA2 lineage. The positive salal along the ditch was removed early fall 2009. In 2010, additional plants were positive on the nursery, and ditch water continued to be positive along the perimeter of the nursery. Composite soil samples collected from along the ditch were also positive in 2010; making this the first location in Washington with evidence that inoculum has spread from a nursery resulting in contamination of water and soil and infection of natural vegetation. With the exception of one NA1 water sample, all samples genotyped in 2010 from the perimeter of the nursery were of the NA2 lineage. This site continues to be monitored by regulatory agencies.

Mystery on the Sammamish: What are the sources of *Phytophthora ramorum* infesting this Washington State waterway?

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Phytophthora ramorum was first detected in Washington in 2003 and has been detected since in 48 nurseries, four streams and three drainage ditches. Genotype analysis indicates that contamination of waterways has typically resulted from spread of inoculum from nearby positive nurseries. However, the source of inoculum associated with the detection of this pathogen in a four-mile-long section of the Sammamish River is a complex situation that remains unresolved. There have been at least nine *P. ramorum*-positive nursery sites in the Sammamish watershed since 2004. The initial detection of *P. ramorum* in the river in 2007 was an NA1 genotype that was consistent with the genotype detected in a holding pond four miles upstream that drains from a positive nursery into the Sammamish. Baiting in 2008, 2009, and 2010 detected additional NA1, NA2, and EU1 genotypes of the pathogen in the river. Positive baits at the mouths of two streams and a drainage ditch that runs through an industrial site into the river in 2009 and upstream in these waterways in 2010, indicate that there are multiple sources of inoculum that have contaminated the river. The NA2 in the river appears to be coming from one of the streams, which drains an area with a NA2 positive nursery approximately three miles upstream from the river. Efforts are underway to identify the sources of inoculum that have contaminated the other stream and the industrial ditch.

LuxR homolog XagR of *Xanthomonas axonopodis* pv. *glycines* is solubilized only in the soybean plant and contributes to the infection process

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Xanthomonas axonopodis pv. *glycines* (*Xag*) is the causal agent of bacterial pustule of soybean. To better understand the virulence factors of *Xag* in the soybean pathosystem a *luxR* homolog, termed *xagR*, encoding a putative transcriptional regulator was studied. Disruption of *xagR* in Xag strain12-2 resulted in a significant reduction of incidence of infection of soybean. While the transcription of *xagR* appears constitutive, XagR accumulates and is solubilized only in soybean plants and could not be induced by plant extracts in culture. Apparently some component(s) in soybean plant are involved in stabilizing XagR from proteolytic degradation, thus increasing protein levels rather than enhancing its transcription. Both *pip* (proline iminopeptidase) and *pro* (extracellular protease) genes as well as biosurfactant production on swarming plates exhibited enhanced expression in *xagR*-over-expressing strains compared with the wild type. XagR over-expressing strains also incited significantly few lesions on soybean.

Host plant and substrate mediated shifts in soil microbial community composition in microplots simulating transitional organic production systems

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Phytopathology 101:S32

Increased pest damage during transition from conventional to organic systems is an impediment to the implementation of organic farming practices. A micro-plot experiment was conducted to determine if damage from soilborne pests can be mitigated through crop production practices that impact soil microbial communities. Three rates of urban plant debris and broiler litter and two planting regimes (continuous tomato or a rotation of sunn hemp (*Crotolaria juncea*) and Japanese millet (*Echinochloa crusgallii*) were established in microplots previously planted to tomato (*Solanum lycopersicum*) for 18 continuous months. Soil in plots was solarized annually. Fungal and bacterial DNA was extracted from soil at periodic intervals, amplified using LH-PCR, and subjected to fragment analysis. Changes in soil bacterial communities were evident following amendments of broiler litter (nitrogen-mediated) or the plant host (rhizosphere-mediated). Changes in soil fungal communities were associated with the addition of urban plant debris (carbon-mediated). After 28 months, all plots were planted to tomato and damage from soilborne pests monitored. An interaction of plant host and urban plant debris significantly impacted bacterial wilt, caused by *Ralstonia solanacearum*. Disease incidence was higher in plots containing urban plant debris, except where sunn hemp/millet was previously planted, indicating a complex association with practices impacting soil microbial communities.

Protein-protein interaction of *Cucurbit aphid-borne yellows virus* using yeast two-hybrid system and bimolecular fluorescence complementation

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In this article, yeast two-hybrid system (YTHS) and bimolecular fluorescence complementation (BiFC) were used to analyze interactions of *Cucurbit aphid-borne yellows virus* (CABYV)-encoded proteins. P0, P1, P1-2, P3, P4, and P5 were tested by YTHS in all possible pairwise combinations, and interaction was detected only for the P3/P3 combination. Results obtained by BiFC further confirmed the P3 self-interaction, and the subcellular localization of reconstituted YFP complexes was observed mainly in nuclei of *Nicotiana benthamiana* leaf epidermic cells. Domains involved in P3 self-interaction were analyzed using deletion mutants by YTHS and BiFC. The results showed that R domain (residues 1–61) in the N-termini interacting with itself and with S domain (residues 62–199) in P3, was responsible for P3 self-interaction. The present work would serve as a molecular basis for further characterization of CABYV proteins, and the regions involved in P3 self-interaction could provide the clue for understanding the capsid assembly pathway of CABYV.

Evidence that recombination plays an important role in the evolution and emergence of new curtoviruses (family *Geminiviridae*)

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Phytopathology 101:S32

Curly top disease is caused by a complex of leafhopper-transmitted curtoviruses (family *Geminiviridae*). A putative new curtovirus (BV3) associated with curly top disease in tomato in California was identified in 2009. A full-length clone of this isolate was infectious, and induced typical curly top symptoms in *Nicotiana benthamiana* plants. The BV3 clone was 2931 nucleotides and had a typical curtovirus genome organization. Total genome sequence comparisons revealed that it is most similar (~96%) to a putative new curtovirus species, Pepper curly top virus, previously identified from pepper in New Mexico. Interestingly, sequence comparisons of BV3 with other curtoviruses revealed high levels of identity (95–100%) with the C1 and C4 open reading frames and right intergenic region of *Beet severe curly top virus* (BSCTV-[US:Cfh]), revealing a recombination event between

these curtoviruses. Host range experiments involving agroinoculation and leafhopper transmission revealed that BV3 has a similar host range to BSCTV-[US:Cfh], including a severe symptom phenotype in sugar beet. A second recombinant curtovirus isolate was identified from beet leafhoppers collected from the Central Valley of California in 2010. The genome of this isolate was composed of *Beet mild curly top virus* (major parent) and BV3 (minor parent), and it may represent another new curtovirus species. Together, these results suggest a more important role for recombination in evolution and emergence of new curtoviruses than previously recognized.

Characterization of the *occF* gene associated with antifungal activity of occidiofungin produced by *Burkholderia contaminans* strain MS14

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Phytopathology 101:S33

Occidiofungin produced by *Burkholderia contaminans* strain MS14 is an octapeptide with a xylose and shows a broad range of antifungal activities to plant and animal fungal pathogens. The 56-kb *occ* gene cluster required for occidiofungin production harbors five nonribosomal peptide synthetase genes (*occA–occE*), two regulatory genes (*ambR1* and *ambR2*), one cyclic peptide transporter gene (*occT*) and other unknown genes. The *occF* gene, 657 bp in size, is located downstream of *occA*. The putative protein encoded by *occF* shares 95.0% and 93.6% identities to glycosyl transferases of *Burkholderia ambifaria* strain AMMD and *B. ubonensis* strain Bu, respectively. It was hypothesized that *occF* adds xylose to the oligopeptide backbone of occidiofungin. To test the hypothesis, a nonpolar *nptII* cassette was inserted into the *occF* reading frame to generate the *occF* mutant MS14KC1 (*occF::nptII*). Plate bioassays showed that antifungal activity of the mutant MS14KC1 against the indicator fungus *Geotrichum candidum* was significantly reduced as compared with the wild-type strain MS14. Mass analysis using matrix-assisted laser desorption/ionization confirmed the lack of xylose in the occidiofungin produced by the mutant MS14KC1. These data suggest *occF* is responsible for addition of xylose to occidiofungin and the presence of xylose is important for the antifungal activity of occidiofungin. This work provides insights for development of biofungicides.

Describe, classify and cultivation of Chinese and America Edible Mushroom 300 species of Inner-Mongolia, Yunan, Tibetan and California and Alaska

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Phytopathology 101:S33

The edible mushroom is the most attractive and popular health food. Those who work in high-tech fields of DNA-based studies do not understand the various names of the mushrooms. Therefore, this study, which requires listening, reading phonetically, and viewing a detailed dictionary, provides new information in the identity of each edible mushroom with clarification of China's ten-thousand-year-old agricultural history of cultivation, taming, and practical uses of edible mushrooms. This five year study includes each species' name (Scientific, English and Chinese), nutritional and medicinal value, and requirements for cultivation techniques including 55 internationally marketed edible mushrooms species, and 245 wild, edible species and varieties found in America (California 49 and Alaska 46) and China (Sino-Japan 38 and Sino-Himalaya 59). Also included are classified indices for over 700 edible and medicinal species classified by the AFOTAL and shared with a total of 263 American species including 45 marketed edible mushrooms species, 43 medicinal, and 23 species that are both edible and medicinal as well. This study continues to aid and examine the ecology and molecular biology and phylogeography system (enen fossils), and seeks to do more to stabilize nomenclature.

The study of Tibetan Platuae forest disease and insects and it's integrated pest management

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Phytopathology 101:S33

The Tibetan (Qinghai-Xizang) Plateau, known as the "world's roof," contains a treasury of information for the natural sciences. The total expedition of the research followed a vast line of about 5,000 km of which the study on the forest disease and insect was 231 km, with 124 forest sample spots areas. The study were done in 26 main virgen forest ecotypes, 62 plantation ecotypes, and 31 nurseries, fruits and tea gardens. More than 851 diseases and fungal specimens and 118 insect specimens were collected. Identification of them was made either in the field or at the Forest pathology Lab,(CAF) and Beijing Agricultural University, with involvement of many experts in forest pathology, mycology, and entomological laboratories. Tibetan are biodiversties fores types and variations of forest pests, however under the condition that the ecological balance of the virgin forests are kept intact, none have caused major damage, showing the Tibetan healthy characteristic of this forest ecosystem. If the breaking of this balance can cause the spread of the pest. Therefore, forest disease and insect management were suggested to be best approached by integrated forest management and especially plan and prevention of pest introduction to nurseries and plantations.

Differential proteins and genes related to *Curvularia lunata* potential virulence variation induced continuously by resistant maize germplasm

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Phytopathology 101:S33

To understand the potential risk of genetic variation of *Curvularia lunata* in maize particularly as resistant maize varieties with the similar genetic background are widely and continuously planted, firstly we continuously carried on subinoculation with weak virulence strain (WS18) to 4–5 leaf age leaves of resistant inbred lines Pob21, Pob 43 and Pob 101, respectively, for eleven generations, then detected differential proteins and genes between re-isolated strain generations using proteomic and transcriptional level assays. Results suggested that as the growth of strain generation, the virulence was gradually enhanced, and up to peak at the six fourth generation. The proteomic analysis showed that among twenty eight differential proteins identified by MS/MS, eleven proteins were up-regulated, and fifteen down-regulated, and two uniques, in which Brn1, Brn2 and SCD as well as HSP 70, peroxiredoxin TSA1 and SOD were all speculated to be involved in pathogen virulence differentiation. SSH was constructed and fourteen differential genes were identified, in which Brn1, ubiquitin, laccase-1 precusor, peroxiredoxin TSA1, SOD, etc., were up regulated association with pathogen virulence variation. We preliminarily selected some of them being identified through gene deletion and mRNA expression analysis, results revealed that Brn1, SCD and SOD were more closely involved in the pathogenic variation induced on resistant maize.

Construction and function analysis of *Trichoderma* transformant with *Metarhizium anisopliae* genes against insects

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Phytopathology 101:S33

Trichoderma is not always effective in practical application particularly when insect pests and diseases occur simultaneously at the same time and space. Aiming to the problem we conducted the transformation of *chit42* or *pr1* cloned from *Metarhizium anisopliae* into *Trichoderma koningi* and examined both genes expression in *Trichoderma* transformants. The average chitinase activity of transformants is approximately 2 times over wild type strain, of which TMC42-4 and TMC42-11 exhibited highest chitinase activity at 6-day of incubation in medium. Maize borer larvae were used to assess lethal effect of transformants to the pest. Among all transformants, TMC42-4 and TMC42-11 exhibited most significant lethal effect to the larvae after two day -feeding. Electron microscope analysis of corn borer mid-gut after feeding by transgenic *Trichoderma* showed that goblet cells enlarged, microvilli of mid-gut began to fall off and mid-gut cell nucleus elongated. Meanwhile, the transformants with *chit42* from *Metahizium anisopliae* still maintained a significant antagonistic activity to *Fusarium verticillioides* and *Fusarium graminearum*, two major maize stem rot pathogens. For further improving transformant inhibition efficiency to maize borer, we linked five chitin-binding domains of different organisms to *chit42*, respectively, then transferred each of fused genes into *Trichoderma* wild type strain. Results suggest that transformants *with chit42-Bm* and *chit42-Dm* showed the highest chitinase activities.

Race and virulence dynamics of *Puccinia triticina* in China during 2000–2006

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Phytopathology 101:S33

Wheat leaf rust, caused by *Puccinia triticina*, is an important foliar disease of wheat in China. The dynamics of races and virulences in the *P. triticina* populations in China during 2000–2006 were studied. Leaf rust samples were collected during surveys of wheat fields and trap nurseries in 17 provinces, and provided by coworkers throughout China. The virulence of single-pustule isolates was determined on near-isogenic Thatcher lines for leaf rust resistance genes *Lr1*, *Lr2a*, *Lr2c*, *Lr3*, *Lr9*, *Lr16*, *Lr24*, *Lr26*, *Lr3ka*, *Lr11*, *Lr17*, *and Lr30*, and races were denominated using the Prt code system. During 2000–2006, 79 races were identified from a total of 613 isolates. Races PHT (23.7%), THT (14.7%), PHJ (11.4%) and THJ (4.2%) were the four common races, all avirulent to *Lr9* and *Lr24*. The frequency of isolates with virulence

to *Lr1*, *Lr2c*, *Lr3*, *Lr11*, *Lr16*, *Lr17* and *Lr26* was over 80% and these isolates were widely distributed in China, while the frequencies of virulence to *Lr9*, *Lr19*, *Lr24*, *Lr25*, *Lr28*, and *Lr29* were 0.2 to 2.5%. The diversity of virulence phenotypes of Chinese *P. triticina* populations appeared to increase from 2000 to 2006. Regional distribution patterns of races and virulences within China and between China and other countries suggest that the populations of *P. triticina* in China are isolated from these countries.

Baseline sensitivity and potential resistance mechanism of *Monilinia fructicola* to SYP-Z048

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Phytopathology 101:S34

SYP-Z048, chemical name 5-(4-chlorophenyl)-2,3-dimethyl-3-(pyridine-3)-oxazoline, is a novel oxazole derivative fungicide inhibiting ergosterol biosynthesis in fungi. In this study, the baseline sensitivity of 100 *Monilinia fructicola* isolates from China to SYP-Z048 was established by measuring mycelial growth inhibition on Yeast Glucose medium. Laboratory mutants resistant to SYP-Z048 were generated and characterized. The sensitivity of mycelium ranged from 0.0034 to 0.0470 µg/ml with a mean EC50 value of 0.0188 µg/ml. Mutants resistant to SYP-Z048 were generated using UV-irradiation, but no mutants were obtained spontaneously. All fitness parameters evaluated in this study pointed to reduced fitness in mutants. Resistant mutants with EC_{50} values greater than 0.3 µg/ml exhibited a single point mutation in the ERG11 gene encoding the sterol 14-α demethylase resulting in an amino acid (aa) change from tyrosine to phenylalanine at position 136 (Y136F). Cross resistance was verified between SYP-Z048 and propiconazole (R2=0.87), but no cross resistance was found between SYP-Z048 and tridemorph, carbendazim, procymidone, azoxystrobin, or pyrimethanil. The lack of ability of *M. fructicola* to generate spontaneous SYP-resistant mutants coupled with reduced fitness of F136Y mutants offers an explanation for the lack of such mutations in field populations. Our results show that SYP-Z048 is a DMI fungicide with potential for brown rot control in stone fruits.

Evaluating alfalfa cutting as a potential measure to enhance abundance of predators to *Aphis gossypii* in cotton-alfalfa intercropping system

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Phytopathology 101:S34

The effects of alfalfa-cutting and non alfalfa-cutting on the population dynamics of *Aphis gossypii* Glover (Homoptera: Aphididae) and several species of arthropod predators were examined in cotton-alfalfa intercropping pattern in north-western China. The objectives of this study were to evaluate the alfalfa-cutting as a potential management technique to enhance abundance of arthropod predators of *A. gossypii*. The entire study area consisted of 50-cm alfalfa strips intercropped with four-row cotton strips. Alfalfa cut twice on June and July, when the population density of *A. gossypii* was gradually increasing in cotton, forced some groups of predator to migrate into adjacent cotton fields from alfalfa. Individual number, species richness, and diversity of predators were higher in alfalfa cut cotton field than in uncut. The population density of *Adonia variegata* (Goeze) (Coleoptera: Coccinellidae), *Pardosa astrigera* L. Koch (Araneae: Lycosidae), *Chrysopa sinica* Tjeder (Neuroptera: Chrysopidae) and *Orius minutus* (L.) (Hemiptera: Anthocoridae) increased 120%, 101%, 61% and 7% in alfalfa-cutting than non alfalfa-cutting respectively. Meanwhile, the population density of *A. gossypii* in non alfalfa-cutting was 2.8 times larger than in alfalfa-cutting. This indicates that alfalfa-cutting induces predator immigration into adjacent cotton fields and helps control cotton aphids. This study provides cotton growers a potential cultural management technique for *A. gossypii* while conserving predators.

Creeping stem cuttings, the possible inoculum source for bacterial wilt of vegetable sweetpotato

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Phytopathology 101:S34

Vegetable sweet potato (*Ipomoea batatas*) (VSP) is an important vegetable in East and South Asia. Durning 2000's, bacterial wilt (BW) caused by *Ralstonia solanacearum* (RS) impaired 30~80% yield of VSP in Taiwan. The asymptomatic creeping stem cuttings (CSCs) were often used as seedling sources for next planting in fields. However, some of such seedlings would show BW symptoms soon after transplants. The asymptomatic CSCs were collected from six infested fields to see if they served as inoculums of BW in fields. By Bio-PCR and tetrazolium tests, 25–88% of asymptomatic CSCs were RS-positive and the RS density reached 1.3×10^3 - 3.5×10^5 CFU/g stem tissue. Such CSCs, 0–55% showed BW symptoms in greenhouse 4 wks after being transplanted in un-infested soil. To study the relationship between disease severity and bacterial density, the RS-free CSCs were inoculated with RS strain RSNC01 by soil inoculation method (5.0×10^5 cfu/g soil). Results demonstrated that the bacterial density was greater than 3.8×10^9 CFU/g stem tissue in CSCs exhibiting stem rot, wilt and death whereas that was up to 4.5×10^7 CFU/g in CSCs showing stunting and wilting of 1–2 leaves. In asymptomatic plants, RS populations between 0 and 3.5×10^5 CFU/g stem tissue were detected. Moreover, no RS was recovered from terminal shoots and erect stems. Thus, the asymptomatic CSCs that were latently infected by RS clearly served as inoculums for BW in fields.

Baseline sensitivity and resistance mechanism of *Monilinia fructicola* to SYP-Z048

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Phytopathology 101:S34

SYP-Z048, 5-(4-chlorophenyl)-2,3-dimethyl-3-(pyridine-3)-oxazoline, is a novel oxazole derivative fungicide inhibiting ergosterol biosynthesis in fungi. In this study, the baseline sensitivity of 100 *Monilinia fructicola* isolates from China to SYP-Z048 was established by measuring mycelial growth. The sensitivity ranged from 0.0034 to 0.0470 µg/ml with a mean EC_{50} value of 0.0188 µg/ml. Resistant mutants were generated by UV-mutagenesis, but no mutant was obtained spontaneously. All fitness parameters evaluated in this study pointed to reduced fitness in mutants. Resistant mutants with EC_{50} values greater than 0.3 µg/ml exhibited a single point mutation in the ERG11 gene leading to an amino acid change from tyrosine to phenylalanine at position 136 (Y136F). No other mutation associated with resistance was detected in putative resistance genes of ERG2, ERG24, and ERG27 encoding$\Delta 8 \rightarrow \Delta 7$ sterol isomerase, 14-α sterol demethylase, C-14 sterol reductase, or 3-keto reductase. Cross resistance was verified between SYP-Z048 and propiconazole ($R^2 = 0.87$). No cross resistance was found between SYP-Z048 and tridemorph, carbendazim, procymidone, azoxystrobin, or pyrimethanil. The lack of ability of M. fructicola to generate spontaneous SYP-resistant mutants coupled with reduced fitness of F136Y mutants offers an explanation for the lack of such mutations in field populations. Our results show that SYP-Z048 is a DMI fungicide with potential for brown rot control in stone fruits.

Semidorminant mutations in *cesA3* leading to the resistance to CAA fungicides in *Phytophthora capsici*

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Phytopathology 101:S34

Phytophthora capsici, a heterothallic pathogen, can cause important diseases of many hosts. CAA fungicides are a group of fungicides used for controlling the oomycete pathogens, such as flumorph, demithomorph, iprovalicarb and mandipropamid. The nucleotide sequences of cellulose synthase genes of sensitive- and resistant strains were analyzed. Amino acid changes linking to the CAA-resistance were just found in the CESA3 gene. An amino acid substitution at position 1105 (Gly to Ala) in the CESA3 gene was correlated to the CAA-resistance, and another amino acid substitution at codon 1109 from Val to Leu was found in high resistant mutants accompanying with the mutation of G1105A. No other mutation correlated with resistance was detected in the CESA1, CESA2 and CESA4 genes. The mutations were verified by genetic cross assay. One parent (838) is a resistant homozygous strain at position 1105 and 1109, and the other parent (22) is a sensitive homozygote. All of F1-progenies from the cross [838(R) × 22(S)] showed resistance to CAA fungicides, suggesting that the resistance of *P. capsici* to CAA fungicides was controlled by semidorminant mutations. The CESA3 gene of *Phytophthora melonis* was also analyzed between resistant- and sensitive strains. A single point mutation at position 1109 (Val to Leu) in the CESA3 gene was found in all CAA-resistant mutants from *P. melonis*.

Searching for small RNAs in Xylella fastidiosa genomes

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Phytopathology 101:S34

Bacterial non-coding small RNAs (sRNAs) have attracted considerable attention due to their ubiquitous nature and their roles in controlling numerous cellular processes including survival, adaptation and pathogenesis. Xylella fastidiosa is an important bacterial pathogen causing many economically important diseases such as almond leaf scorch, citrus variegated chlorosis and

Pierce's disease of grapevine. However, little is known about small RNA in this bacterium, despite of the fact that several whole genome sequences of X. fastidiosa strains have been published. To fill in this gap, a research project was initiated to search for small RNAs in Xylella fastidiosa. The complete genome sequences of four X. fastidiosa strains (9a5c, M12, M23, and Temecula1) were selected for in silico analysis to scan for small RNA using the sRNAscanner program (PLoS ONE 5:e11970). Candidate small RNA genes were identified in all of the four X. fastidiosa strains with 46 for 9a5c, 50 for M12, 49 for M23, and 47 for Temecula1. Size of candidate small RNA ranged from 40 to 350 bp. Results from BLAST analysis showed that 34 small RNA genes were shared by all four X. fastidiosa strains. Species-, subspecies- and pathotype-specific small RNAs were also identified.

Genetic structure of *Waitea circinata* var. *circinata* on creeping bentgrass and annual bluegrass putting greens in southern California

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Phytopathology 101:S35

Brown ring patch is a new disease of annual bluegrass, rough bluegrass and creeping bentgrass in the U.S. caused by *Waitea circinata* var. *circinata* (Wcc). An initial study found significant haplotype diversity (n = 17) and a lack of population structure from 42 Wcc isolates broadly sampled across the U. S. This is not consistent for a newly introduced pathogen. In this study, soil samples were collected from two putting greens at a course in La Jolla, CA for 3 years to obtain distinct pathogen populations. In total, 116 Wcc isolates were recovered from the 465 soil cores. Amplified fragment length polymorphism was used to analyze the genetic structure. Based on 53 loci, most isolates were found to be unique genotypes. After clone-correcting the data set and analyzing 10 loci with allele frequencies between 0.29 and 0.86, no significant pair-wise population differentiation was found based on Fst analyses ($-0.08 < \text{theta} < 0.06$), consistent with gene flow occurring between greens and between years. In contrast, multilocus disequilibrium analyses based on the index of association were only consistent with a random mating model from 3 out of the 6 populations. However, high genotypic diversity and few clonal genotypes found between years suggest that the pathogen may be sexually reproducing in the field and that inbreeding could account for the gametic disequilibrium detected.

Development of expressed sequence tag-derived SSR markers for *Puccinia striiformis*, the stripe rust pathogen

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Phytopathology 101:S35

Puccinia striiformis causes stripe rust on wheat, barley, and many grass species. Co-dominant microsatellite markers are more suitable for studying population structures of this dikaryotic fungus. In this study, we characterized simple sequence repeat (SSR) loci based on three previously developed expressed sequence tag (EST) libraries for *P. striiformis* f. sp. *tritici* (*Pst*), the wheat stripe rust pathogen. SSR loci were isolated by screening the EST sequences using the Simple Sequence Repeat Identification Tool. By scanning 3,311 unique EST sequences, filtering out those with repeat sequences less than 18 bp, and screening those with suitable flanking sequences, 46 were selected for designing primers using the Primer3 program. The 46 primer pairs were tested on races PST-1, PST-21, and PST-127 of *Pst* and race PSH-45 of *P. striiformis* f. sp. *hordei* using the M13 tailing and fluorescent capillary electrophoresis on an ABI3730 genotyper. The three PST races represent the most diverse races of the wheat stripe rust pathogen from 1960s to 2007 in the U.S. Thirty-four primer pairs produced repeatable polymorphic bands and 19 of them generated co-dominant markers among the four races. The lengths of the amplicons ranged from 130 to 506 bp containing 5 to 13 di-, tri-, or tetra-nucleotide repeats. The SSR primers should be useful in studying the population structure and evolution of the pathogen.

Aspects of popcorn disease occurrence on mulberry fruits in Korea

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Phytopathology 101:S35

Occurrence of popcorn disease on mulberry fruits was surveyed at Jeollabukdo location in Korea from 2009 to 2010. The diseased fruits turned grayish white and changed to hard and black sclerotia during overwintering after falling onto the ground. A popcorn disease was occurred up to 90 percent from late may to late June. Incidence was 20~90 percent when a mulberry tree was cultivated in the raising outdoors but was occurred weakly in the vinyl plastic hothouse. Incidence of popcorn disease according to the height of mulberry tree was high three times near by the ground (0~70 cm) than that of 140 cm upper side on the three height. Apothecia produced from overwintered sclerotia in the fields of mulberry trees were observed in late April. Two types of apothecia were produced from the sclerotia, which were cup-shaped or clump-shaped. The fungus with cup-shaped apothecia was identified as *Ciboria shiraiana*, and that with clup-shaped apothecia as *Scleromitrula shiraiana. C. shiraiana* and *S. shiraiana* occurred at the ratio of about 7 vs. 3 in the fields.

Deletion of the N terminus of *Papaya ringspot virus* larger coat protein disrupt viral systemic infection

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The NIa protease of *Papaya ringspot virus* (PRSV) is responsible for the processing of at least six cleavage sites in the C-terminal part of the polyprotein. According to the cleavage rule of NIa, two consensus cleavage sequences (VYHE/S and VFHQ/S) exist in the region between NIb protein and coat protein (CP) and resulted in the formation of two in-frame heterologous N-terminal CP with 20-amino-acid apart. The purpose of our study is to characterize the role of the two CP in PRSV during virus infection. Five CP mutated viruses were constructed, including $CP_{Q/S\text{-}Q/S}$, $CP_{E/S\text{-}E/S}$, $CP_{N\Delta10}$, $CP_{N\Delta15}$, and $CP_{N\Delta20}$. Plasmids contained wild type genome ($CP_{E/S\text{-}Q/S}$) or the five CP mutants were inoculated into systemic host *Carica papaya* for examining the symptom expression and virus accumulation. Plants inoculated by $CP_{ES/QS}$ showed typical symptoms at 10 d.p.i., while plant inoculated by $CP_{Q/S\text{-}Q/S}$ and $CP_{N\Delta10}$ expressed symptoms at 14 d.p.i. and the other mutants were unable to cause any symptoms. Western blot and ELISA analyses using an antiserum against PRSV showed that all the mutant viruses, except for $CP_{E/S\text{-}E/S}$, were existed in the inoculated leaves, while only wild type, $CP_{Q/S\text{-}Q/S}$ and $CP_{N\Delta10}$ mutants were detectable in the systemic leaves. Our preliminary results suggested that the mutations in the N terminus of larger CP did hamper the ability of PRSV infection in plants and the 20 amino acids at the N terminus of the larger CP play a critical role in viral systemic infection.

Diversity of the mating type locus in *Sclerotinia sclerotiorum* in relation to formation of apothecia

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Phytopathology 101:S35

Sclerotinia sclerotiorum is a homothallic fungus and carries both alpha box (MAT1-1) and HMG box (MAT1-2) genes together at a single MAT locus. We investigated the presence of these genes for *S. sclerotiorum* isolates from lettuce. In addition, we also investigated the MAT locus diversity among the eight sibling ascospores harvested from a single ascus. Primer sets from the literature that previously resulted in amplification of MAT1-1 and MAT1-2 genes in *S. sclerotiorum* were employed in this study. We found that the MAT1-1 primers failed in approximately 25 to 50% of the isolates depending on the sampled field. Likewise, among the eight sibling ascospores, only 4 ascospores showed the MAT1-1 amplicon, suggestive of segregation. In contrast, the MAT1-2 specific primers produced amplicons for all isolates, including all eight sibling ascospores. There was a correlation between the lack of MAT1-1 amplification and the formation of apothecia in culture. Whereas nearly all MAT1-1-positive isolates formed apothecia, only 28% of the isolates that were MAT1-1-negative produced apothecia. All eight sibling ascospores produced apothecia. We are currently using DNA sequencing to investigate the MAT locus variability in relation to apothecia formation.

Analysis of gene expression during infection of field pea roots by *Fusarium graminearum*

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Phytopathology 101:S35

Field pea (*Pisum sativum*), an important rotational crop with cereals is greatly affected by root rots. *Fusarium graminearum*, commonly known as a cereal pathogen, has recently been associated with this disease. A study was conducted to elucidate mechanisms associated with *F. graminearum* infection on this crop. *F. graminearum* genes expressed exclusively during infection of field pea roots were identified and compared to genes expressed during infection of cereals. Fungal gene expression in artificially infected field pea roots and *F. graminearum* grown in culture was assessed using the Illumina RNA-Seq technology. A total of 3237 *F. graminearum* genes were found to be differentially expressed *in planta*. Among these, 1627 genes were upregulated and 1610 were downregulated. The upregulated genes included homologs of genes encoding virulence factors in *Nectria haematococca* such as, an ABC transporter homologous to the *NhABC1* gene and several pisatin demethylase homologs similar to the *PDA1* gene. Other upregulated genes

with known functions included those involved in cell wall degradation like endoglucanases, xylanases, and pectate lyases. Expression of six upregulated genes was confirmed by RT-PCR to validate the inferences from the sequencing results. Comparison of genes expressed during this interaction with those common between crown rot of wheat and head blight of barley suggests significant differences in expression patterns on this host.

Specific detection of the causal agent of bacterial blight, *Pseudomonas syringae* pv. *pisi* in the seeds of peas by nested PCR and real-time TaqMan PCR

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Phytopathology 101:S36

Pseudomonas syringae pv. *pisi*, a casual agent of bacterial blight of common peas, cowpea, and other leguminous cultures, is disseminated by the infected seeds. In this study, we have developed nested PCR and real-time TaqMan PCR for detection of the bacterium from the pea seeds. Primers pisi-JH-F and pisi-JH-R amplified a 306 bp fragment only from *Pseudomonas syringae* pv. *pisi* strains, while no amplification was occurred from the other plant bacterial pathogens. Nested-PCR with primers pisi-JH-F-ne and pisi-JH-R-ne amplified a 104 bp fragment only from the *Pseudomonas syringae* pv. *pisi* strains with 1000 fold more sensitive than 1st PCR. The primers did not amplified any non-specific DNA from the seed extracts of 8 different beans and peas and DNA isolated from the seed extracts. Real-time PCR using TaqMan probe-pisi, which was designed inside amplicon of the nested PCR, amplified only from *Pseudomonas syringae* pv. *pisi* strain. Detection limit of the real-time TaqMan PCR was similar to the nested PCR's with the artificially inoculated pea seeds. We believe that the PCR assays developed in this study are very useful to detect *Pseudomonas syringae* pv. *pisi* from the leguminous seeds.

Use of silver nanoparticles for control of seedborne diseases

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Phytopathology 101:S36

Optimal control of bacterial or fungal seedborne pathogens can be achieved by seed treatment to disinfect at the seed stage, because they are impossible or difficult to control after infection. Conventional seed treatment methods using fungicide, hot water or bleach have limited applications due to narrow control spectrum of target pathogens, harmful chemical exposure or contamination. Silver has highly effective antimicrobial properties but low toxicity to humans and plants. If silver is transformed into a nanoparticle, its antimicrobial activity is intensified, making it useful in eliminating seedborne pathogens. The objective of this study was to evaluate antimicrobial activities of silver nanoparticles to control fungal (*Fusarium moniliforme*) and bacterial (*Burkholderia glumae*) pathogens in rice and their potential phytotoxicity to affect rice seed germination and seedling growth. Silver nanoparticles were chemically synthesized and their antifungal and antibacterial properties tested. Silver nanoparticles significantly reduced the fungal and bacterial pathogens both on growth media and rice seeds at concentrations of 0.15 – 150 ppm. Seed health after treatment was evaluated by germination rate, root and shoot lengths at 7 d after germination and seedling height at 3 wk after germination. No adverse silver effect on seeds was observed. These data suggest that silver nanoparticles can be used for seed treatment for control of various seedborne pathogens.

The transcription factor Amr1 induces melanin biosynthesis and conidium production but differentially suppresses virulence in Alternaria brassicicola

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Alternaria brassicicola is a successful saprophyte and necrotrophic pathogen with a broad host range. Some consider that, like many opportunistic plant pathogens, A. brassicicola is in a continual process of adaptation as a parasite. To better understand pathogenesis mechanisms in A. brassicicola, we screened targeted gene knockout mutants corresponding to over 200 predicted transcription factors. We discovered mutants of a gene Amr1 with an unexpected increase in virulence that produced consistently larger lesions than the wild type. Lesions produced by the wild type were of various sizes, whereas lesions caused by the mutants were less variable than the wild type. The amr1 mutants were melanin-deficient in all tissues and structural genes associated with the melanin biosynthesis pathway were not induced. In contrast, amr1 mutants expressed higher amounts of several hydrolytic enzyme-coding genes in planta and grew faster than the wild type in the presence of pectin in an axenic medium. This study demonstrates that a gene important for survival in nature negatively regulates virulence by suppressing the expression of genes including a subset of those corresponding to cell wall-degrading enzymes. We speculate that the functions of this gene are important for A. brassicicola to be a successful saprophyte and opportunistic plant parasite.

The land plant-specific NbPSL1IP protein plays a key role in plant antiviral defense by interacting with *Potato virus X* RNAs and proteins

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Potato virus X (PVX) contains *cis*-acting elements including stem-loop 1 (SL1) RNAs at the 5′ region that are required for PVX RNA replication, encapsidation, and translation. A two-dimensional electrophoresis Northwestern blot analysis was used to identify 24 tobacco host proteins that interact with SL1 RNAs. The functions of one of these, NbPSL1IP, which binds to both SL1(+) and SL1(-) RNAs, was further characterized. A Blast search identified 24 land plant-specific NbPSL1IP homologous proteins. Phylogenetic analysis demonstrated that duplication of PSL1IPs has occurred less often in eudicots than in monocots or bryophyta. qRT-PCR revealed high induction of *NbPSL1IP* expression upon PVX infection. PVX RNA accumulation and movement were reduced by overexpression of *NbPSL1IP* and increased by silencing of *NbPSL1IP*, indicating a function of *NbPSL1IP* in antiviral defense. Subcellular localization studies demonstrated that *NbPSL1IP* is associated with microtubules and plasmodesmata. In addition, PVX infection altered the subcellular localization of NbPSL1IP from microtubules to endoplasmic reticulum. The BiFC assay showed that NbPSL1IP interacts with multiple PVX proteins including capsid protein, TGB1, and TGB2. The land plant-specific NbPSL1IP inhibits PVX RNA accumulation and movement by interacting with *cis*-acting elements and PVX movement proteins and by re-localizing in response to PVX infection.

Diversity and pathogenicity of *Fusarium* species associated with grain mold of sorghum in Korea

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Sorghum (*Sorghum bicolor* Moench) is cultivating at present throughout Korea as a food and feed crop, which was traditionally grown on a small scale. Grain mold symptoms of the plant were frequently observed during disease surveys in Korea from 2007 to 2009. The symptoms were highly variable. Severely infected grain is fully covered with mold and partially infected grain may look normal and discolored. Fifty-five isolates of *Fusarium* species were obtained from the disease symptoms of the plant collected from several locations in the country. Out of the isolates, 25 were identified as *Fusarium thapsinum*, 14 as *F. proliferatum*, 8 as *F. graminearum*, 5 as *F. equiseti*, and 3 as *F. incartatum* based on their morphological and cultural characteristics. Elongation factor 1 alpha gene sequences of the isolates were used for phylogenetic analysis. Analyses of the sequences revealed that the isolates were confirmed to be identical with related species of NCBI GenBank. Pathogenicity tests showed that three dominant species, *F. thapsinum*, *F. proliferatum* and *F. graminearum* were strongly virulent to grains of sorghum. The present study is the first report of sorghum grain mold caused by *Fusarium* species in Korea.

Detection of *Citrus leprosis virus* cytoplasmic type utilizing the polyclonal antibodies specific to the movement and coat proteins

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Citrus leprosis virus cytoplasmic type (CiLV-C) is an economically important citrus pathogen in citrus growing areas in South and Central America. CiLV-C is considered as one of the major exotic pathogens for introduction into the U.S. citrus industry. Detection is usually done by symptoms and reverse transcription polymerase chain reaction (RT-PCR). A standard serological diagnostic method is needed for the detection of CiLV-C. Polyclonal antibodies were developed against expressed proteins of the putative coat protein gene p29 from segment RNA1 and the putative movement protein p32 from segment RNA2. To achieve this, the p29 and p32 gene sequences of CiLV-C were synthesized, cloned into pDEST-17 vector and BL21-AI competent cells were transformed. The p29 and p32 expressed proteins were purified using His tag affinity chromatography after induction with L-

arabinose. The purified protein (purity >85%) was injected into rabbit for polyclonal antibody production. The antibody titer was determined by using direct and indirect ELISA and used for detection and identifications of leprosis infected plants from different geographical origins.

Improving PCR-based detection of *Xylella fastidiosa* in blueberry with a cost-effective DNA extraction procedure

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Phytopathology 101:S37

Commercial nucleic acid extraction/isolation kits are used widely because they are rapid (~30 min) and simple. However they may not be suitable for plant tissues rich in polyphenols and/or polysaccharides, which are well-known PCR inhibitors. The high concentration of such inhibitors in blueberry tissue and xylem sap prompted us to evaluate various methods of DNA extraction to improve detection of *Xylella fastidiosa* (Xf), the causal agent of bacterial leaf scorch, an emerging disease. From healthy plants, leaf petioles (100 mg) were sampled and Xf suspensions added to final concentrations of 0, 0.6, 6, 60, 600, and 60,000 cells per qPCR reaction. In addition, petioles of symptomatic leaves from diseased plants were also sampled. DNA templates (100 µl) were obtained via three different plant kits (GenElute, Sigma-Aldrich; PowerPlant, Mo Bio; and DNeasy, Qiagen), two soil kits (PowerSoil and PowerBiofilm, Mo Bio), Terra PCR Direct (Clontech), FTA cards (Whatman), and a modified CTAB protocol (adapted from doi:10.1016/j.viromet.2008.09.008). Using the CTAB procedure, all Xf samples were detectable with qPCR. Extracts from Terra PCR Direct and on FDA cards failed to amplify even from severely diseased samples. The other commercial kits allowed qPCR-based detection greater than 600 Xf cells per reaction. Although relatively time-consuming (~1.5 h extraction), the sensitivity of the CTAB procedure permitted detection of Xf at 0.6 cells per reaction at low cost.

Double *fliD* and *xagP* mutants of *Xanthomonas axonopodis* pv. *glycines* and their roles on host and nonhost plant

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Phytopathology 101:S37

We carried out a genetic and functional characterization of the pectase lyase and hook associated protein 2 encoded by *XagP* and *fliD* of *Xanthomonas axonopodis* pv. *glycines* (Xag) strain 12-2 respectively. *Xagp* has been reported to play a role in activating HR induction, where *fliD* functions as a capping structure at the distal end of the filament essential for motility. The inactivation of *fliD* led to reduce disease-associated pustule lesions on susceptible soybean cv. Spencer and still exhibited HR in tobacco leaves. The absence of *xagP*, expression of HR-like cell death was induced in cv. Spencer, but not in tobacco. Double mutation of these two genes also resulted in HR-like cell death in Spencer with no expressed HR in tobacco leaves. Complementation to the corresponding deficient mutants restored Xag motility, pectolytic activity, HR, and virulence. This suggests a link between *xagP* and *fliD* in full virulence on soybean but not in HR on either host or nonhost plants.

A new endophytic fungus from *Citrus medica* var. *sarcodactylis* and its application on controlling damping-off and anthracnose of *Brassica rapa*

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Phytopathology 101:S37

The endophytic fungi are new sources of bi-agent in management of plant diseases. A fungal isolate CB10 from *Citrus medica* var. *sarcodactylis* was identified as *Appoharknessia* sp. based on morphological and molecular characteristics. This fungus showed high efficacy in the inhibition of the mycelial growth of several plant pathogens including *Botrytis cinerea*, *Colletotrichum higginisianum*, *Fusarium oxysporum* and *Pestalotiopsis psidii*. A preliminary test on the control of anthracnose of Brassica rapa caused by *C. higginisianum* in greenhouse indicated that the disease incidence of *B. rapa* cv. San Fong No.2 was reduced by 37.5% four days after treatment with 100 µg/ml CB10 mycelial extraction (ME). Moreover, CB10 ME increased the survival rates by 38.9% of seeds (cv. San Fong No.2) in *Rhizoctonia solani* infested soil after seeds were soaked in 10 µg/ml ME. The bioactive compounds were identified by liquid chromatography/mass spectrometry/ mass spectrometry (LC/MS/MS) after purification by column chromatography. The results showed five major compounds identified as m/z 195.1, 313.4, 341.4, 579.6 and 607.6. Based on data base of Spectral Database for Organic Compounds (SDBS), the five major compounds were identified as 2-hydroxy-5-thiocyanatobenzoic acid, methyl trans-3-cyano-2-styryl-1-azulenecarboxylate, methyl N-(4-((3-butylureido) sulfonyl)-alpha-methyl-benzyl) acetamide, azocarmine G, and diosmin. The diosmin was previously reported as one of flavonoid compound inhibit pathogenic mycelial growth and induce plant resistance.

Methods for introduction of nonpathogenic *Fusarium oxysporum* into cucumber plants for better control of Fusarium wilt disease in Taiwan

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Phytopathology 101:S37

The nonpathogenic *Fusarium oxysporum* (*Fo*) has been demonstrated to delay the symptom expression of Fusarium wilt of cucumber (*Fusarium oxysporum* f. sp. *cucumerinum*) in greenhouse or field trials. For improving the inoculation system, three methods, by seed coating, substrate infestation or hypocotyl cutting, were compared for the efficacy of controlling Fusarium wilt of cucumber. The nonpathogenic isolate Fo276 that showed high potential in controlling the disease was used as an inoculum source and its distribution in inoculated cucumber tissues were analyzed. Results indicated that the hypocotyl cutting inoculation could reduce the severity of Fusarium wilt of cucumber by 64% whereas seed coating and substrate infestation only decreased the severity of Fusarium wilt by 20 and 41%, respectively. It was obvious that Fo276 provided better control of the disease when introduced by hypocotyl cuttings. Results of the distribution study indicated that Fo276 was restricted in peg tissue and mainly colonized in epidermis or cortex cell by substrate infestation and seed coating whereas Fo276 could colonize in hypocotyl tissue and vesicular tissues by hypocotyl cutting inoculation. Thus, the highly effective colonization of nonpathogenic *Fo* in hypocotyl and vesicular tissues via hypocotyl cutting may play a key role in a higher reduction of the disease severity.

Optimization of *Maize fine streak virus* (MFSV) protein expression in Drosophila S2 cells

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MFSV is a negative-sense RNA virus in the family Rhabdoviridae that is transmitted by the leafhopper G. nigrifons. The virus replicates in both its maize host and insect vector. In order to determine whether Drosophila S2 cells can be used as a system to study MFSV replication, we tested conditions for optimal expression of MFSV proteins, a replicon construct and the T7 RNA polymerase. Two of the three proteins required for synthesis of the MFSV genomic RNA, MFSV N and P, could be expressed from pMT/V5-His-Topo vector in S2 cells for at least 4 days after induction with CuSO4. Experiments are underway to assess MFSV L protein expression in S2 cells. Preliminary results suggest that the L protein can be detected only when expressed from linear DNA fragments. The replicon construct carrying the MFSV transcriptional initiation and termination sequences flanking an antisense GFP cDNA sequence downstream of a T7 promoter was inserted into the same vector. The transcript accumulated in transfected cells for at least 4 days. As expected, the GFP protein did not accumulate in transfected cells in the absence of the MFSV components and T7 polymerase. Expression of constructs encoding the T7 polymerase indicated that the protein could be detected in S2 cells for at least 4 days when fused to a nuclear localization signal peptide (NLS), but did not accumulate when lacking the NLS. Our results indicate that the proteins and constructs required for MFSV replication can be expressed in S2 cells.

Review of the development of fludioxonil for post harvest decay control on various tropical fruit crops

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Post harvest decay of tropical fruit including mango, papaya, and pineapple represents one of the most important challenges facing growers worldwide, particularly for transportation of these crops to distant markets. Fludioxonil (branded as Scholar® and Graduate®, Syngenta) has been widely tested and labeled for control of various post harvest decays of citrus, stone fruit, pome fruit, kiwi, pomegranate, and sweet potato. It has a broad spectrum of activity against critical storage decay fungi including *Penicillium* spp., *Botrytis cinerea*, *Lasiodiplodia* spp., *Alternaria* spp., *Colletotrichum* spp., *Rhizopus stolonifer* and several others. Research on post harvest activity of fludioxonil on tropical fruit was initiated in 2002. Results from mango and papaya trials in South Africa, and pineapple trials in Hawaii have demonstrated that fludioxonil offers both long lasting control of postharvest decay, as well as being a resistance management tool, enabling rotation of this new mode of

action with other registered products. This paper reviews studies conducted over the past eight years on various tropical fruits.

Seed storage duration and relationships with seed quality
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Two cultivars differing in seed quality were placed in four storage environments varying from ideal to poor. Seed were sampled every two weeks and tested for standard germination, accelerated aging, and with the Seed Vigor Imaging System (SVIS). Seed were also planted in the field and emergence determined at 2 and 4 weeks. Standard germination differed between the two cultivars, but did not change significantly throughout the test. Accelerated aging and SVIS remained relatively constant until the middle of July and then began to decline. The change in these parameters was greater for the poorer quality seed lot and was especially severe under the poorest storage conditions. Stands were generally higher in first two months with the higher quality seed lot, but stands of all seed lots declined sharply by the middle of July no matter how they were stored. Correlations of the seed quality assays and field performance were inconsistent in the SVIS test when data were analyzed by planting for the first emergence count. However, accelerated aging data did have a significant positive correlation with field emergence for every planting at the time of the first stand count. No correlations of the quality tests and field emergence for the data collected at the second stand count were consistently significant throughout the plantings.

Sexual reproduction of *Pseudoperonospora cubensis*
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The oomycete *Pseudoperonospora cubensis* attacks members of the *Cucurbitaceae*, especially cucumber and melon in which it causes severe foliage damage. It propagates clonally by sporangia. Sexual propagation was not reported nor the germination of, or infection with, oospores. Here we report on the sexual reproduction of *P. cubensis* under controlled conditions in the laboratory. When field isolates were inoculated singly onto detached leaves in growth chambers at 20°C, sporangia were produced but not oospores. However, when pairs of selected isolates were mixed and inoculated onto detached leaves, oospores were formed in the mesophyll within 6–11 days. Oospores were round, light-brown, 40 µm in diameter. Oospores were produced in *Cucumis sativum* and *Cucumis melo* but not in *Cucurbita pepo*, *Cucurbita moschata* or *Cucurbita maxima.* Oospores-containing leaves were homogenized in water, the homogenate was dried twice to kill sporangia, re-suspended in water and inoculated onto detached leaves in growth chambers at 20°C. Downy mildew lesions carrying sporangia appeared within 7–20 days in leaves of *Cucumis sativum*, *Cucumis melo and Cucurbita moschata.* Some F1 progeny isolates were pathogenic to cucurbit species towards which their parents were not pathogenic such as *Luffa cylindrica* and *Citrullus lanatus.* This is the first report showing sexual reproduction of *P. cubensis* in the laboratory.

Black root rot of soybean: An emerging problem in Arkansas
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Phytopathology 101:S38

Several new or previously considered minor soybean diseases have recently emerged in Arkansas to damaging levels. Plant pathologists in Arkansas have documented two "first identifications" of soilborne fungal pathogens during 2008. One of these is black root rot (BRR) of soybean caused by the pathogen *Thielaviopsis basicola* synanamorph *Chalara elegans*. This disease is most severe early in the growing season when soils are cool (16–20°C), especially on soils with a history of cotton production. Late season foliar symptoms bear a resemblance to those of other better known soybean diseases such as stem canker and sudden death syndrome. In order to survey the distribution of BRR in Arkansas 167 symptomatic samples were collected from soybean fields throughout the soybean production areas during 2009 and 2010. Soybean roots were processed and plated onto carrot-based selective media. Plates were observed for development of *T. basicola* and readily identified miscroscopically by the characteristic dark mycelium and chlamydospores. In 2009, BRR was identified in eight counties (Chicot, Desha, Drew, Jefferson, Lee, Monroe, Phillips and Poinsett). In 2010, BRR was identified in an additional seven counties (Arkansas, Ashley, Clay, Jackson, Lincoln, Lonoke and White). Hundreds of thousands of acres of production ground in the last few years has shifted to soybeans from fields previously farmed as "cotton ground". Efforts to monitor the distribution of this emerging disease across Arkansas continue.

Use of real-time and nested PCR to detect *Phytophthora ramorum* in infested nursery container mixes and soils
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Phytophthora ramorum has been shown to be present in field soil and container mixes (i.e., substrates) at ornamental plant nurseries. *P. ramorum* can be recovered from a substrate using a baiting bioassay, which involves culturing of infected bait pieces on selective medium (PARP-V8). Results may not be available for 2–4 weeks. Techniques for the rapid detection of *P. ramorum* in substrates are needed to give regulators a better tool to prevent the spread of this quarantined pathogen. Two or three replicate aliquots from each of 23 substrate samples were baited with leaf disks from *Camellia japonica* and *Rhododendron catawbiense*. Some of the bait pieces from each aliquot then were embedded in PARPH-V8 to isolate *P. ramorum*, and DNA was extracted from other bait pieces for molecular detection of the pathogen. DNA was examined by both real-time and nested PCR using the ITS gene target following the USDA-APHIS protocol. Of the 23 substrate samples, six were found to be positive for *P. ramorum* by both real-time and nested PCR and by isolation. In one substrate sample, *P. ramorum* was detected by nested PCR and not by real-time PCR or isolation. Overall, there was agreement among detection assays for 22/23 substrate samples (96%). Real-time and nested PCR appear to be as reliable as isolation on selective medium for detecting *P. ramorum* from leaf pieces used as soil baits. In addition, these two detection assays greatly reduce the time required to obtain results.

The role of lipopolysaccharide in virulence and host specificity of *Xylella fastidiosa*
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Phytopathology 101:S38

Xylella fastidiosa (*Xf*) is a Gram-negative, xylem-limited bacterium that causes disease in a wide range of hosts, including grape, almond, and oleander. Both almond and grape isolates can cause disease in grapevine. However, grape isolates cannot cause disease in almonds, indicating a fundamental difference between these two isolates. Moreover, the oleander strain cannot infect grape or almond and vice versa. The molecular mechanisms that determine this host specificity are poorly understood. We are investigating the role of the abundant cell surface polysaccharide, lipopolysaccharide (LPS) in the interaction between *Xf* and its grape host. Because LPS is displayed on the cell surface, it mediates interactions between the bacterial cell and its surrounding environment. LPS is comprised of conserved lipid A-core component and a variable O-antigen portion. O-antigen has been implicated in virulence and host specificity in many bacterial species. By targeting key genes involved in O-antigen biosynthesis, we will determine if O-antigen is an important virulence factor for disease development in grape. More specifically, we are investigating the contribution of O-antigen to surface attachment and mature biofilm formation, two critical steps for successful infection of the host xylem. Additionally, we will determine if O-antigen contributes to the high level of host specificity observed for this pathogen.

Global food security short courses to enhance urban forestry education and training at Southern University and A & M College
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Phytopathology 101:S38

There is a need to provide more post baccalaureate training and experience in plant health management at U.S. land grant universities to counter the threat of exotic or naturally occurring plant pathogens to ensure our national security and global competitiveness of U.S. Agriculture. The objective of the global food security short courses are to enhance urban forestry education and training in global food security by providing students with firsthand experience in a multidisciplinary, setting with various universities, state and federal agencies involved in plant disease surveillance, rapid identification and detection, and mitigation of high consequence plant pathogens. Training activities have included field trips to New Orleans, LA and Huston, TX to visit the USDA APHIS PPQ Plant Inspection Stations, U. S. Customs and Border Protection agricultural inspection at the Port of New Orleans, LA and Huston, TX. Students visited collaborators in Pennsylvania to learn more about the Plum Pox outbreak and how this disease impacted industry, also; students visited the BSL-3 containment facilities at the USDA ARS Lab Ft. Detrick, MD to see and discuss research projects on sudden oak death, and

other pests and pathogens of national concern. The impacts of the Plant Biosecurity Short course are over 36 graduate students, 200 undergraduate, and 50 K-12 students have received training in surveillance, detection, identification, and response to high consequence plant pathogens.

Using pathogen dispersal characteristics to improve biological control of Canada thistle with the rust fungus *Puccinia punctiformis*
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Phytopathology 101:S39

The noxious weed Canada thistle, *Cirsium arvense*, causes extensive problems in pasture, field crops and natural areas worldwide. The rust fungus *Puccinia punctiformis* is a promising biological control agent that reduces Canada thistle infestations through fatal, systemic infections. Successful augmentative biological control with *P. punctiformis* aims to establish severe, self-sustaining epidemics with natural spread of the pathogen. To better understand and predict the behavior of epidemics under different conditions and improve biological control, we performed a series of experiments to evaluate dispersal characteristics of the various *P. punctiformis* spore types. Dispersal gradients, measured by capturing spores at varying distances from a point source, were different between the two spore types. Specifically, teliospores exhibited a steeper gradient than urediniospores. Comparisons of spore terminal velocities in a particle settling tower indicated that teliospores had a mean fall velocity almost three times that of urediniospores. The timing, effect of environmental conditions, and quantity of spore release was also investigated with spore trapping from diseased field patches. The significance of all these dispersal characteristics will be discussed in the context of applying pathogen predictive modeling to improve weed biological control.

Ecological and ecological effects on inundate release *Trichogramma dendrolimi* to control Asian corn borer in northern China
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Phytopathology 101:S39

Trichogramma dendrolimi has been used over 40 years as a prevalent method for control Asian corn borer (*Ostriniae furnacalis*) population on the north part of china. A large scale inundate release on long-term basis resulted in the population suppression of the corn borer under the economical threshold in the mountainous region and the hilly area as well as the flat area with rich vegetation. Stabilization period can be maintained for 4–6 years if the release last over 10 years. The suppression of the corn borer population under the economical threshold in plain area also be attained by means of the cover release for the whole area of the dispersion radius of Asian corn borer (4 km) in large scale and in consecutive year release. Tactics on control corn borer in secondary generation occurrence area of corn borer is the combination of inundate release to control first generation of corn borer and inoculate release to control second generation of corn borer. Release time was minimized to one time by mixing the wasps which was on the different develop stage to one egg-card to make the adult emergence successionally to cover whole egg stage period of the pest.

Characterization of Poculum sp. isolated from warm-season turfgrass in Florida
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Phytopathology 101:S39

Fungi were isolated from warm-season turfgrass in Florida with symptoms that differed from typical dollar spot, in that infection centers were larger in diameter, and affected grass had a distinctly lighter color. Isolates produced cultures with distinct substratal stroma morphology, size (0.5 – 2.0 mm), and pigmentation different than the dollar spot fungus, Sclerotinia homoeocarpa, but similar to descriptions of Poculum spp. To further differentiate and determine the phylogenetic placement of these presumed Poculum spp., we conducted a multi-locus sequence analysis with these isolates and the closely related species of Poculum henningsianum and S. homoeocarpa. Three loci commonly used for fungal speciation were subjected to neighbor joining and parsimony analyses and included ITS, β–tubulin, and EF1–α. Strongly supported lineages in the phylogenies were consistent with groupings of isolates based on morphological features. Seven out of eight isolates shared higher sequence identity to P. henningsianum (93.1%) than to S. homoeocarpa (84.7%), but one of the eight had a higher identity to S. homoeocarpa (93.8%) compared to P. henningsianum (86.9%). These data indicate the isolates belong to a previously undescribed species of Poculum closely related to S. homoeocarpa and P. henningsianum in the Rutstroemiaceae.

Rhizoctonia web blight development on azalea in relation to duration of leaf wetness
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Phytopathology 101:S39

Rhizoctonia web blight, caused by binucleate *Rhizoctonia* spp., is an annual problem in the southern United States on container-grown azaleas (*Rhododendron* spp.). Temperature has been the primary variable for predicting web blight development in the field, with only minor predictive contribution due to moisture variables. To better define the influence of moisture on web blight development, plants were maintained in a greenhouse in open-topped, clear plastic chambers with 0, 4, 8, 12, 16, and 20-h cycles of 10 sec. mist at 30-min intervals, all terminating at 9:30 A.M. Binucleate *Rhizoctonia* AG-U infested barley was secured in netting at the base of terminal leaf clusters, and a proportional leaf blight incidence per stem recorded as a repeated measure over 6 weeks. Temperature, relative humidity (RH), and leaf wetness (LW) were recorded in each chamber. A similar day (29°C) and night (22°C) temperature range occurred in all experiments. Both LW duration and assessment time were always significant, with a significant interaction in 12 of 14 experiments. Web blight severity was positively correlated with increased LW duration. Disease incidence was highest mostly with 20 h LW, which sometimes was not different from 16 and 12 h LW. Eight or fewer hours LW usually resulted in disease incidence not different from 0. When data from all experiments were combined, estimates of maximum disease linearly increased with increased hours LW and hours RH > 75%.

Pycnidial development and pycnidiospore germination of Botryosphaeriaceae species as influenced by temperature
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Phytopathology 101:S39

Two botryosphaeriaceous grapevine pathogens (*Diplodia seriata* and *Lasiodiplodia theobromae*) were grown at eight different temperatures to determine the effect on pycnidial development and subsequent pycnidiospore germination. Pycnidial formation and spore development were evaluated at 48 hour intervals beginning seven days after inoculation. Germination efficiency was then assessed for pycnidiospores harvested from these developed pycnidia. Pycnidial formation and pycnidiospore development were significantly affected by temperature. *D. seriata* pycnidia and pycnidiospores developed more quickly than those of *L. theobromae* at all temperatures. Optimal temperatures for this development ranged between 20 and 30°C for *D. seriata* and 25°C for *L. theobromae*. After two hours of incubation at 25°C, germination success was greater for pycnidiospores formed at 15 and 20°C for *D. seriata* and at 20°C for *L. theobromae*. Overall spore germination rates were higher in *L. theobromae* than in *D. seriata*, and highest in spores formed at 30°C for both species. Results of this study show that these species were capable of producing pycnidia and mature pycnidiospores under a broad range of temperatures and that spores produced at all temperatures were capable of subsequent germination. These results give an indication of the environmental conditions that influence the epidemiology, survival and reproduction mechanisms in these species, and may assist in generating forecasting models.

Ecology of *Bacillus amyloliquefaciens* on wheat florets in relation to biological control of Fusarium head blight
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Phytopathology 101:S39

The TrigoCor strain of *Bacillus amyloliquefaciens* is a promising biological control agent (BCA) for Fusarium head blight (FHB) of wheat, caused by the fungus *Fusarium graminearum*. We are using TrigoCor as a model to understand why it, like many BCAs, provides consistent FHB control in the greenhouse but not in the field. Using dilution plating, we periodically quantified *Bacillus* populations from wheat heads for 14d post-*Bacillus* application in the greenhouse and in two NY fields. Although *Bacillus* populations were fairly stable on heads in both environments, the population level in the greenhouse (10^8 CFUs/head) was significantly higher than in the field in 2009 (10^6-10^7 CFUs/head) and 2008 (10^4-10^6 CFUs/head). In 2010 field trials, we increased the amount of *Bacillus* applied per head, resulting in levels comparable to those recovered from the greenhouse. Despite these high *Bacillus* levels, there was still insufficient FHB control, suggesting that population levels alone do not explain biocontrol. We used LC to measure levels of key *Bacillus*-produced antifungal compounds on wheat heads from both greenhouse and 2010 NY field trials. Although the levels of compounds on heads decreased rapidly by 3d post-application in both environments, the quantity per head was significantly higher in the greenhouse than in the field. Inadequate levels of antifungal metabolites on wheat heads, perhaps in

conjunction with low *Bacillus* population levels, may limit FHB control in field situations.

Standardization of protocols to test wheat (*Triticum aestivum* L.) for reaction to blast in a biocontainment laboratory

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Phytopathology 101:S40

Growth medium, spore age, and inoculum density are essential factors for determining host responses to a plant pathogen. The standardization of these factors is important to obtain adequate and reproducible disease assessments. We are testing U.S. wheat cultivars for reaction to the exotic disease blast, caused by the fungus *Magnaporthe oryzae*, in a Biosafety level 3 lab. Although several protocols have been published, it was necessary to adapt those to a biocontainment environment. Four culture media (potato dextrose, V8, oat meal, and corn meal agar), three spore ages (7, 14, and 21 days), and two levels of spore hydration (non-dried and dried) were used to study their effect on appressoria formation by isolate T-25. Over 99% of hydrated and then dried spores did not germinate regardless of growth medium or age, and spores from 14- and 21-day-old cultures had low germinability. The preferred method for production of large amounts of infective spores was the use of 7-day-old cultures from V8 or oat meal agar. Hydrated conidia and conidia that had been dried for 1 min., 15 min., 30 min., or 1 hr, before being rehydrated, were tested for infectivity. In general, dried, and then rehydrated conidia lost their germinability and did not infect wheat. The optimum inoculum density and volume was then determined on three cultivars showing different levels of susceptibility. The preferred inoculum density was 20,000 spores per ml with an inoculation volume of 1.0 ml per wheat head.

Extracellular trapping of bacteria in plant defense responses: Dynamics and specificity

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Phytopathology 101:S40

Major diseases infecting crops are caused by soilborne pathogens. Chemical control of these pathogens has been severely restricted due to health and environmental concerns. A natural plant-based extracellular material provides effective defense for plant root tips which house root meristems. This complex consists of polysaccharides and proteins and has been shown to adhere to and immobilize soilborne pathogens, yet the underlying mechanism has remained obscure. Vertebrate defense has been found to involve production of ‘extracellular traps’ by cells which use extracellular DNA (exDNA) to immobilize and kill bacteria, fungi, and protozoa. Protection of root tips occurs by a similar process. Root tips of most plants produce thousands of healthy ‘border’ cells that protect root meristems from injury and infection by immobilizing toxins, bacteria, fungi, and nematodes. Border cells appear to employ exDNA in a manner directly analogous to that of white blood cells. This new discovery will make it feasible to explore the production, function, and application of these specialized cells in disease resistance. The parallels between plant and mammalian defenses also may provide opportunities not only to harvest materials that can be used in medicinal applications but also to precisely define the mechanisms by which trapping occurs. High speed microscope digital video imaging will be used to illustrate the timing and specificity of trapping of diverse species of bacteria.

Localization of *Phytophthora plurivora* effector protein citricolin in *Fagus sylvatica* roots by light and fluorescence laser scanning microscopy

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Phytopathology 101:S40

Elicitins are a group of effector proteins released by Phytophthora species. Citricolin is the elicitin released by *P. citricola*, recently renamed as *P. plurivora*, an aggressive root pathogen of *F. sylvatica* (European beech). Citricolin was purified to homogeneity from liquid culture and specific antibodies were gained in order to localize the peptide in infected root tissue in combination with a red fluorescent secondary antibody. Roots of *F. sylvatica* seedlings were infected with 5×10^5 *P. plurivora* zoospores. After 2, 4 and 8 dpi plants were harvested and root samples were analyzed by light and fluorescence laser scanning microscopy. Light microscopy showed that 2 dpi, the pathogen mainly colonized the root epidermis and 2 days later (4 dpi) only some few hyphae were found within the cortex. However, the vascular cylinder was highly infected at that time. In contrast to light microscopy, fluorescence microscopy images clearly showed that the peptide citricolin was already found inside the cortex 2 dpi and its occurrence increased at later time points. From our data we conclude that the P. plurivora effector protein citricolin was able to enter host cortex tissue in the absence of the pathogen and in contrast to the literature, citricolin was not only found in the apoplast but also inside the cytoplasm, probably using the plant machinery to be translocated.

Potassium phosphite blocks root colonization of *Phytophthora plurivora* in the phloem of *Fagus sylvatica* seedlings

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Phytopathology 101:S40

F. sylvatica seedlings were inoculated with *P. plurivora*, an aggressive root pathogen and the effect of K-phi on pathogen-colonization is described here. Leaves were sprayed with 0,5% K-phi four days before roots were inoculated with 5×10^5 zoospores. After 2, 4 and 8 dpi, root samples were analysed by light microscopy. No morphological changes were recorded on control and K-phi treated plants. At 2 dpi, encysted zoospores and mycelia were visible colonizing epidermis. Only some few hyphae were found in the cortex for both K-phi treated and control plants. However, at 4 dpi the vascular region was highly colonized manly within the phloem, whereas the presence of pathogen in cortex was still low. At 8 dpi the whole root system was colonized; the cortex tissue was highly infected and severely disrupted. At that time Oogonia were found. Nonetheless, in plants treated with K-phi, *P. plurivora* was mainly restricted to the vascular region and only some few hyphae were found in the cortex. No mortality was recorded for K-phi treated plants, while non-treated infected plants showed mortality up to 66%. We conclude that once the pathogen access the roots it grows directly towards the phloem (sugar sink) and from there colonizes the whole tissue later on. When K-phi is sprayed on leaves it is transported to the whole plant through phloem cells. At the time the pathogen reaches the phloem, K-phi acts as a local fungicide killing the pathogen and avoiding further colonization.

Potassium phosphite protects European beech (*Fagus sylvatica*) seedlings against *Phytophthora plurivora*

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Phytopathology 101:S40

Potassium phosphite (K-phi), a salt of phosphorous acid, was recently described to have an ability to protect plants against certain Phytophthora species. In this work, we describe the effect of 3 concentrations of K-phi treatment on beech seedlings infected with *P. plurivora*, an aggressive root pathogen. Leaves were sprayed with 0,5; 0,25 and 0,05% K-phi four days before roots were inoculated with 5×10^5 zoospores. Non-inoculated control plants showed a slightly decrease in water uptake and photosynthesis with increasing K-phi concentration probably due to local leaf necrosis after the treatment. However, no mortality was observed. In contrast, all infected plants, including those treated with 0,05; 0,25% K-phi exhibited a strong decrease in water uptake and CO2-assimilation rates, as well as a strong wilting of leaves. About 66% of these plants died after 8 days of infection. Remarkably, all inoculated seedlings treated with 0,5% K-phi did not show any mortality, any wilting symptom and all physiological parameters were comparable to control plants. Furthermore, *P. plurivora* was successfully re-isolated from infected roots and the mycelium was visible on fine roots. These results clearly show that K-phi at 0,5% can protect beech seedlings from infection by *P. plurivora*, but not in lower concentrations. Nevertheless, it is still ambiguous whether K-phi acts as a local fungicide or as a resistance inducer. Experiments are ongoing to clarify this question.

Management of aflatoxin contamination of corn in Oklahoma

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Phytopathology 101:S40

Aflatoxin contamination caused by *Aspergillus flavus* is an important constraint to corn production. Afla-Guard, an atoxigenic strain of *A. flavus*, was evaluated for control of aflatoxin contamination at various application timings, rates, and formulations. In the timing study, the granular formulation (11.2 kg/ha) was applied at growth stage intervals from V3 to VT, and the wettable granule (WG) formulation (25 g/ha) was applied at VT. In the rate study, granules were applied from 5.6 to 22.4 kg/ha, and the WG formulation at 25 and 50 g/ha at VT. Plots were inoculated with a toxigenic strain 7-d after VT. Aspergillus ear rot incidence exceeded 50% for all treatments, but treatments applied from V9 to VT had higher levels of ear rot (80 to 100%) than the non-treated check ($P = 0.05$). Aflatoxin levels were high, exceeding 1100 ppb for the non-treated check in both trials. Treatments reduced aflatoxin levels from 59 to 87% for granules applied at V9 to VT ($P = 0.05$),

but not at V3 or V6. When applied at VT, all rates of each formulation reduced aflatoxin levels by 64–85% ($P = 0.05$). However, increasing rates of either formulation did not further reduce aflatoxin level. Kernels with bright greenish yellow fluorescence ranged from 3–5% and did not differ among treatments. However, kernel fluorescence was positively correlated ($P = 0.05$) with ear rot incidence (r = 0.32 to 0.41). Treatment effects on yield were not significant. Afla-Guard was effective in reducing aflatoxin contamination in corn, but did not reduce levels below 20 ppb.

Residual efficacy of fungicides for brown patch management

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Phytopathology 101:S41

Brown patch, caused by *Rhizoctonia solani*, is a serious disease of creeping bentgrass on golf course turf. In many parts of the Midwestern U.S., disease-related damage is avoided by applying fungicides preventively during periods of hot humid weather, at 14- to 28-day intervals. Fungicides sometimes fail to provide adequate control for the entire application interval, suggesting that chemical protection is depleted sooner than expected. Our objective was to investigate the nature of the depletion of fungicide protection from turf. A bioassay was conducted with five fungicides (azoxystrobin, flutolanil, metconazole, polyoxin D, and pyraclostrobin) applied to field plots of creeping bentgrass maintained at fairway height. Samples (4.25 inch diameter cup-cutter plugs) were collected periodically (0, 3, 7, 10, 14, 17, and 21 days after treatment), inoculated with sorghum seed culture of *R. solani*, and incubated in a controlled environment for 48 hrs. For each sample date, the extent of fungicide protection was determined by measuring diameters of symptomatic turf on treated and untreated turf plugs. Although the shape of the depletion curves differed among fungicides, in general, protection declined rapidly 7–14 days after treatments were applied. Understanding the length of time that effective concentrations of fungicides remain within the turf may improve scheduling fungicide applications for brown patch control.

***In silico* simulation of massively parallel sequencing as a diagnostic tool for bacterial phytopathogens**

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Phytopathology 101:S41

Increasing importation of commodities from countries abroad increases the risk of introduction of exotic plant pathogens. Although individual pathogen assays are available, current screening methods have limited ability to detect multiple plant pathogens concurrently. The advent of massively parallel sequencing (MPS) technology allows for the creation of a single assay to detect simultaneously, any and all microbes in a sample, including pathogens that have been genetically modified. In this project, we created bioinformatic pipelines, streamlined PC programs, for mock sample databases generation used in simulating 454 runs, query "probe" design and BLAST searches. Pathogen specific queries, ranging in lengths from 20 nt to 140 nt, were created for detection of the bacterial select agents *Xylella fastidiosa* 9a5c, *Xanthomonas oryzae*, and *Ralstonia solanacearum* race 3 biovar2, as well as for *Candidatus Liberibacter* asiaticus (not a select agent). Bioinformatic pipelines generated between 20 to over 6000 unique queries for each target bacterium. The query sets were used to BLAST mock sample databases with one host for all pathogen sequences at various ratios. All four bacterial pathogens were readily detectable *in silico*, suggesting that MPS technology has advantages beyond those of existing pathogen detection assays. This research merges bioinformatics and plant pathology for addressing national security needs in the agriculture industry.

The occurrence of late blight in 2010 following the 2009 epidemic

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Phytopathology 101:S41

Late blight caused by *Phytophthora infestans* (Mont.) de Bary is one of the most destructive diseases of potato and tomato worldwide. Late blight epidemics in the United States in 2009 were severe due to widespread inoculum and weather that was conducive to disease. This study attempted to determine the genotype composition of late blight in the following year's population. A total of 55 isolates of *P. infestans* were collected from major crop production areas in the U.S. during 2010. Isolates were characterized by their pathogenicity, mating type, and *in vitro* metalaxyl sensitivity. They were also subjected to molecular genotyping. Before 2007, isolates collected from potato and tomato crops were mainly the US-8 or US-11 clonal lineages, respectively. However, *P. infestans* populations in the U.S.A. underwent a significant genetic shift in 2007–2009; isolates with unique genotypes and epidemiological parameters including increased aggressiveness were detected in Florida and throughout the northeastern region of the U.S. Summer 2010 was one of the hottest and driest on record for much of the south and east. Although there were far fewer outbreaks in 2010, four of these novel genotypes were again identified and caused damage in tomato and potato crops.

Characterization of the fungal community in the tomato phyllosphere

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Phytopathology 101:S41

While much is known about the diversity and activity of foliar pathogens, much less is known about fungal populations inhabiting healthy leaves. In this study we investigated fungal community composition in the tomato phyllosphere across four cultivars, three tissue types, and two sampling times. Fungi were isolated from healthy leaves and fruit and categorized using amplified ribosomal DNA restriction analysis (ARDRA) of the ribosomal internal transcribed spacer (ITS) region. Isolates representing each unique ARDRA profile were then selected and their ITS sequences sequenced. From a collection of 273 isolates, 30 distinct genomovars were observed, though only five of these individually represented more than 5% of the collection. These five included species of Cladosporium, Mucor, Epicoccum, and Alternaria. None of these predominant groups showed any significant variation by cultivar or field position. However, Epicoccum and Mucor were more prevalent at the later sampling time, and one group of Mucor was only found on mature leaves and fruit at the second sampling time. The activities of the isolates are currently being investigated in greenhouse bioassays for pathogenicity and biocontrol activity.

Nutritional requirements and possible alternate hosts of *Xylella fastidiosa* that causes pear leaf scorch in Taiwan

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Phytopathology 101:S41

Xylella fastidiosa pear leaf scorch strain (XF-PLS) infects Hengshen pear (*Pyrus pyrifolia*) and causes leaf scorch and eventual death of the infected trees. Recent study using randomly amplified polymorphic DNA (RAPD) revealed that XF-PLS was genetically distinct from other *X. fastidiosa* strains, suggesting XF-PLS may harbor unique genetic factors associated with the pathogenicity to Asian pear. To better characterize XF-PLS, its nutritional requirements were conducted by single ingredient elimination from a PD2-based culture medium. Results showed that XF-PLS could grow in a medium without hemin chloride or trisodium citrate dehydrate, whereas it essentially required magnesium sulfate, disodium succinate, and bovine albumin serum for growth. Alternate hosts for XF-PLS were checked by stab-inoculation of ca. 3×10^8 cfu/ml bacterial suspension into the stem of tested plants. Inoculated plants were kept in a greenhouse at 28°C. Petioles above the inoculation site were harvested every 3 weeks post inoculation for 3 months and were thin-sliced in PD2 broth for bacterial recovery or ground in SCPAP buffer for PCR detection with XF-PLS specific primers PLS-F and PLS-R. Based on the amplification of a 416-bp DNA fragment by PCR and the recovery of XF-PLS colonies on PD2 agar medium, XF-PLS could multiply and migrate up in the vascular tissues of periwinkle and tobacco, two previously reported alternate hosts of Pierce's disease strain.

Comparison of "*Candidatus* Liberibacter asiaticus" populations from Brazil, China, and U.S. at two non-related genomic loci

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Phytopathology 101:S41

Huanglongbing (HLB, yellow shoot disease) is a highly destructive disease in citrus production worldwide. "*Candidatus* Liberibacter asiaticus" is associated with HLB. Information about population diversity of "*Ca.* L. asiaticus" from

different geographical regions is important for HLB epidemiological research and disease control. In this study, DNA samples of "*Ca.* L. asiaticus" from Brazil, China, and U. S. were collected. Variation among bacterial population was evaluated using sequences at two non-related genomic loci, CLIBASIA_01645 and CLIBASIA_05610. The former has a hyper-variable region with different tandem repeat numbers (TRNs) and the latter has single nucleotide polymorphisms (SNPs). At the CLIBASIA_01645 locus, the Brazil population is predominated by TRN>10 strain, contrasting to the U. S. population with TRN<10 strains being predominant and the China population with highly heterogeneous TRN genotypes, as previously reported (Phytopathology 100:567-572). At the CLIBASIA_05610 locus, all studied China strains, Brazil strains and the TRN>10 U. S. strains were identical, whereas the TRN<10 U. S. strains have unique substitution at 9 positions. By combining the results from these two different molecular markers, it is concluded that 1) China and U. S. populations of "*Ca.* L. asiaticus" are distinct; 2) Florida has two different "*Ca.* L. asiaticus" populations; and 3) the Brazil population of "*Ca.* L. asiaticus" was unique and highly homogeneous.

A highly sensitive and robust single-tube nested PCR assay for the detection of *Pineapple mealybug wilt associated virus* (PMWaV-2)

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Phytopathology 101:S42

An assay was developed for the detection of *Pineapple mealybug wilt associated virus-2* (PMWaV-2), which is an important factor in the etiology of mealybug wilt of pineapple. The assay combines reverse transcription of RNA isolated from pineapple with a specific and very sensitive, single, closed-tube nested polymerase chain reaction (PCR). The external and nested primer pairs amplified a segment of the coat protein gene of the virus. These external primers were designed to anneal at higher temperatures than the nested primers to prevent primer competition in consecutive amplification reactions. To further reduce potential competition, the external primers were used at one-thousandth the concentration of the nested PCR. The specificity and sensitivity of this assay are much greater than PCR procedures using only a single primer-pair. A Taqman® probe was designed for use in quantitative PCR to directly detect and quantify the PCR amplification products in a single tube assay. The advantages of the single-tube assays using both conventional and quantitative PCR are reduced handling time and prevention of cross contamination compared to regular nested PCR in which the reactions are carried out in two separate tubes. The new assay will be used to study the mechanisms of virus-plant-insect interactions.

Identification of fungi associated with trunk diseases of grapevine (*Vitis vinifera*) in Chile

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Phytopathology 101:S42

Grapevine trunk diseases have become prevalent in Chile. Symptoms include external canker lesions and internal wedge shaped discoloration with hard (HN) and/or soft necrotic (SN) tissue, vascular streaks (VS) and line necrosis (LN) at the margin of necrotic tissue. Shoot stunting, leaf malformation and chlorosis have also been observed. A total of 413 trunk samples obtained during surveys of central Chile (extended near 900 kilometers along a north to south axis) between 2009 and 2011 were plated on PDA plus tetracycline, streptomycin and Igepal for 14 days at 20°C. Cultures were identified morphologically and/or molecularly using the ITS1-5.8S-ITS2 of rDNA. *Phaeomoniella chlamydospora* was most frequently isolated and along with *Diplodia seriata* was isolated from all four symptom types. *Eutypella leprosa* was isolated from HN, VS and LN. *Inocutis jamaicensis* and Hymenochaetaceae sp. were isolated from SN, VS and LN. *Dothiorella sarmentorum*, *Neofusicoccum parvum* and *Seimatosporium* sp. were only isolated from HN. *Phaeoacremonium* sp. and *Cryptovalsa ampelina* were only isolated from VS. Experiments showed that all isolates were pathogenic in grapevines. Based on these results, grapevine trunk diseases appear to be associated with a fungi complex in Chile with *Pa. chlamydospora* being the most frequently isolated pathogen. Interestingly, *Eutypa lata*, a commonly found pathogen worldwide, has not been identified in Chile.

Interactions between Fusarium root rot pathogens and *Heterodera glycines*, on soybean roots

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Phytopathology 101:S42

Fusarium species are ubiquitous in soil and can cause important soybean diseases including root rot. In some cases, root rot can be exacerbated by other pathogens, such as the soybean cyst nematode (SCN). It is unclear whether there are significant interactions between SCN and species of *Fusarium* causing root rot on soybean, and the impact of these interactions has not been described. In order to determine whether SCN infestation enhances root rot disease in soybean, seedlings of SCN-susceptible and SCN-resistant cultivars were grown in soil infested with *Fusarium* alone and in combination with SCN under greenhouse conditions. Two isolates from each of eight *Fusarium* species were tested. There were significant interactions between SCN and some *Fusarium* isolates causing root rot. Root rot severity, shoot dry weight, and root dry weight differed among *Fusarium* isolates and there were significant interactions between effects of SCN and Fusarium isolates for these variables. Co-inoculation with SCN resulted in greater root rot severity on plants inoculated with six isolates representing five *Fusarium* species (*F. oxysporum*, *F. graminearum*, *F. semitectum*, *F. solani* and *F. sporotrichioides*) on both soybean cultivars. In general, SCN - *Fusarium* interaction effects for root rot ($p = 0.0004$), and shoot dry weight ($p = 0.02$) were greater in the SCN-resistant than in the susceptible cultivar. SCN appears to influence Fusarium root rot, but only for particular *Fusarium* species.

Distribution and frequency of isolation of *Fusarium* species associated with soybean roots in Iowa

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Phytopathology 101:S42

Species of *Fusarium* are known as common and widespread fungi that can cause damping-off and root rot diseases in soybean. However, the most important pathogenic species and their overall impact are unclear. In order to characterize the distribution and frequency of *Fusarium* species associated with soybean roots, we conducted a three-year root survey in 98 Iowa counties. Ten plants were collected from 3 fields per county at both V2-V3 and R3-R4 growth stages. Soybean root pieces were surface sterilized and placed onto a *Fusarium* selective culture medium. *Fusarium* colonies were identified to species based on cultural and morphological characteristics. Species identification was confirmed for selected isolates by amplification and sequencing of the translation elongation factor (TEF) gene. Twelve *Fusarium* species were identified; *F. oxysporum*, *F. solani*, *F. acuminatum* and *F. graminearum* were the most frequent and widespread species. Some species (*F. semitectum*, *F. subglutinans*, *F. virguliforme*, and F. poae) were recovered from a low percentage of fields. Most of the species have been reported on soybean before, but some have not previously been associated with soybean roots such as *F. subglutinans*, *F semitectum* and *F. sporotrichioides*. Species prevalence among fields differed regionally within and between years. Differences in species frequency were found between growth stages; greater species diversity occurred in roots at V2-V3 stages compared to R3-R4 stages.

Root-knot nematode genomes encode suites of plant peptide hormone mimics

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Phytopathology 101:S42

Our complete genome of the root-knot nematode (RKN) *Meloidogyne hapla* provides a powerful platform to study plant-metazoan interactions. We are particularly interested in nematode encoded genes that mimic plant regulatory molecules, especially members of the CLE and CEP families of peptide hormones. Using a double-affine Smith-Waterman algorithm we established that *M. hapla* encodes eight CLEs, nine CEPs and RALF. Unlike the native peptide ligands which are secreted as pro-proteins and require regulatory cleavage, the *M. hapla* mimics presumably are secreted directly into the host apoplast as active hormones, pointing to a direct role in the parasitic interaction. Alignment of family members has revealed striking sequence diversity amongst *M. hapla* CLEs and predicts both A and B types documented in model plants. Logoplot analysis of the *M. hapla* CLEs shows high conservation of residues required for function in native plant peptide hormones. Collectively this suggests that RKN encodes a comprehensive array of plant regulatory functions. As is the case for plant CEPs these *M. hapla* genes exhibit only limited sequence diversity, although they are clustered in a region of the genome that is hyper variable between wild isolates. Transcription of the nematode mimics was confirmed by qPCR, and using a bioassay we examined the affect of the nematode peptides on roots. These experiments demonstrated dose dependent inhibition of growth, thus establishing physiological relevance.

Management of onion purple blotch with bioformulations and fungicides

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Purple blotch [*Alternaria porri* (Ellis) Neerg.] is a serious disease of onion causing up to 30 per cent yield loss and under favourable conditions causes total crop failure. For the management of purple blotch seven fungicides, two neem formulations and four bioagents were evaluated both *in vitro* and *in vivo* conditions. Under *in vitro* evaluation, solid and liquid media of PDA were poisoned with various concentrations of fungicides and neem products and for antagonistic organisms dual culture technique was adopted. The fungicides *viz*., tebuconazole, propiconazole and hexaconazole were effective in complete inhibition of *A. porri* even at 50 ppm level. In pot culture, foliar spray of tebuconazole 0.15% registered the least severity of purple blotch (41.8%) followed by hexaconazole 0.1% and propiconazole 0.1% as against 85.6% in untreated check. Similar trend was observed in field experiment with tebuconazole registering the least severity of purple blotch (26.7%) followed by mancozeb, propiconazole and hexaconazole. Among the bioformulations, azadirachtin 1% formulation at 0.2% registered the lowest disease severity of 41.1% followed by azadirachtin 0.03% formulation at 0.2% with 43.4% and PGPR (TNAU formulation) at 0.5% with 47.8% as against untreated check with 64.4%. The highest bulb yield of 13.1 t/ha was recorded in tebuconazole followed by mancozeb (12.8 t/ha), propiconazole (12.7 t/ha) and hexaconazole (12.6 t/ha) which were on par. The untreated check registered the lowest bulb yield of 8.8 t/ha.

Genetic diversity and characterization of geographic distribution and of Beogomovirus in Yunnan, China

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Phytopathology 101:S43

Begomoviruses is a group of circular single-stranded DNA viruses that causesd economically significant diseases in tropic and subtropics area. The present study describes the characterization of distribution and genetic diversity of begomocviruses in Yunnan province between 2002 and 2008. A total of 1370 samples were collected from 4 different regions of begomoviruses in Yunnan, 42 full-length begomoviruses genome sequences have been amplified by PCR or RCA. Sequence analysis revealed that 42 isolates were belonged to 20 species of the genus Begomovirus, including 10 new report species globally, 7 new report species and 3 recorded species. Base on the different components of viral genome, four phenotypic groups of begomoviruses were identified in Yunnan: (1) single DNA-A component; (2) DNA-A and DNA-B component; (3) DNA-A and betasatellite component; (4) DNA-A, betasatellites and alphasatellites component;According to survey about begomoviruses regions, there are four heavily endemic areas with begomoviruses: (1) the valley of Golden Sand River in north of Yunnan; (2) the basin of Yuan River and Red River in south-central of Yunnan; (3) the basin of Lu River and Ruili River in west of Yunnan; (4) the basin of Lancang River in the south of Yunnan. On the basis of the present study, this viral complex has been particularly devastating in different areas of Yunnan and the situation has progressively worsen to the point where it is a major constraint on the production of many crops in Yunnan province, China.

Comparative transcriptome analyses of *Fusarium oysporum* f. sp. *cubense*

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Phytopathology 101:S43

Fusarium oysporum f. sp. *cubense* (FOC), the causal agent of Panama disease of banana is a highly destructive and genetically diverse pathogen. Despite its economic importance, genomic information of FOC is poor and no transcriptomic analyses have been reported so far. By using 454 sequencing technology, we generated >2.5 million expressed sequenced tags (ESTs) from four FOC strains representing four different Vegetative Compatibility Groups and the four described races that infect banana: race 1 (R1), race 2 (R2), subtropical race 4 (SR4) and tropical race (TR4). These ESTs were obtained from libraries prepared from mRNA extracted from three physiological conditions (mycelia, conidia and germinated conidia), which were pooled at a 2:2:1 ratio. Most genes are represented in all libraries, but in silico comparative analyses identified a set of unique ESTs for each race (689 for R1, 974 for R2, 296 for SR4 and 555 for TR4), which constitute excellent candidates for future plant-pathogen interaction studies and functional analyses. In subsequent analyses, a 40x sequencing coverage of FOC (TR4) genomic data from NCBI was assembled using a de novo assembly methodology. Preliminary analyses show a high colinearity of EST and genomic data that significantly contributes to the quality of the assembly. Potential applications of these data will be further discussed.

Characterization of three new isolates and extended experimental host range of *Phytophthora capsici* in Brazil

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Phytopathology 101:S43

Phytophthora capsici is an important pathogen affecting many host species. Here we report the association of *P. capsici* with new diseases on snap bean pods, gherkin (*Cucumis anguria* L.) and strawberry fruits in Brazil. In addition, we evaluated a set of plant species as potential hosts of *P. capsic*i at both seedling and fruit stages. Test plants (87 accessions of 68 species) were evaluated for their reaction to four isolates (obtained from snap bean, *Capsicum*, gherkin, and strawberry fruits). Seedlings were inoculated by placing 3 mL of the zoospore suspension (2 × 104 zoospores/mL) into the soil around the collar area. The C. annuum cultivars 'Ikeda' and 'SCM-334' were used as susceptible and resistant controls, respectively. Fruits were inoculated by placing mycelial plugs on the fruits after toothpick injury. All isolates were identified as *P. capsici* according to their morphology and by the sequences of their ITS regions. At the seedling stage, 36% of the accessions were found to be susceptible in contrast with 85% in the fruit evaluation. New solanaceous and non-solanaceous hosts were found in both assays. This updated P. capsici host list includes new reports for Brazil such as strawberry, gherkin, tobacco, snap and common bean plants as well as apple and pear fruits.

Increasing the sensitivity of PCR for the detection of foodborne pathogens in fresh produce

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Contamination of fresh produce by human pathogens, such as *E. coli* O157:H7 and *Salmonella*, is a serious threat to human health and to the fresh produce industry. Early detection of the pathogens is critical in assuring the safety of fresh produce, from preharvest certification of field lots to quality control of the final market product. The aim of this study was to evaluate the effect, on PCR sensitivity, of adding a 5′ AT-rich overhanging sequence (flap) to the design of primers specific for the detection of *E. coli* O157:H7 and *Salmonella*. Specific primers targeting the *rfb*E O157 and *inv*A genes for *E. coli* O157:H7 and *Salmonella*, respectively, were synthesized with or without a 12-bp 5′ AT-rich overhanging sequence. PCR sensitivity assays were conducted using purified E. coli O157:H7 and *Salmonella* genomic DNA, crude cell lysates, and genomic DNA/crude cell lysates spiked with DNA extracted from surface wash water from tomato and jalapeno peppers. PCR amplicons were eluted and quantified using a nano drop spectrophotometer. Amplicon band intensities were significantly greater when primers contained the flap, and the yield of PCR amplification for *Salmonella* and *E. coli* O157:H7 was increased by 20 and 23%, respectively. Improvement in the efficiency of PCR detection has potential applications in routine food safety monitoring, foodborne disease epidemiology and management, and in biosecurity and microbial forensics applications, as fewer target pathogens can be detected in less time.

Citrus-CTV molecular interactions: What is the host side of the story?

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Tristeza caused by Citrus tristeza virus (CTV) is a unique example where cross protection (CP) has been successfully used. However, in most instances, CP has broken down over time. Lack of knowledge of the molecular regulatory mechanisms of CP is the major factor and hampers efforts to improve and increase sustainability of CP. There are multiple anecdotal reports on the virus strains involved in CP, but molecular events in citrus that result in CP are unknown. Deep sequencing (Illumina) analysis of small interfering RNAs (siRNAs) extracted from sour orange (SO) severely affected by seedling yellows (SY) and from the cross protected SO showed atypical accumulation of virus-derived siRNAs in the 3′ end of the CTV genome. Contigs from these datasets identified sequences highly homologous to the 282 Kb Ctv resistance locus of *Poncirus trifoliata*, providing strong evidence that this region is involved in the siRNA pathways. Similarly, a total of 50 miRNA families have significant sequence presence, of which 8 miRNA families showed differential expression. Analysis of Citrus mRNA (EST) targets for these miRNAs in the cross protected SO plant and the SO showing strong SY and healthy untreated SO control show that there are 433 targets that are common to both the treated plants (absent in control), 873 targets that

are only in the cross protected SO plant and 753 targets in the SO showing strong SY, suggesting dynamic rewiring of the miRNA-mRNA interaction network.

Potato virus and phytoplasma diseases in Yunnan, China

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In recent years, virus and phytoplasma diseases are the important constraints to potato production in Yunnan, China. Field surveys were conducted from 2006 to 2010. A total of 415 potato plant samples, including 302 samples showing virus-like symptoms and 113 samples showing phytoplasma-like symptoms, were collected from potato-growing regions in Yunnan. A total of 184 seed potato samples, including 45 potato seedling, were collected from the local seed potato-produced bases. All the samples were tested using the DAS-ELISA kits purchased from Agdia(US). The results showed the overall virus infection rate was as high as 65.8%. Infections by Potato virus S (PVS), Potao leaf roll virus (PLRV), Potato virus Y (PVY), Potato virus A, Potato virus X, Potato virus M were 233 (38.9% of total), 97 (16.2%), 92 (15.4%), 56 (9.3%), 52 (8.7%), 10 (1.7%), respectively. 264 samples were infected with more than one virus. PVS-PLRV was the most frequently detected in the mixed infection (7.5%), followed by PVS-PVY (7.0%). Phytoplasma samples were detected by nested-PCR using the reported primer pairs, and then sequenced. Sequence analysis showed at least three Candidatus phytoplasma species infecting potato in Yunnan, including Canddatus Phytoplasma asteris, Candidatus Phytoplasma trifolii and Candidatus Phytoplasma fragariae. Among 45 potato seedling tested by RT-PCR using Potato spindle tuber viroid (PSTVd) specific primers, and sequence analysis, 8 samples were infected by PSTVd.

An elution-independent collection device for rapid sampling of microorganisms and nucleic acids for PCR assays

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Phytopathology 101:S44

A novel elution-independent collection device (EICD), Oklahoma State University patent pending reference number 2010.26, was designed for rapid collection of microorganisms and recovery of nucleic acids. The easy-to-implement EICD collects fluid specimens by contact and lateral flow. Minute pieces (1.2 mm diameter) of a built-in soluble element dissolve directly in commercial PCR mixtures without an intermediate elution step, thereby streamlining PCR based assays. More than 27 different materials from 5 different manufactures were assessed for one-step RT-PCR assays (without an intermediate RNA extraction step). The resulting EICD prototype is effective i.e. on sap from tobacco plants infected with *Tobacco mosaic virus* (TMV) and bacterial suspension (*Erwinia tracheiphila*), as well as whole ground insects (*Liposcelis brunnea*). All samples were ground in PBST buffer, and bacterial and insect samples were microwaved 30 seconds before loading. Control treatments consisted of NA extracted with commercial kits. Positive amplifications of each specific target were obtained regardless of whether extraction was done. Samples collected using the EICD prototype were ready for PCR processing within 3 minutes, far less time than the 10–30 minutes required using commercially available kits. This EICD can be a rapid sampling choice in molecular-clinical diagnostic applications for medical, veterinary, plant health biosecurity, forensics and food quality.

Aflatoxin producing potential and community structure of *Aspergillus* section *Flavi* in almond orchards of the Central Valley of California

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Aflatoxins are highly carcinogenic secondary metabolites produced by *Aspergillus* section *Flavi*. Several nut crops including almonds, pistachios, and walnuts are affected by aflatoxin contamination. The aflatoxin-producing ability of *Aspergillus* soil communities constitutes a major risk factor for aflatoxin contamination in almond orchards. Over 2,100 *Aspergillus* isolates from 28 almond producing orchards located in the northern, central, and southern Central Valley (California) were isolated from soil samples during 2007 to 2010. The aflatoxin-producing potential of over 800 *A. flavus* L-strain isolates was also determined. Results indicate that *A. flavus* L-strain was the most common, followed by *A. parasiticus*. Although the incidence of the highly aflatoxin-producing *A. flavus* S-strain was low (5%) in 2007, it increased to 28% in 2010, which could increase the risk of aflatoxin contamination in almond orchards and other nut crops. Preliminary data indicate that the aflatoxin-producing potential of the L-strains increased from 63% of toxigenic isolates in 2007 to 80% in 2008. The L-strains isolates from the northern orchards showed the highest aflatoxin-producing potential in both years. Incidence of atoxigenic *A. flavus* communities decreased in all regions of the valley from 2007 to 2008. The incidences of aflatoxin-producing isolates in the *A. flavus* communities in the orchards pose a risk of aflatoxin contamination of nut crops in California. The almond industry has taken a number of measures pre- and post-harvest to assure control and compliance with aflatoxin standards. These measures include: 1) Good agricultural practices like insect pest management and product handling; and 2) Sorting of insect damaged kernels.

Genetic diversity of *Candidatus* Liberibacter asiaticus strains from Thailand based on DnaA and TufB genes

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Huanglongbing (HLB) is considered as one of the most destructive diseases of citrus in the world because it causes rapid decline of infected trees. The HLB widespread throughout Asia and some parts of North and South America is *Candidatus* Liberibacter asiaticus (LAS). Two additional HLB bacteria are *Ca.* L. americanus (LAM) and *Ca.* L. africanus (LAF) which are reported only in South America and Africa, respectively. Genetic diversity among LAS strains from Thailand and other countries was investigated by analyzed dnaA and tufB genes. Total 45 strains were collected from different hosts and geographical regions. PCR were amplified from total DNA by primers dnaAF; 5′-CCCCTCTCCGCCGCCAACAT-3′, dnaAR; 5′-ACTGGCCTCGTTGAA GCCCA-3′ for 334 bp of dnaA gene and tufBF; 5′-TCCTGGGCATGCT GATTATG-3′, tufBR; 5′-CGCACGATTTACTCCACGAA-3′ for 539 bp of tufB gene. PCR products were sequenced and analyzed. Phylogenetic tree of dnaA sequences showed most of Thai strains were difference from strains of China and Brazil. Three Thai strains were closely related to strains from Asian countries such as Vietnam, Indonesia, the Philippines and Taiwan. Other 19 Thai strains were separately in 4 other groups. Phylogenetic tree of tufB gene showed more conserved among strains from all regions except some strain of Thai, China and Brazil which were diverse. The results indicated that dnaA gene was correlated to geographical regions whereas tufB gene was less genetic diversity.

The dynamics of ABA biosynthesis by *Cercopora zeae-maydis*

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Gray leaf spot, caused by *Cercospora zeae-maydis* is an economically important yield-limiting foliar disease of maize. During infection, maize produces the phytohormone, abscisic acid (ABA), which regulates stomatal aperture and host response to pathogen attack. Interestingly, we recently observed that *C. zeae-maydis* produces ABA in culture, suggesting a possible function in pathogenesis, given that *C. zeae-maydis* infect the host through the stomata. However, the role of fungal ABA biosynthesis during infection is poorly understood. Through degenerate PCR, we have identified a putative ortholog of the *Botrytis cinerea ABA3* gene, which is being disrupted by split marker homologous recombination for functional characterization. To elucidate the importance of ABA in fungal pathogenesis, the regulation of ABA biosynthesis in response to metabolic and environmental cues is being determined by direct LC-MS measurement of ABA production and expression of the putative *ABA3* gene by qPCR. This work will shed light on the regulation of ABA biosynthesis at the molecular level and serve as a foothold for further elucidation of the function of ABA produced by fungi during pathogenesis.

Distribution of *Arabis mosaic virus* (ArMV) on grapevines and roses in Western and Eastern Azarbaijan Provinces, Iran

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Phytopathology 101:S44

Arabis mosaic virus (ArMV) belongs to the plant virus genus *Nepovirus* in the family *Secoviridae*. ArMV is transmitted by several species of the nematode vector in genus *Xiphinema*, and has a very wide natural host range in crop plants and ornamentals. The objective of this study was to determine the distribution of ArMV through the vineyards and rose plantations in Western and Eastern Azarbaijan Provinces of Iran. To achieve this aim a total number

of 1043 samples including 251 rose and 792 grapevine leaf samples were collected randomly from the gardens in several districts of Western and Eastern Azarbaijan provinces during the years 2008 to 2009. Using Dot-immunobinding assay and Double-antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA), all samples were tested for the presence of ArMV. Results showed that ArMV was distributed through the rose plantations and vineyards of East Azarbaijan with the relative incidence rate of 30.3% and 46.6%, respectively and 30.2% and 42% in rose and grapevine of West Azarbaijan Province respectively. Using specific primers for the coat protein gene of ArMV, extracted RNA samples were tested for the presence of ArMV by RT-PCR method. DNA fragments of 440 bp and 519 bp were amplified from the serological positive ArMV samples. To our knowledge this is the first precise study on the ArMV distribution through the different rose plantations and vineyards in several districts of West and East Azarbaijan Provinces of Iran.

Protect U.S.: Community-based invasive species education for small farmers and the general public

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Phytopathology 101:S45

The community invasive species network (www.protectingusnow.org) is concerned with protecting the U.S. from exotic, invasive species. Protect U.S. is a collaborative partnership between the National Plant Diagnostic Network (NPDN), Regional Integrated Pest Management (IPM) Centers, United States Department of Agriculture, Animal and Plant Health Inspection Service, Plant Protection and Quarantine (USDA-APHIS-PPQ), National Institute of Food and Agriculture (NIFA), the National Plant board, the Department of Homeland Security (DHS) your local Land Grant University Cooperative Extension Service, and other organizations involved in exotic species extension and regulatory activities. Target audiences for Protect U.S. educational content include small farmers, the general public, and K-12 audiences. Protect U.S. content may be integrated into NPDN First Detector training, but is delivered in a simplified format for audiences not traditionally reached through this program. The Protect U.S. website officially launched in September of 2010, and a national train-the-trainer webinar occurred in February of 2011. By December of 2011, e-learning modules and scripted powerpoints (for educators) will be available on at least 11 invasive species, plant biosecurity, and pest and pathogen topics. E-learning includes interactive quizzes, games, and certificates of completion. Project outcomes, including the number of learners completing modules, web statistics, webinar feedback, and opportunities for module authorship will be presented.

Biogeographic diversity analysis of *Erwinia amylovora* using multi-locus variable number of tandem repeats analysis (MLVA)

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Phytopathology 101:S45

Fire blight is a chronic, major threat to sustainable pome fruit production. Phytosanitary control strategies have relied on inoculum source assumptions. We applied recent genome sequencing to develop MLVA for deep resolution of strain genotypes as a tool to enable inoculum reservoir source-tracking. Genome-wide DNA repeats were identified starting with the complete sequence of CFBP 1430. Six selected repeats regions of 6-18 bp were amplified in multiplex PCR with labeled primers allowing determination of the number of repeats at each locus with capillary electrophoresis. MLVA analysis was applied to a large number of *E. amylovora* isolates, representing global and regional diversity of the pathogen. Globally, higher diversity was observed than in previously employed genotyping methods and more than 92 haplotypes identified based on chromosomal repeats in over 600 isolates. Shannon's diversity index for individual loci ranged from 0.18 to 0.61. Two large groups with further sub-groupings were recognized among global isolates with further biogeographical stratification. This study demonstrates the potential of MLVA for further development as a tool for epidemiological studies, geographical surveillance, and source-tracking of *E. amylovora*.

Spontaneous Gac mutants in *Pseudomonas* biological control strains: Are they cheaters or mutualists?

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Phytopathology 101:S45

Many bacterial biological control agents produce secondary metabolites that influence plant-microbe interactions. These are often controlled by regulatory networks such as the GacA/S two component system. The genetic stability of GacA/S is influenced by growth conditions: spontaneous Gac mutants often become the majority in nutrient-rich media. Natural populations often contain variants with defective Gac systems. These mutants have a decreased metabolic load but do not displace the wild type. How does natural selection maintain the wild type in the presence of a mutant with enhanced growth? One hypothesis is that Gac mutants are 'cheaters' that do not contribute to the public good: favored within groups but selected against between groups. An alternative hypothesis is that Gac mutants have a mutualistic interaction with the wild type: each variant benefits by the presence of the other. *Pseudomonas chlororaphis* strain 30-84 Gac mutants do not produce phenazines, which inhibit pathogens and are critical for biofilm formation. We tested the predictions of these hypotheses by quantifying interactions between the wild type and Gac mutant within growing biofilms. We found that the wild type and Gac mutants interact mutualistically. Our results suggest that the persistence of alternative Gac phenotypes may be due to the stabilizing role of local frequency-dependent selection in structured environments and may be a conserved strategy which improves overall success.

On-farm research activities to implement methyl bromide alternatives: An area wide initiative update

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Phytopathology 101:S45

On-farm research (OFR) is an important mechanism to enable growers to implement alternatives in strawberry production systems and solve disease and weed management challenges. In 2009 to 2010, three strawberry trials were conducted. For each of the sites, the fumigation material evaluated was based on the need and desire of the specific grower according to farm specific pest complexes. All sites included the use of virtually impermeable films (VIF) to cover the raised beds combined with reduced rates of fumigants. Fumigants used in the strawberry trials included: Methyl Bromide (50% methyl bromide+50% chloropicrin; MB) at 240 lb/A, Pic-Clor 60 shank applied at 188 lb/A and drip applied at 250 lb/A, Inline (60% 1,3-dichloropropene+33.3% chloropicrin) drip applied at 26 gal/A, Telone C-35 shank applied at 294 lb/A, MIDAS (50% methyl iodide+50% chloropicrin) shank applied at 150 lbs/A and metam sodium drip applied at 75.0 gal/A. Treatments including MIDAS, Telone C-35, Pic-Clor 60 and Inline were applied using reduced rates under VIF mulch. Products were applied using grower equipment under grower selected conditions with a minimum of 10% of the total acreage fumigated with a MB alternative. In most cases, the OFR was arranged in a RCBD and growers collected harvest data semi-weekly. Alternatives were found to work as well or better than the MB treatment and provided confidence to growers to transition away from MB.

Sensitivity of *Magnaporthe grisea* to isprothiolane, iprobenfos and tricyclazole

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Phytopathology 101:S45

In this study, the sensitivity of *Magnaporthe grisea* to three fungicides was determined by mycelium growth rate method. 82 *M. grisea* isolates were collected from disease nurseries of various villages and cities in Fujian province, where there was no history of fungicide applications. Baseline sensitivities of *M. grisea* to isoprothiolane and iprobenfos were established with a mean EC_{50} (effective concentrations for 50% inhibition of mycelial growth) of 2.4369 µg/mL and 6.9168 µg/mL, respectively, which showed an unimodal distribution. 141 *M. grisea* isolates from fields were conducted to monitor the development of resistance to isoprothiolane and iprobenfos. The results suggested that the frequencies of sensitive, low-resistant and intermediate-resistant isolates to isoprothiolane were 14.89%, 81.56% and 3.55%, to iprobenfos were 42.55%, 53.19% and 4.26%, respectively. A total of 223 *M. grisea* isolates were collected from disease nurseries and fields as described above, and the sensitivity to tricyclazole was detected in vivo. All the 223 isolates were sensitive to tricyclazole with EC_{50} ranging from 1.085 to 47.61 µg/ml, which showed a skewed unimodal distribution and no resistant subpopulation among these strains.

Wheat powdery mildew researches in China

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Phytopathology 101:S45

Wheat powdery mildew (*Blumeria graminis* f. sp. *tritici*) is one of the most important diseases in China. The occurrence once reached 12 million ha in

1990 and 1991 and the losses caused were 1.44 and 0.77 million tones, respectively. The area of occurrence has been some six million ha since 2001 and the grain loss around 0.3 million tones (http://www.ppq.gov.cn/nongqing_xiaomai.asp). The Institute of Plant Protection, Chinese Academy of Agricultural Sciences, has worked on wheat powdery mildew since 1980. The work includes the epidemiology and forecasting of the disease, the biology and the genetics of the pathogen, the virulence and fungicide resistance of the population, the inheritance of host resistance gene(s) and their SSR markers and the chemical control in order to tackle the issue posed by wheat mildew. The results on the epidemic studies in Henan, Gansu, Shaanxi, Beijing, etc, the dynamics of virulence and fungicide resistance of the pathogen populations, the inheritance of some avirulence genes and the preliminary genetic linkage map of wheat mildew constructed with AFLP markers and avirulence, wheat resistance against mildew and the chromosomal localization of gene(s) from Chinese landraces, and the screened chemicals that can be used as the alternatives to triazols will be discussed.

The generation of *Pepino mosaic virus* infectious clones; investigating the link between genotype and phenotype

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Phytopathology 101:S46

Pepino mosaic virus is one of the most important pathogens affecting tomato production worldwide. It also has the potential to infect a range of other host species; the outcomes of such new interactions are unknown with the potential to be devastating. A large disparity in symptomatology exists between highly homologous (>99%) strains and little is known about how viral genotype relates to phenotype. A yeast recombination method was used to construct an infectious clone and chimeric viruses contained within a pYES2.1 vector under control of a SP6 promoter. RNA transcripts generated from the SP6 promoter were infectious on a range of indicator plants including *Nicotiana benthamiana*, *Nicotiana glutinosa*, *Nicotiana tabacum*, *Datura stramonium* and *Lycopersicon esculentum*. Three weeks post inoculation 100% of plants tested positive and sequencing RNA from the infected plants confirmed no cross-contamination had occurred. Both ELISA and real-time PCR testing showed all clones had titres equal to wild-type. The clone of the EU strain can move systemically in *N. glutinosa* while the chimeras cannot. Distinct visual differences indicate an element in the 3′ end of the infectious clone is responsible for the symptoms of extreme leaf bubbling in *N. benthamiana*, severe stunting in *L. esculentum* and distinct mosaics in *D. stramonium*. Further investigation will underpin the exact amino acid changes contributing to the changes in symptomatology with the aim of directly linking genotype to phenotype.

***Clonostachys rhizophaga* can delay and reduce emergence of chickpea but does not consistently induce wilt in Washington State**

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Phytopathology 101:S46

Clonostachys rhizophaga, first reported in 2009 as causing severe chickpea wilt in Syria (100% mortality on cv ICC 12004), was isolated from post-harvest chickpea debris in eastern Washington in 2003, 2008 and 2009. Isolates CP98B and CP08C6 were identified as *C. rhizophaga* on the basis of morphology and beta-tubulin sequences (99% similarity to type strain CBS 202.37). In 2009, conidial suspensions (10^6 conidia/ml) were applied to chickpea seed (ICC 12004) in replicated greenhouse trials conducted at 22–30C days & 16C nights, with two sets of mock-inoculated controls. The experiment was repeated in 2010. In 2009, neither isolate induced wilt, both initially inhibited emergence ($P < 0.04$), but later only emergence in CP98B differed from both controls ($P < 0.09$). In 2010, emergence with CP08C6, but not with CP98B, was reduced compared to controls ($P < 0.03$). One plant of the CP98B treatment and one of CP08C6 wilted, with *C. rhizophaga* being re-isolated. In 2011, two replicated trials were conducted in a growth chamber, with daytime temperatures of 22–34C days (34C for minimum of 4 hr) & 18C nights, to more closely approximate Syrian field conditions, or those of eastern Washington under modest climatic warming. Neither isolate had affected emergence nor induced wilt when trials were terminated after pod formation. In spite of 99% similarity, our isolates differ behaviorally from Syrian isolates; or conditions in Syria were more favorable to disease development.

Genotypic diversity of *Verticillium dahliae* impacting potato and mint

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Phytopathology 101:S46

Verticillium dahliae, causal agent of Verticillium wilt, can infect >200 hosts. Isolates from mint and potato usually belong to different vegetative compatibility groups (VCGs) which may result in genetic differentiation among *V. dahliae* isolates from these hosts. Eighty-six isolates from peppermint, scotch spearmint, and native spearmint and 65 isolates from potato, seed potato, and seed potato tare soil were characterized for VCG, mating-type, and multilocus microsatellite haplotype to determine the relationships among *V. dahliae* isolates affecting potato and mint. All isolates from mint and potato were mating-type *MAT1-2* and belonged to VCG 2B and VCG 4, respectively. Genetic diversity was greater among isolates from potato ($H_{obs} = 0.87$) than mint ($H_{obs} = 0.22$) and 88% of mint isolates were of one haplotype. A single haplotype accounted for 93% of isolates from seed potatoes and was sampled from potato in several states. Principal coordinate analysis (PCoA) clustered potato and mint haplotypes into distinct groups and analysis of molecular variance (AMOVA) indicated the two groups were significantly different ($P = 0.02$). Although genetic differences between VCG 4A and VCG 4B accounted for 53% of the total variation, VCG 4 subgroups from potato were not differentiated using PCoA or AMOVA ($P = 0.34$). Minimum spanning network analysis suggests *V. dahliae* isolates from mint and potato are genetically distinct but the potential for gene flow exists between VCG 4A and 4B subgroups.

Progress on Industry Pest Information Platform (iPIPE)

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Phytopathology 101:S46

Several years ago, the American Seed Trade Association (ASTA) proposed the Industry Pest Information Platform (iPIPE) to share survey and diagnostic data between industry, government and universities. Recently, USDA-APHIS-PPQ has been able to provide support for the initiative through a project funded by Section 10201 of U.S. Farm Bill. The aim of the project is to create a common architecture for information exchange and to enhance the early detection of exotic plant pests. The iPIPE pilot phase will be part of the NAPPFAST system, an information tool developed by NC State University, PPQ and the information technology company ZedX. The pilot phase includes 10 emerging diseases on corn and soybean including *Puccinia polysora* (southern corn rust) and *Clavibacter michiganensis* subsp. *nebraskensis* (Goss wilt). ASTA companies submit survey and diagnostic data which is displayed anonymously at a county resolution in the iPIPE portion of NAPPFAST. Registered users of iPIPE can view pest observations in an interactive map and overlay weather data, NAPPFAST risk maps and HYSPLIT atmospheric trajectories. The benefits of iPIPE include an improved information network for tracking emerging and exotic plant pests and eventually better information for permitting and certification. As the system matures, it is expected to reach out to other key partners including the National Plant Diagnostic Network.

Development of a qPCR assay for quantification of *Verticillium dahliae* in spinach seed

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Phytopathology 101:S46

Verticillium wilt, caused by the soilborne fungus Verticillium dahliae, is an important disease of lettuce and other specialty crops in the Salinas Valley of California. Although spinach is not affected by Verticillium wilt in commercial production, spinach seed infected with V. dahliae from locations in the U.S. Pacific Northwest and Europe and planted in Salinas Valley increases inoculum density and potentially introduces exotic strains that may contribute to Verticillium wilt epidemics. A sensitive, rapid and reliable method for quantification of the fungi in seed may help to curtail the spread of V. dahliae via spinach seed. The objective of this research is to develop a qPCR assay to detect and quantify V. dahliae in spinach seed. We employed Cyber Green detection methodology for qPCR, and conducted parallel NP10 plate assays to determine actual seed infection in multiple seed lots. The qPCR assay reliably detected V. dahliae in spinach seed, showing a sensitivity of detection of 20 infected seeds per 1000 (2% infection level). In two seed lots examined at infection levels ranging from approximately 0.5% to 75%, the relationship between percentage of seed infected and seed pathogen DNA content was highly significant ($R2 > 0.97$, $p < 0.001$). We are currently testing several seedlots to determine threshold qPCR parameters that can be used as a guide to predict the percent seed infected from seed pathogen DNA content.

Potato IPM program: Taking the research to the farm
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Phytopathology 101:S47

Potatoes are the largest agricultural commodity in the State of Maine, generating a total economic value of over $500 million, and producing employment for over 6,000 individuals. The University of Maine Cooperative Extension Potato IPM program is a multidisciplinary program that assists growers in the management of potato pests by providing specific and timely scientific information. Information gathered through multiple sources, including direct observation, traps, weather data, and prediction models, is delivered to potato growers in Maine and around the globe through electronic and standard newsletters, websites, and via telephone message centers. The Potato IPM program impacts approximately 56,000 acres of commercial potatoes and employs 18 program aids who assist in producing over 1.5 million data points that help IPM scientists track potential pest outbreaks and provides potato growers and industry professionals with current information on specific and timely treatments, which can be used to minimize pesticide applications and maximize potato yield and quality. In 2009, the UMaine Cooperative Extension Potato IPM Program produced an estimated $26 million positive impact on the Maine potato industry.

Coat protein expression strategy of oat blue dwarf virus
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Phytopathology 101:S47

Oat blue dwarf virus (OBDV) was the first marafivirus (family *Tymoviridae*) to be sequenced and for which an infectious clone has been reported. Although sequence data are now available for multiple marafiviruses, precise details of the expression strategy of these viruses remain undocumented. Translation experiments with OBDV suggest that a large 224-240 kDa polyprotein encoded by much of the genome is post-translationally processed into its functional components, in agreement with its tymoviral lineage. ORFs for two 21-25 kDa coat proteins (CP) are encoded near the 3′ terminus and are coterminal with the large polyprotein ORF. By analogy with tymoviruses, a marafibox is presumed to serve as a promoter for a subgenomic RNA encoding the CP, yet the expression strategy for the CPs has to date been poorly investigated. The smaller (major) CP appears to be the product of direct translation of a subgenomic RNA, while the larger (minor) CP may be a cleavage product derived from a larger precursor or also may be expressed directly from a subgenomic RNA. Using an infectious clone of OBDV, we have developed a series of mutants to dissect and analyze the OBDV CP expression strategy. Preliminary results from protoplast experiments are consistent with a coat protein expression strategy involving proteolytic cleavage to produce the minor CP and direct translation of a subgenomic RNA to produce the major CP.

Field application of asafoetida and seaweed for the management of root diseases of watermelon and eggplant
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Phytopathology 101:S47

Water melon (Citrullus lanatus (Thunb.) Matsum. & Nakai) and eggplant (Solanum melongena L.) are highly susceptible to root rotting fungi Fusarium solani, F. oxysporum, Macrophomina phaseolina and root knot nematode (Meloidogyne spp.) causing huge losses each year in Pakistan. In field experiments, application of asafoetida, a medicinal gum from Ferula assafoetida and seaweeds Spatoglossum variabile, Stokeyia indica and Melanothamnus afaqhusainii showed significant suppressive effect on root rotting fungi Fusarium solani, *M*acrophomina phaseolina and root knot nematode Meloidogyne incognita) attacking watermelon and eggplant and improved plant growth in soil naturally infested with root rotting fungi and artificially infested with root knot nematode. Length of vine of watermelon, shoot length of eggplant and fresh shoot weight were higher in seaweed and asafoetida treated plants as compared to control or Topsin-M, a fungicide, treated plants. Seaweed and asafoetida treated plants also showed earlier fruiting than control or fungicide treated plants. At farmer's field seaweed showed similar suppressive effect on F. solani and M. phaseolina and root knot nematode on water melon in soil naturally infested by these pathogens. Application of seaweed produced healthy plants and number of fruits and weight were significantly higher in seaweed and asafoetida treated plants. Asafoetida and seaweeds offer a non-chemical means of disease management.

A non-structural, p17 protein of *Potato leafroll virus* co-localizes in plant phloem tissue with virus capsid protein
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Phytopathology 101:S47

Potato leafroll virus (PLRV) is a phloem-limited, positive-strand RNA virus. The ca. 6-kb PLRV genome encodes eight open reading frames (ORFs), of which ORFs 0-2 encode proteins involved in genome replication and silencing suppression, and ORFs 3-5 encode proteins involved in particle assembly, systemic movement, and aphid transmission. A non-structural, p17 protein encoded by the ORF 4 was previously identified as a host-dependent movement protein of PLRV. The two structural proteins, capsid protein (CP, ORF 3) and p80 (ORF 5), were known to be indispensable for the PLRV transmission by aphids. In a phloem-limited virus, systemic movement is a pre-requisite for a successful acquisition of the virus by its aphid vector from the vascular system, and recently p17 was suspected to be involved in aphid transmission of PLRV. Here, we have studied tissue localization of the p17 protein in *Nicotiana benthamiana* plants infected with wild-type PLRV and a series of p17 mutants. Tissue localization was determined using p17-specific antibodies in a tissue immuno-binding assay. In plants infected with PLRV, p17 was found exclusively in the phloem, co-localizing in the same vascular tissue stained with antibodies specific to the PLRV capsid protein. Implications of p17 tissue localization for its possible role in PLRV aphid transmission will be discussed.

New method for establishing a network of operational warning of Septoria leaf blotch disease in winter wheat
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Phytopathology 101:S47

A mechanistic model, PROCULTURE, based on commonly available meteorological data and assessing in real time the risk of progression of septoria leaf blotch disease on winter wheat has been developed in Belgium and the Grand-Duchy of Luxemburg (GDL) to limit fungicide use. However, the reliability of meteorological stations used for the warning system varies according to the distance to the fields. A weather analysis based on the Fourier transform highlighted a great difference in the intraday variation between two sites in the GDL (Everlange and Reuland). The correlation between these two sites is very high for the hourly temperature ($R = 0.96$), and for the hourly relative humidity (RH) ($R = 0.86$), ($P < 0.05$). However, the intraday variation (<11 hours) highlights contrasts for a given meteorological parameter. Hence, the correlation between temperature or RH decreased respectively from 0.96 to 0.43 and from 0.86 to 0.30. The comparison between infection conditions given by PROCULTURE using the Fourier transform, shows: (i) a positive but weak correlation between temperature at Reuland and Everlange ($R = 0.64$), (ii) a good correlation between RH for these two sites ($R = 0.86$), and (iii) a contrasted difference for rain ($R = 0.27$), ($P < 0.05$). This Fourier transform based method enables to take into account the RH and temperature variation related to topography levels in the warning system and to understand and explain the variation in disease expression between a plateau and a valley bottom or between North and South slopes.

Regional-based typology of the main fungal diseases affecting winter wheat in the Grand-Duchy of Luxembourg
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Phytopathology 101:S47

Despite its small territory size, the Grand-Duchy of Luxembourg (GDL) has several microclimates that result in a variability of disease severity between the South (Gutland) and the North (Oesling). Septoria leaf blotch disease of wheat is an important disease in the GDL. Over 2003–2009, the severity was strong in Gutland (51% on average over the last two upper leaves at the late milk growth stage) and low in the Oesling (16% for the same leaves). For the years 2006, 2008 and 2009, the disease severity was less than 6% in the Oesling while it exceeded 40% in the Gutland. The second fungal disease that has become economically important is the wheat leaf rust. Over the same period, the Gutland and the Oesling showed consistently the highest and lowest disease severity respectively. In 2003 and 2007, the Gutland showed the highest disease severity with 66% and 57% respectively, whereas the lowest severity (<1%) was observed in the Oesling. Another important disease

is wheat powdery mildew. The 2003 and 2009 cropping seasons showed the highest disease severity with 15% and 40%, respectively, in the Oesling whereas less than 1% severity was registered in the Gutland. Fusarium head blight was also present in the eastern part of the Gutland showing the highest prevalence and severity in 2007 and 2008 (8.5% and 8.3% respectively). These prevalence and severity percentages were significantly higher compared to the Oesling (% prevalence % severity, p = 0.049 and p = 0.012, respectively, Tukey's test).

Deciphering the putative role of *AoMDV1* in Ochratoxin A biosynthesis in *Aspergillus ochraceus*

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Phytopathology 101:S48

Ochratoxin A (OTA) is a mycotoxin produced by several species of *Aspergillus* and *Penicillum* including *Aspergillus ochraceus*, an important OTA producer in cereals. Since OTA is nephrotoxic, teratogenic and carcinogenic for both humans and animals, several countries have established action levels to regulate its presence in some agricultural products. The *AoMDV1* gene, encoding a protein involved in mitochondrial division, has been previously disrupted in *Aspergillus ochraceus* and the resulting *Ao*Δ*MDV*1 transformant was found not able to produce OTA. In an attempt to understand the involvement of *AoMDV*1 in OTA biosynthesis and identify additional genes regulating the biosynthesis of OTA in *A. ochraceus*, a cDNA-AFLP differential display screening was performed with the *Ao*Δ*MDV1* transformant (MDV9) (OTA^-) and a wild type strain of *A. ochraceus* (OTA^+) NRLL 5175. Twenty five transcript-derived fragments (TDFs) were up-regulated in the OTA^+ strain compared to the *Ao*Δ*MDV1* strain. They included TDFs with high sequence homology to genes involved in the regulation of signal transduction, the biogenesis and metabolism of mitochondria and peroxisomes and in mediating stress response. A Yeast two hybrid screen was also used to identify interacting partners of AoMDV1 under conditions conducive to the biosynthesis of OTA.

Volunteer stream monitoring for invasive *Phytophthora* species in western Washington

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Phytopathology 101:S48

To supplement state agencies in their monitoring for *Phytophthora ramorum*, the sudden oak death (SOD) pathogen, a community-based stream monitoring program was initiated in 2010. This project expands on the streams currently being sampled by the WA Dept. of Natural Resources (WADNR) as part of the national *P. ramorum* survey and on nursery surveys by WA State Department of Agriculture (WSDA) to allow for early detection of *P. ramorum* and other invasive *Phytophthora* species, as well as examining the biodiversity of *Phytophthora* spp. in stream ecosystems. This project provided an opportunity to increase public awareness of waterborne plant pathogens and the damage they cause. The baiting process involved placing rhododendron leaves in mesh bags and deploying them in streams for two weeks. After bait retrieval the leaves were cultured on selective media and colonies of *Phytophthora* isolated onto V8 agar. *Phytophthora* species were identified using molecular and cultural methods. Several species of *Phytophthora* and *Pythium* were identified from stream samples and no *P. ramorum* was found in 2010. Volunteers included Master Gardeners, high school, community college, university students, and other individuals. Lecture and lab sessions were taught to introduce students to plant pathology, *Phytophthora* diseases, and laboratory methods. Some students worked on group projects related to *Phytophthora* in the lab at WSU-Puyallup. The program was expanded in 2011 with more baiting sites and student involvement.

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Phytopathology 101:S48

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Mapping partial resistance to *Pythium irregulare* in the soybean accession PI 424354

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Phytopathology 101:S48

Pythium irregulare causes damping-off of soybean and has emerged as an important soybean seedling pathogen in Ohio. The objective of this research was to identify major quantitative trait loci (QTLs) in the plant introduction PI 424354 that confers resistance to *P. irregulare*. Two $BC_1F_{2:3}$ populations were used in this study including: 224 recombinant inbred lines (RILs) of OHS 303 (partially susceptible) x (Williams (susceptible) x PI 424354) and 128 RILs of Dennison (susceptible) x (Williams x PI 424354). Seeds from each RIL were planted into a colonized sand-cornmeal mixture. Data was collected from 2-week old seedlings for percent germination, total weight (g), root weight (g), and a root rot score using an ordinal scale. Based on the analysis of the phenotype data for both populations, there was a significant difference between lines ($P < 0.0001$) for root weight and root rot scores, and the data for both populations fit the model for quantitative resistance. A combination of SNP data from the BeadXpress and SSR will be used to construct the genetic map and identify the QTL(s). These results suggest that this soybean accession can be an important source of partial resistance in developing germplasm for breeding new cultivars with more durable resistance to *P. irregulare*.

Mapping partial resistance to *Fusarium graminearum* in 'Conrad' soybean

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Phytopathology 101:S48

Fusarium graminearum has emerged as an important soybean seedling pathogen in Ohio. The objective of this research was to identify the major quantitative trait loci (QTLs) conferring resistance to *F. graminearum* in a Conrad (Resistant) x Sloan (Susceptible) $F_{6:8}$ population. A rolled-towel assay was used to phenotype 262 recombinant inbred lines (RILs). Twenty seeds from each RIL were placed on a germination towel and inoculated with 2.5×10^4 macroconidia/ml, the towels were rolled and placed in a bucket in the dark. The experimental design was a randomized block design, in which blocks and replications of the RILs were set up over time. At seven days after inoculation, the lesion and plant lengths were measured for each seedling, and the proportion of the seedling affected (lesion length/plant length) was calculated as a measure of disease severity. The mean disease severity for each RIL was then analyzed using best linear unbiased predictor. Based on the analysis of the phenotype data, the data fits the model for quantitative resistance. A total of 208 SSR and SNP markers were screened and a map of 172 markers was developed using JoinMap, and QTLs were identified using MapQTL. Conrad is a major source of partial resistance to *Phytophthora sojae*, this comparison of two root pathogens for overlapping QTLs associated with resistance will provide key clues to both basal resistance and pathogen specific resistance in this cultivar.

Partial saturation of potted ornamentals reduces Pythium root rot on flooded floor greenhouses

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Phytopathology 101:S48

Sub-irrigation of potted ornamentals has become a useful means for conserving and recycling water and fertilizers. However, a major concern for growers has been the potential for spread of disease organisms namely *Pythium* spp. We observed that current ebb&flow watering systems operate slowly and allow the root medium to approach saturation during each watering cycle. Growers have no ability to restrict the amount of water provided to the plants. We designed a flooded floor system to rapidly deliver and drain water, thus providing a partial saturation (PS) of the rooting medium. Less water is absorbed by the pots and therefore, there was less water, fertilizer, and inoculum leaching out. When poinsettias, chrysanthe-

mums, and geraniums were equally inoculated with virulent *Pythium* spp., placed on flooded floors along other plants and exposed to a conventional saturation (CS), the percentage of root rot was 63, 58, and 25%, respectively. When these crops were similarly inoculated and exposed to PS that produced an average 10% less volumetric water content, the amount of root rot was reduced to 41, 43, and 5%, respectively. The water stress resulting from PS treatment reduced biomass and stem height by 10 to 20% compared to CS. In general, crop quality was improved by PS, because plants were more compact, so they could be held at production spacing for a longer time before quality declined.

University of Arkansas Soybean Disease Screening Project
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Phytopathology 101:S49

Since 1990, Arkansas has maintained one of the most comprehensive soybean disease screening programs in the southern U.S., thanks to the Arkansas Soybean Promotion Board. A combination of inoculated field nurseries and greenhouse test are used to screen all varieties entered into the official University of Arkansas Variety Testing Program (OVT) each year to obtain disease ratings to all the major diseases in Arkansas. Our disease ratings are reported in the Arkansas Variety Testing Report, Arkansas Soybean Update, and the SOYVA variety selection program. Currently, the University of Arkansas has developed field nurseries at the Southwest Research and Extension Center and at the Newport Research Station for evaluating frogeye leaf spot, aerial web blight, and stem canker. Supplemental inoculum of a specific pathogen is applied throughout the growing season to help ensure adequate disease pressure for evaluations. Plots are visually rated at the R5 growth stage using a 0 – 9 scale. Soybean cyst (races 2, 3, 5, 9 and 14), root knot nematode, and reniform nematode resistance screenings are conducted in greenhouses at the Southwest Research and Extension Center and the Crowley Warren laboratory on the University of Arkansas Fayetteville Campus. Diseases cost $250,000,000 per year in lost yield and quality statewide, by some estimates. This program has provided producers comprehensive information on the resistance package for each variety, lowering the risk and economical loss due diseases and nematodes.

Seasonal synchrony between pheromone trap catches of the bean bug, *Riptortus pedestris* and the timing of invasion into soybean fields
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Seasonal catches of the synthetic pheromone of the bean bug, *Riptortus pedestris* (Heteroptera: Alydidae), captured in traps containing the synthetic pheromone were investigated under different field conditions from 2005 to 2007. In soybean fields, the number of bugs attracted to the pheromone traps increased after flowering and peaked at 9–13 days after flowering. After these attraction peaks, the populations of adult bugs and nymphs increased in soybean fields. In traps located in grassland, however, only small numbers of bugs were caught during the soybean flowering stages (from mid August to early September). The sex ratios of adults caught in the pheromone traps differed among soybean growth stages. Before flowering, more males were caught than females. After flowering, trapped females increased in number and the female ratio exceeded 0.5 throughout the flowering periods. These results suggest that attraction to the pheromone may be affected by the host plant phenology, and that females in particular respond strongly to the pheromone during flowering of the host plant soybean.

Green leaf volatile-induced direct defenses against insect herbivores
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Green leaf volatiles (GLV), which are rapidly emitted by plants in response to insect herbivore damage, are now established as volatile defense signals. Receiving plants utilize these molecules to prime their defenses and respond faster and stronger when actually attacked. Here, we report that GLV also have a strong direct defense capacity, which, when activated, result in significantly reduced damage in corn (*Zea mays*) seedlings. By studying effects of GLV on global gene expression we found that within 1 h many genes directly linked to direct defenses were up regulated. Furthermore, bioassays confirmed this by showing that GLV-pretreated plants, when challenged with Beet armyworm caterpillars incurred significantly less damage when compared to controls. Consequently, jasmonic acid levels and emission of herbivore-induced volatiles were dramatically reduced. Also, in a choice-feeding assay we found that caterpillars avoid GLV-pretreated plants. We also tested other potential volatile defense signals like methyl jasmonates, methyl salicylate, and ethylene. Compared to GLV we found no significant effect on caterpillar performance measured as volatile release. Additionally, gene expression analysis showed that none of these compounds had any significant effect on defense gene activation. These results clearly demonstrate that GLV exposure induces direct defenses in corn seedling and provide immediate protection against insect herbivore damage.

Yam virus diseases: A threat to a food security crop in West Africa
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Phytopathology 101:S49

Yam is one of the most important tuberous crops of West Africa. Millions of people depend on yam tuber for food and income, thus yam is a food security crop in West Africa. Yam production is adversely affected by virus diseases which reduce tuber yield and restrict international movement of germplasm. Information on disease incidence and distribution is necessary to determine research priorities and control strategies. Yam leaves from tubers collected from five West African countries were tested by ELISA and/or IC-PCR/IC-RT-PCR for *Yam mosaic virus* (YMV), *Yam mild mosaic virus* (YMMV), *Cucumber mosaic virus* (CMV), and yam-infecting badnaviruses (undifferentiated). Yam tubers from Nigeria and Ghana, and yam leaves from field surveys in Nigeria, Ghana, Benin and Togo were also tested. All the tubers (100%) and 69.9% of the leaves were positive to at least one of the viruses tested for. The yam-infecting badnaviruses had the highest incidence followed by YMV, YMMV and CMV. Incidence of mixed infection was higher in the leaves (30.9%) than in the tubers (16.3%) and the most frequent mixed infection observed was YMMV and yam-infecting badnaviruses. These results indicate that yam-infecting badnaviruses are an emerging viral threat in the yam system in West Africa and highlight the urgent need to produce yam varieties with multiple virus resistance particularly with resistance to yam-infecting badnaviruses.

Occurence of citrus quick decline in California
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In the summer of 2009 trees were rapidly declining and dying within a few weeks on an estimated 150–200 acres of orchard in Tulare County, at the San Joaquin Valley of California. Bud union symptoms included "honeycombing" or "inverse stem pitting" on the side of the bark. These symptoms were observed on sweet orange, grapefruit, and tangerines on Sour Orange (SO) rootstock. Additionally, roots showed grayish brown to purple lesions in the bark of large scaffold roots, which is characteristic of dry root rot symptoms caused by *Fusarium solani.* Leaf, shoot, and bark material were tested for *Citrus tristeza virus* (CTV) using Enzyme-Linked Immunosorbent Assay (ELISA), and Reverse Transcription Polymerase Chain Reaction (RT-PCR). Roots were plated onto different culture media in order to isolate fungal and bacterial pathogens. Fungal and bacterial cultures were further processed for molecular identification. To assess the girdling at the bud union by CTV, presence of starch in roots was determined by dipping roots in 2% potassium iodide and 0.2% elemental iodine solution. Molecular and ELISA analyses of plant samples showed that declining trees were consistently infected with CTV and *F. solani*. *F. solani* and CTV were never recovered from healthy looking trees. The roots of declining trees were also starch depleted, indicating plants stressby CTV induced girdling at the bud union. We hypothesize that interactions between CTV and *F. solani* potentially play role in the quick decline problem in Tulare County.

Identification of different species causing Botryosphaeriaceae canker in citrus reveal *Neofusicoccum mangiferae* with *Scytalidium*-like synanomorph
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Phytopathology 101:S49

Branch/trunk cankers of citrus caused by members of the Botryosphaeriaceae can sometimes lead to serious decline or death of branches and whole plants. A study was conducted to determine the different species causing the disease on citrus and their geographical distribution in California. Orchards in six citrus growing California counties- Fresno, Riverside, San Diego, San Luis Obispo (SLO), Tulare, and Ventura - were studied. Infected samples were collected from which isolates were obtained and identified using morphology and molecular methods with three markers - Internal Transcribed Spacer [ITS], Beta Tubulin, and Translation Elongation Factor. Nine different species (*Dothiorella viticola*, *D. iberica*, *Neofusicoccum mangiferae*, *N. mediterraneum*, *N. luteum*, *N. australe*, *N. parvum*, *N. luteum*, and *Diplodia mutila*) have been identified and tested to be causing citrus canker. All ages of trees and commonly used rootstocks - Carrizo, Volkameriana, and Sour

Orange - were infected. During morphological identification of *N. mangiferae*, multiple conidia types were found including septate conidia with a dark central region as well as *Scytalidium*-like synanamorph. These conidia types were initially described by Sutton and Dyko (1989). Although isolates of the organism have been studied in the Netherlands, South Africa, Australia, etc, the conidia types have not been found elsewhere.

First report of *Raffaelea canadensis* showing laurel wilt disease symptoms on avocado in California

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Phytopathology 101:S50

Laurel wilt disease caused by the fungus *Raffaelea lauricol*a and vectored by a non-native redbay ambrosia beetle, was first detected in Georgia in 2003. Laurel wilt has caused extensive mortality of native redbay in Georgia, Florida, South Carolina, and recently Mississippi. During a survey in 2010 in Temecula, CA, avocado orchard with a history of root rot, an avocado (cv. Hass) tree, was found showing typical laurel wilt disease symptoms. The crown was declining, and it exhibited dead branches without leaves. Black-to-brown discolored sapwood under the bark and many ambrosia beetle exit holes within 1 to 1.5 m up the bole were also observed. A *Raffaelea* sp. was consistently isolated from symptomatic tissues plated onto cycloheximide-streptomycin malt agar and incubated at room temperature for two weeks. Small subunit (18S) sequences of rDNA were amplified using primers NS1 and NS4. A BLASTn search of all sequences revealed high homology to *Raffaelea canadensis*. Pathogenicity testing was conducted by pipetting 50 µl of a 10^5 conidia per ml suspension using two isolates into 2 mm diameter holes on each of two avocado (cv. Hass) trees (10–15 cm DBH). Sterile water was used as a control in five 2 mm diameter holes on each tree. *R. canadensis* was consistently re-isolated from necrotic tissue but not from control treatments. To our knowledge, this is the first report of *R. canadensis* associated with ambrosia beetle (*Xyleborus* sp.) causing wilt on avocado in California.

Perception by growers and consultants of the importance of corn diseases

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Phytopathology 101:S50

To improve our knowledge about perceptions of corn disease management for successful corn production, a multi-state survey was conducted in Illinois, Iowa, Ohio, and Wisconsin in 2010. An equal number of surveys were sent out to randomly selected growers and consultants (n = 188 for each group and state for a total of 1,504 surveys). The valid response rate was 47%. Preliminary data analyses have been conducted. In both grower and consultant groups, maximizing profit and/or yield were considered the two most important components of successful corn production. In a series of questions (n = 13) about the relative importance of different production practices, foliar fungicides were ranked from 9th to 13th by consultants and 12th to 13th by growers, yet a high percentage of consultants have recommended the use of fungicides (>50%). Disease resistant hybrids, however, were ranked 6th to 9th and 5th to 7th by consultants and growers, respectively. Weeds were considered more important for managing than insects or diseases. Among different disease categories, stalk rots were considered a significant risk to annual corn production, although there was some variation in responses across states and groups. However, most consultants and growers considered themselves only good (mid-rating) at disease diagnostics. These results suggest that further efforts are needed to improve knowledge of the impact of corn diseases on an annual basis.

Does one size fit all for delivering corn disease-related information?

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Phytopathology 101:S50

With advanced technologies like Twitter, blogs, and Facebook, are there better ways we can package educational material to meet the needs of our clientele? To improve our knowledge about the use of information and technology, a multi-state survey was conducted in Illinois, Iowa, Ohio, and Wisconsin with corn growers and consultants in 2010. An equal number of surveys were sent out to randomly selected growers and consultants (n = 188 for each group and state for total of 1,504 surveys). The valid response rate was 47%. Preliminary findings indicated that consultants typically spend 30–45 minutes per meeting with their grower clients. Communications using telephone or email were <6 times and <3 times per month, respectively. Consultants are more likely to use the internet to gather information, although internet use is >50% for both groups. Consultants also are more likely to bookmark ag-related materials. For consultants, email is a valuable tool and they typically sign up to email lists from University extension and industry. Both University extension and seed dealers were considered valuable sources of information. The use of newspapers, radio, and TV only were considered slightly important. When considering the amount of time spent with clients by consultants, there is great potential for providing information to key clientele more effectively through the use of advanced technologies.

Design and validation of queries for the detection of *Phytophthora ramorum* in simulated metagenomes

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Phytopathology 101:S50

The main goal of this investigation was to validate a queries design method for the detection of Straminopile plant pathogens using massively parallel sequencing as a diagnostic tool. Unique pathogen diagnostic queries were obtained from the genome of *Phytophthora ramorum*. A PERL script, based on a modified Tools for Fingerprint Identification (TOFI) script, was used to obtain *P. ramorum* diagnostic queries of 20, 40, 60, 80, 100, 120, and 140 bp. Mock Sample Databases (MSD) containing the pathogen and the host genomes mixed at various ratios were simulated using MetaSim, a sequencing simulation program. Finally, a BLAST of all queries against the MSD was performed, and numbers of pathogen query hits and matches assessed. The numbers of queries obtained for *Phytophthora ramorum* were: 97075 (20 bp), 141799 (40 bp), 54346 (60 bp) 20573 (80 bp), 218 (100 bp), 3437 (120 bp), and 1401 (140 bp). Queries with 40 bp length showed the highest number of matches at all pathogen abundance values under 25%. The number of hits and matches increased as the length of queries decreased, with the exception of 20 bp queries that generated less matches than 40 bp queries. At pathogen abundance values of more than 0.5%, the percentage of positive hits was beyond 95% of total hits. Queries 40 bp lengths blasted against MSDs with pathogen abundance between 0.01% and 0.5% needed 580 reads to reach 10 matches, suggesting good potential for high-throuput diagnostics.

Design and validation of queries for the detection of *Puccinia graminis* in simulated metagenomes

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Phytopathology 101:S50

The main goal of this investigation was to validate a queries design method for the detection of fungal plant pathogens using massively parallel sequencing as a diagnostic tool. Unique pathogen diagnostic queries were obtained from the genome of *Puccinia graminis*. A PERL script, based on a modified Tools for Fingerprint Identification (TOFI) script, was used to obtain P. graminis diagnostic queries of 20, 40, 60, 80, 100, 120, and 140 bp. Mock Sample Databases (MSD) containing the pathogen and the host genomes mixed at various ratios were simulated using MetaSim, a sequencing simulation program. After blasting all queries against MSDs with variable pathogen concentrations, hits and matches were assessed. The numbers of queries obtained for P. graminis were: 594209 (20 bp), 175895 (40 bp), 59986 (60 bp), 21790 (80 bp), 8108 (100 bp), 3131 (120 bp), and 1294 (140 bp). Queries with 20 bp length showed the highest number of matches at all pathogen abundance values under 25%. At pathogen abundance values of more than 0.5%, the percentage of positive hits was beyond 87% of total hits. The number of hits and matches increased as the length of queries decreased. The number of reads needed to reach 10 matches increased as pathogen abundance decreased. Queries 20 bp lengths blasted against a MSD with pathogen abundance between 0.5% and 0.01% needed 85 reads to reach 10 matches. Queries 20 bp long showed the most potential for diagnostics, reaching 98.28% of positive hits.

Chemical management of Fusarium wilt of watermelon in the eastern U.S.A.

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Phytopathology 101:S50

Yield loss due to Fusarium wilt is a re-emerging problem in watermelon in the U.S. because of the increasing production of triploid cultivars, which lack host

resistance, and the emergence of the virulent race 2 of *Fusarium oxysporum* f. sp. *niveum*. One potential management strategy is the use of soil applied fungicides to reduce Fusarium wilt. The U.S. national program, inter-regional project 4 (IR-4), initiated trials of soil applied chemicals to manage Fusarium wilt of watermelon in 2007. Subsequent field trials in Maryland, Indiana and Delaware in 2008 and 2009 indicated that the fungicides acibenzolar-S-methyl, prothioconazole and thiophanate-methyl may be effective soil-applied fungicides. Prothioconazole alone or in combination with other fungicides reduced Fusarium wilt in 2009 in Maryland. In Indiana, however, prothioconazole reduced wilt only when used alone or with thiophanate-methyl. One additional trial was conducted in 2010 in Maryland. Acibenzolar-S-methyl alone or in combination with prothioconazole and thiophanate-methyl reduced wilt in that location. While acibenzolar-S-methyl, prothioconazole and thiophanate-methyl fungicides have shown promise in reducing wilt in some environments, the variation in efficacy across tests and over environments indicates that further evaluation is necessary.

First report of "*Candidatus* Phytoplasma asteris" (Group 16Sr1) infecting fruits and vegetables in Islamabad, Pakistan

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Phytopathology 101:S51

Nearby fruit and vegetable fields in Islamabad, Pakistan were surveyed for phytoplasma infection. "Candidatus Phytoplasma asteris" (Group 16Sr1) was found infecting mango, citrus, loquat, geranium, periwinkle, radish, blackberry and potato. Results suggest that a polyphagus vector may be involved in phytoplasma transmission to these plant species, which are first host records of 16Sr1 phytoplasma infection in Pakistan.

Morphological, pathological, and molecular characterization of lupin anthracnose and its relationship with tamarillo anthracnose in Ecuadorian Andes

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Phytopathology 101:S51

Anthracnose, caused by Colletotrichum acutatum, is a serious problem of lupin (Lupinus mutabilis) in Ecuador and worldwide, tamarillo (Solanum betacea) and many other hosts. Morphological features, host specificity test, specie-specific and ITS-PCR sequence analyses were used to characterize Colletotrichum isolates from lupin and tamarillo. All Colletotrichum isolates from lupin and tamarillo tested positive with C. acutatum-specific polymerase chain reaction (PCR). Colony diameter, spore shape, and insensitivity to benomyl grouped the lupin and tamarillo anthracnose isolates closer to C. acutatum. Host preference test demonstrated a positive cross-reaction among C. acutatum from lupin and tamarillo. Isolates were more virulent when they were inoculated on their own host. The phylogenetic relation among isolates of lupin and tamarillo was compared with other Colletotrichum isolates from hosts around the world. Comparative analysis with a range of reference ITS sequences identified the isolates from lupin and tamarillo anthracnose as C. acutatum. Analysis of internal transcribed spacer (ITS) sequences of Colletotrichum isolates clustered lupin and tamarillo isolates from Ecuadorian Andean zone into two separe subgroups. Molecular analyses indicated that the C. acutatum from Andean lupin is distinct from other C. acutatum lupin populations around the world. Project financially supported by TELFUN project /WUR and ESPE University, Ecuador. Recognition to Marco Rivera, Pablo Landazuri and Cynthia Rosas.

Detection of tospoviruses infecting Hymenocallis littoraris and Hippeastrum vittatum in Kunming, China

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Phytopathology 101:S51

The leaf samples of Hymenocallis littoraris (Jacq.) Salisb. and Hippeastrum vittatum (L, Her) Herb., showing chlorotic ringspot symptoms, were collected from Kunming, capital of Yunnan province, China. In order to confirm the infection of tospovirus, the samples were tested using both electromicroscopy and ELISA. The quasi-spherical particle 80–85 nm in diameter were observed in the dips of diseased leaves of both H. littoraris and H. vittatum through negative staining method. Tospovirus-like particles were found to exist single in line in metaplasm in H. vittatum and multiply in mass in the vesicle of endoplasmic reticulum in H. littoraris through observation of ultrathin section. The sap of diseased leaves had a positive reaction to the compound antibody of Watermelon silver mottle virus (WSMoV)/Groundunt bud necrosis virus (GBNV). The results confirmed that H. littoraris and H. vittatum were infected by tospoviruses.

Population genetic analysis of Leptographium longiclavatum a pathogen associate with the mountain pine beetle Dendroctonus ponderosae

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Phytopathology 101:S51

The mountain pine beetle (MPB) and its fungal symbionts (Leptographium longiclavatum) have destroyed over 16 million ha of pine forests in Canada, the largest epidemic in recorded history. The fungal symbionts could play an important role in the epidemics by reducing the tree defence response following the beetle colonization. We investigated the genetic structure of L. longiclavatum isolated from various populations in western North America using microsatellite markers. Based on Bayesian clustering inference, we found that there are two clusters that are concordant with geographic origin. One cluster comprises individuals from Northern sites where the beetle-fungus complex has recently established, and a second cluster is found along the Rocky Mountains. This distribution pattern is best explained by geographic origin and is concordant with the patterns observed in the beetle and with Grosmannia clavigera, another important, pathogenic fungal symbiont of the MPB. The general agreement in north-south differentiation of L. longiclavatum and G. clavigera populations, as well as the MPB suggests the dependence of fungal dispersal on their bark beetle vector and similar demographic processes in these two fungi. This information is important for disease management and surveillance.

A new broad-spectrum fungicide for use in row crops

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Phytopathology 101:S51

Priaxor™ is a new broad-spectrum premix fungicide under development in the United States by BASF Corporation containing a 2:1 ratio of the active ingredients pyraclostrobin and fluxapyroxad. Priaxor has been submitted to the EPA for potential registration on several major row crops including soybean, corn, wheat, barley, and canola. Research has indicated Priaxor is highly effective at controlling several important diseases of row crops including gray leaf spot (Cercospora zeae-maydis) and northern corn leaf spot (Exserohilum turcicum) in corn as well as brown spot (Septoria glycines) and frogeye leaf spot (Cercospora sojina) in soybean. Priaxor has demonstrated excellent disease control of the Septoria diseases of wheat (Septoria tritici and S. nodorum) in research trials. In barley, high levels of control have been achieved on net blotch (Pyrenophora teres) and scald (Rhynchosporium secalis). The combination of two active ingredients with different modes of action will reduce the risk of fungicide resistance development in the target pathogens. Trial results from 2009 and 2010 will be presented. EPA registration is expected in 2012.

Early activation of defense genes in kumquat by the citrus canker pathogen

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Phytopathology 101:S51

Citrus canker, caused by *Xanthomonas citri* subsp. *citri* (Xcc), is an economically important disease that affects various citrus types around the world. During previous work we showed that kumquat [*Fortunella margarita* (Lour.) Swingle] was resistant to citrus canker, the result of an active defense response. In this study we compared the induction in kumquat and susceptible grapefruit (*Citrus paradisi* Macf.) of a number of defense genes associated with PAMP-triggered immunity (PTI, or basal defense) and effector triggered immunity (ETI, or R-mediated resistance), the latter often leading to systemic acquired resistance (SAR). Our results showed that, generally, defense genes were induced earlier and to higher levels in the resistant kumquat after inoculation with Xcc. In addition, three genes belonging to the NPR1 family were differentially expressed between the two citrus types. This is important because in other species these genes are central in the induction of SAR and function either as positive or negative regulators of a number of downstream genes. We also compared the response of the two citrus species when the peptide Flagellin22 from Xcc (a PAMP) was used instead of the pathogen. The response to this treatment was less intense than the response observed after Xcc inoculation. Our results indicate that an earlier and enhanced activation of defense genes may play an important role in the defense mechanism of resistance to citrus canker observed in kumquat.

Efficacy of OMRI-certified fungicides and chitosan to manage early blight and septoria leaf spot in tomato

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Phytopathology 101:S51

Field studies to evaluate the efficacy of OMRI-certified and other materials for control of early blight and septoria leaf spot of tomato were conducted in Lexington, Kentucky in 2009 and 2010. Nine fungicides as well as ammonium bicarbonate and chitosan were evaluated in an organic production system. The most effective fungicides to manage septoria leaf spot and early blight of tomato were copper-based fungicides. None of the biological-based products (Sonata® and Serenade Max®), plant-based extracts (Trilogy® and Regalia® SC), chitosan, ammonium bicarbonate nor horticultural lime sulfur provided a significant ($P. > 0.05$) reduction in disease severity However, in spite of significant ($P < 0.05$) disease control in plots treated with copper-based products, no significant ($P. > 0.05$) improvement in yield over the untreated control was observed during the first two experiments, in which the initial symptoms of foliar disease were observed after fruits were set. In the third field trial, in which initial symptoms were observed before fruit set, Serenade Max®, Bordeaux mixture, Regalia® SC, water-soluble chitosan and lime sulfur improved yield, although none provided significant disease control.

Characterization of new races (races 11 and 12) and several novel strains of spinach downy mildew pathogen *Peronospora farinosa* f. sp. *spinaciae*
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Phytopathology 101:S52

Spinach downy mildew disease, caused by the obligate pathogen *Peronospora farinosa f. sp. spinaciae* (Pfs), is the most destructive disease for spinach production. New races of this pathogen have been emerging at a rapid pace during the last two decades as a result of the high-density, year-around spinach production in California. Up to 2007, 10 races of Pfs had been identified and the spinach resistance locus RPF2 provides resistance to races 1-10. Race 11 was identified in 2008 and could overcome the resistance of race 1-10 resistant cultivars. Spinach resistance loci RPF1, RPF3 and RPF6 can provide resistance to this race. Race 12, recently sanctioned by the International Working Group on Peronospora (IWGP), was identified in 2009 that could overcome the resistances of RPF1 and RPF2. The RPF3 locus was effective for race 12. In 2010, a novel deviating strain, UA0510C, was found to be virulent to RPF2 and RPF3 containing cultivars, and only the RPF1 locus was effective to this isolate. Another novel deviating isolate, UA4410, and race 12 caused identical disease responses on differentials, but UA4410 could be distinguished from race 12 by its ability to infect a number of additional cultivars including Pigeon, Zebu, Finch, and Celesta. A total of 150 spinach cultivars and breeding lines have been evaluated for resistance to races 10, 11, and 12 as well as deviating isolates UA510C and UA4410.

Partial biochemical characterization of caspase 3-like activity involved in *Solanum tuberosum-P. infestans* interaction
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Phytopathology 101:S52

The induction of at least eight caspase-like activities has been described under different stress conditions and during PCD in plants. The aim of this work was to determine if caspase-like proteolytic activity is involved in *P. infestans* (*Pi*) induced PCD in *Solanum tuberosum* leaves. Protein extracts from *Pi* infected leaves were prepared and proteolytic DEVDase activity was measured at different times after infection. Results obtained show that DEVDase activity was increased in a 70 and 80% at 24 and 48 h after *Pi* infection, respectively. In order to characterize the activity detected, the effect of caspase and general inhibitors was analyzed. Only Ac-DEVD-CHO (50 µM), a specific caspase-3 inhibitor and PMSF, a serine protease inhibitor (2.5 mM) were able to reduce the activity in a 95 and 50% respectively. Additionally, the effect of several ions on the potato caspase-3 like activity was determined. DEVDase activity was sensitive to CaCl2 (500 mM) losing 40% of activity and to ZnCl2 (10 and 100 mM) which enhanced it 6 folds. All concentrations assayed of NaCl (0 to 500 mM) had no effect. It is known that animal caspase-3 and plant DEVDase activities are active at neutral pH. In contrast, this caspase-3 like activity is active at acidic pH, suggesting activation after cytoplasm acidification or a vacuolar/intermembrane mitochondrial localization. This work constitutes the first evidence and characterization of caspase-like activity during *S. tuberosum-Pi* interaction.

Transcriptome analysis of a wheat cultivar infected by different chemotypes of *Fusarium graminearum*
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Fusarium head blight (FHB), caused by species of the fungus Fusarium, is a worldwide disease of wheat (*Triticum aestivum* L.). FHB disease can cause animal feed refusal/sickness and illness in humans by producing mycotoxins that consist of nivalenol (NIV), deoxynivalenol (DON), 3 deoxynivalenol (3ADON), and 15 deoxynivalenol (15ADON). *F. graminearum* isolates with a 3ADON chemotype are displacing the predominant 15ADON isolates in many parts of North America. 3ADON isolates have been found to produce significantly higher levels of DON than those with a 15ADON chemotype. To get a clear insight to the host genes involved in defense response when fusarium infects and to analyze the expression profiles of these genes in both; 3 ADON-infected plants and 15 ADON-infected plants, suppressive subtractive hybridization (SSH) method and quantitative real-time PCR (qRT-PCR) were carried out. Twenty up and down-regulated genes were identified. Of the genes that had matches to known genes present in the NCBI database, several had roles related to plant defense and stress tolerance. Five putative defense-related genes were confirmed by qRT-PCR. Several-fold higher induction of the putative genes in the 3 ADON-infected genotypes "Sumai3" compared with a control, indicates a putative role in the resistance response to Fusarium graminearum. Additionally, the expression profile of the two infected plants (by 3ADON & 15 ADON) varied between sampling times post inoculation.

Field efficacy of novel fungicides for the control of *Sclerotium cepivorum* in California
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White rot of alliums, caused by the pathogen *Sclerotium cepivorum*, is a devastating disease of onion and garlic worldwide. A combination of sclerotia stimulants and fungicides is currently recommended to control the disease. A field study was conducted in Tulelake, California, to determine the efficacy of five different fungicides for the control of white rot on onion, including two novel fungicides: penthiopyrad (LEM17) and fluopyram (Luna Privilege.) The other fungicides investigated were tebuconazole (Folicur,) boscalid (Endura,) and fludioxonil (Switch.) Disease was measured as incidence of kilograms of diseased and healthy bulbs at harvest. Means separation was determined using the Tukey test. Tebuconazole and penthiopyrad were most effective; both treatments reduced white rot incidence by over 50%. Fluopyram was moderately effective, and reduced incidence by 30%. Fludioxonil and boscalid did not significantly influence disease severity. No phytotoxicity was observed in any of the treatments. These data provide evidence of potential new chemical treatments for the control of *S. cepivorum*.

Development of non-invasive inoculation methods of tomato fruit with *Geotrichum candidum* to improve post-harvest disease management strategies
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Geotrichum candidum is the causal agent of sour rot in tomato and other fresh produce. This disease is a limiting factor of tomato production on the Eastern Shore of Virginia and can cause major losses in the field and especially during post-harvest handling. Infections by *G. candidum* are most prevalent during periods of wet harvest conditions or abrupt temperature changes and if improper post-harvest handling procedures are employed. There are currently no in-field treatments targeted to prevent sour rot infections so post-harvest treatments are used to prevent further losses. Currently, the methodology utilized for screening materials on harvested tomato fruits is invasive and involves severe wounding to inoculate with *G. candidum*. This practice does not accurately reflect natural fruit infections and response to post-harvest treatments may differ under these artificial methods. This compelled us to develop a method of infection without wounding. Bartz (2000) showed that tomatoes cooled with *Rhizopus* water suspensions developed infection, though the majority of studies focus on bacterial cells entering tomatoes and there is a lack of work on internalizing fungal pathogens. As a result, a vacuum pressure method of internalizing *G. candidum* spores was developed to internalize spores into tomato fruit to accurately imitate infected fruit for further management studies.

Influence of weather factors on panicle blast in upland rice in Brazil
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Panicle blast (*Magnaporthe oryzae*) is responsible for severe grain yield losses in upland rice. A field experiment was conducted with rice cultivar BRS Bonança to identify the meteorological factors that determine the panicle blast severity. The lay-out was a split plot design with three replications. The treatments consisted of 12 weekly plantings and five levels of nitrogen. Twenty panicles per plot which emerged at the same time (50% flowering),

totaling 720 were tagged. Panicle blast was assessed 15 days after tagging the panicles (PB1) and 10 days before harvest (PB2) using a five grade scale. The variable levels of panicle blast obtained at different plantings at highest nitrogen level (N240) were used to relate to mean temperatures and precipitation. A correlation matrix was developed utilizing, two, three and four day averages of weather variables and PB1 and PB2. The results showed that there was no influence of precipitation on panicle blast severity. The correlations with mean temperature of 4 days, beginning 7 days after panicle emergence explained the variation in PB1. The following multiple regression equation was developed to estimate the panicle blast severity (PB1): $\hat{y} = 155.62 + 0.54\ x_1 - 4.75\ x_2$ ($R^2 = 0.50$) where x_1 = minimum temperature and x_2 = maximum temperature. There is further need to test the validity of this equation in other upland rice cultivars.

An analysis of plant disease and vector threats under future climates

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Climate change will have a progressively negative effect on crop yields with further reductions arising through increased severity of plant disease, particularly vector-borne disease. While some pathogens' life cycles will be limited by increasing temperatures, other climatic, such as elevated (e) CO_2, may provide more favourable conditions for some pathogens. Field based experiments from the Australian Grains Free Air Carbon Dioxide Enrichment (AGFACE) experiment and closed environment chambers investigated the effects of elevated (e) CO_2 on wheat pathogens such as wheat stripe rust (*Puccinia striiformis*), crown rot (*Fusarium pseudograminearum*) and *Cereal Yellow Dwarf Virus* vectored by the aphid Rhopalosiphum padi. *P. striiformis* disease progress and fecundity was not affected by eCO_2 but high temperatures during the growing season may limit development and survival of the diseasae in some regions. Conversely, eCO_2 will increase *F. pseudograminearum* biomass while saprophytic fitness remains stable, potentially leading to rapid colonization of stubble harvest and an increased incidence of crown rot in future climates. Preliminary investigations of *R. padi* grown in eCO_2 indicated no significant differences in development but reductions in fecundity for nymphs. Using an electrical penetration graph (EPG) to study feeding behaviour it was found *R. padi* probed less but spend more time ingesting from wheat grown under eCO_2 potentially leading to a lower incidence of virus transmission.

Synergy in biorational insecticides used on collard greens, *Brassica oleracea*, infested with diamondback moth, *Plutella xylostella*

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The diamondback moth, *Plutella xylostella*, (Lepidoptera: Yponomeutidae) is widely distributed and is perhaps the major pest of crucifers. The pest status of the diamondback moth has risen as it has become resistant to most conventional and biorational insecticides such as *Bacillus thuringiensis* and Spinosad®. In order to slow down the resistance capacity of the diamondback moth, we evaluated various mixtures of Agroneem Plus®, Spinosad®, Actara® and jalapeño pepper extract using laboratory bioassays with a view to obtaining mixtures that could be adopted by small vegetable growers in North Carolina. These mixtures were applied to collard greens using a leaf dip laboratory assay with 3rd instar larvae and also using a greenhouse test. The mixtures were evaluated for their effect on larval fitness, oviposition deterrence over 48 hours and ovicidal activity. Our results indicate a possible interaction between the methanolic extract of jalapeño pepper and Spinosad®, whilst Agroneem Plus® and Actara® also may exhibit interactions. Spinosad® at the recommended rate killed 100% of the exposed larvae. These mixtures are being further evaluated to determine their ability to delay resistance in diamondback moth populations.

Interaction between powdery mildew (*Blumeria graminis*) and triticale (*xTriticosecale*) in Germany as a model for pathosystem analysis

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Phytopathology 101:S53

Triticale, the intergeneric cross between wheat and rye, was highly resistant to powdery mildew before 2004, but has revealed an increasing susceptibility since then. Breeding of cultivars with durable resistance requires precise knowledge about virulence structure of pathogen populations and genetics of host resistance. The German triticale mildew population was monitored by analysing 694 single-pustule isolates in 2007–2010 with a new differential set of 20 triticale cultivars. Data revealed a high diversity with 272 pathotypes and high Simpson indices (0.95–0.97) within years. Most of the isolates (80%) were highly complex with at least 14 virulences. In detached leaf segment tests, only 16 out of 826 (2%) breeding lines and the triticale cultivar Grenado exhibited complete seedling resistance. The same genotypes showed effective adult-plant resistance at six field locations in three years. Analysis of primary triticale produced by crossing durum and aestivum wheats with defined race-specific resistance genes and rye inbred lines showed that the outcome is not predictable and expression of resistance genes is strongly affected by the rye genome. By genetic mapping of race-specific resistance genes, a dominant monogenic inheritance has been identified in each of six triticale lines. In conclusion, the use of race-specific resistances seems to have a restricted durability only.

Could viruses of wheat prevent supply meeting demand?

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Global wheat production must increase by 50% by 2050 to meet projected requirements. While Defra statistics show that the yield of wheat in the UK increased from 1940 until 2000, it then began to plateau. It has been suggested that this could be due to virus infections suppressing yield. Exploring this possibility is important, and begins with high throughput diagnostics to assess the prevalence of viruses in UK wheat. Real-time PCR is well suited to this, and assays have been developed for a selection of viruses that have been found in wheat, and other related plants in the UK in the past. Thus far approximately 600 samples of wheat from across the UK have been screened for six of these viruses. Just five samples were positive. Assays are being developed for the remaining viruses to complete this work. Since there is such a low prevalence of these known viruses it has been hypothesized that the problems are due to as yet unidentified viruses. Therefore the project will move in an exciting new direction by exploiting next generation sequencing methods to investigate the complete virome present in wheat and its surrounding environment (weeds, hedgerow plants and possible vector samples). In comparison to specific assays, which are biased and only include viruses that are currently identified and characterised, a comprehensive picture of the hidden diversity in each sample will be produced; this information may hold the key to current and future threats to wheat production.

Comparison of the ergot alkaloid synthesis (*EAS*) gene cluster among Clavicipitaceous fungi

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Genome sequence analyses of several fungi belonging to family Clavicipitaceae allowed comparison of ergot alkaloid synthesis (*EAS)* gene clusters. These fungi exhibit diverse associations with their host plants, from symbiotic mutualism with grasses (e.g., *Epichloë /Neotyphodium* spp. in Poaceae) and morning glories (*Periglandula* spp. in Convolvulaceae), to ergot (*Claviceps* spp.) infection of cereals. Animal toxicosis due to ergot alkaloids (EA) in *Neotyphodium coenophialum*-infected tall fescue have a major impact on U.S. livestock production, with annual losses close to $1 billion. Characterization of the *Claviceps purpurea EAS* gene cluster identified the presence of eight core genes requited for the production of the simplest EA (clavines and lysergic acid), and additional flanking genes encoding nonribosomal peptide synthetases (*lpsA*, *lpsB* and *lpsC*) are required for the more complex ergopeptines with enhanced pharmacological activities. The presence, order and orientation of the *EAS* genes as well as the length of the clusters were compared, and in most Clavicipitaceae the gene cluster arrangement was similar to the *C. purpurea*, and a novel gene (*easP*) has been identified in *EAS* clusters of *P. ipomoeae* and *C. paspali*. *Neotyphodium coenophialum*, *Epichloe festucae* and *E. glyceriae* shared a different gene arrangement of 11 *EAS* genes. *Epichloe brachyelytri* isolate E4804 had putatively functional genes only for the initial four enzyme steps of the pathway.

Ergot alkaloid gene expression studies in a grass-endophyte association
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Many epichloid endophytes (*Epichloe* and *Neotyphodium* spp.) are systemic symbionts of cool-season grasses, providing protection against vertebrate and invertebrate herbivores by producing mycotoxins. Among these compounds are the ergot alkaloids (EA) responsible for livestock toxicosis. The outcome of the EA pathway is a complex profile of alkaloids consisting of the pathway end product and several intermediates that can accumulate at levels comparable the end products. These endophytes grow vegetatively throughout aerial tissues of infected plants without morphological differentiation. However, there is a possibility that fungal hyphae in different tissues have different gene expression patterns. To test this hypothesis, we conducted a reverse-transcription-quantitative PCR study of the *N. lolii* x *E. typhina* hybrid isolate Lp1 in perennial ryegrass to measure the expression of six EA biosynthesis genes (*dmaW*, *easC*, *easD*, *easA*, *cloA*, and *lpsA*) in grass pseudostems, center leaves, outer leaves, leaf blades, and developing seeds. For each gene, tissue samples from at least three individual plants were analyzed. Possible relationships between gene expression levels and ergot alkaloid profiles were examined. Within the same plant tissue all examined genes revealed similar expression profiles, whereas expression levels differed widely among different tissues. There was no definite correlation between the differences in gene expression and the profile of alkaloids accumulating in different tissues.

High levels of natural resistance against selected DMI fungicides in populations of *Fusicladosporium carpophilum* but not *Alternaria* spp. from almond
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Scab caused by *Fusicladosporium carpophilum* and Alternaria leaf spot caused by *Alternaria* spp. are common and economically important diseases of almond in California. To replace the overused QoIs where widespread resistance has developed in both pathogens, newly registered DMIs fungicides have proven very effective in managing these diseases. Baseline sensitivity studies were conducted to aid in monitoring of fungicide sensitivities of field populations. *F. carpophilum* exhibited a wide and continuous range of sensitivities for difenoconazole (EC_{50} 0.002 to 5.455 µg/ml, mean 0.283 µg/ml), metconazole (EC50 0.013 to 3.85 µg/ml, mean 0.496 µg/ml), and propiconazole (EC_{50} 0.038 to 6.701 µg/ml, mean 0.755 µg/ml). Generally, isolates less sensitive to one compound were also less sensitive to the other compounds. Thus, many of the isolates were naturally resistant to these fungicides. All isolates of *Alternaria* spp. were determined to be sensitive against the three fungicides. EC50 values ranged from 0.007 to 0.076 µg/ml (mean 0.017 µg/ml) for difenoconazole, 0.014 to 0.224 µg/ml (mean 0.045 µg/ml) for metconazole, and 0.028 to 0.172 µg/ml (mean 0.108 µg/ml) for propiconazole. To maintain a high level of disease control with the use of these fungicides, resistance management with strict rotations of fungicides with different modes of action will need to be done in integrated programs.

Fluopyram fungicides for the control of diseases of horticultural and row crops
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Phytopathology 101:S54

Fluopyram is a new fungicide active ingredient in development worldwide by Bayer CropScience. Application for registration is pending with the Environmental Protection Agency with an expected registration summer or fall of 2011. In addition to Luna Privilege (fluopyram solo), premixes were developed with four other fungicidal active ingredients: Luna Sensation (+ trifloxystrobin), Luna Experience (+ tebuconazole), Luna Tranquility (+ pyrimethanil), and Propulse (+ prothioconazole). The mixtures have demonstrated excellent crop safety and outstanding control of a broad range of major foliar and fruit diseases such as powdery mildew, brown rot blossom blight, early blight, gummy stem blight, gray mold, scab, and others. Luna is providing improved crop quality at harvest and during storage/transportation. Biological profile, efficacy trial results, resistance management/mode of action, and pending use labeling will be presented.

Bacteria associated with creeping bentgrass (*Agrostis palustris* L.) disease syndrome in southern & southeastern United States during the summer of 2010
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During the summer of 2010, the Clemson University Commercial Turfgrass clinic received samples of bentgrass putting greens from 15courses from southern & south eastern United States. The samples were submitted on suspicion of *Pythium* infection. Additional samples were received and described as "healthy" turf. On the samples suspected of having disease, symptoms were varied from yellowing of lower leaves to wilt and dessication of entire plants. Microscopic observations revealed streaming of bacteria from both infected yellow leaves and newly emerging leaves. There was no evidence of *Pythium* oospores or zoospores. Following surface disinfestation, bacteria were isolated by maceration of infected leaves in nutrient broth and streaking the suspension on nutrient agar (NA). Pure cultures of predominant bacterial colonies growing on plates were established on NA. Based on color, and morphology of bacteria on several media we were able to distinguish 16 different bacterial morphologies. Pathogenicity testing of each culture was conducted on creeping bentgrass (*Agrostis stolonifera L.*) cv. Penn G-2. There were 11 pathogenic isolates belonging to 10 different bacterial morphologies. Sequence analysis of 16S rDNA of pathogenic bacteria revealed the highest similarity (>98%) to *Xanthomonas translucens* pv *poae*, *Acidovorax avenae* subsp *avenae*, and a similarity of (>93%) to *X. campestris* pv *campestris* and *X. oryzae* pv *oryzae* for all the 11pathogenic isolates.

Modelling of *Guignardia* pseudothecium maturation and ascospore dispersal in citrus orchards
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Phytopathology 101:S54

Ascospores are considered the most important inoculum source citrus black spot (CBS), caused by *Guignardia citricarpa*, but pseudothecium maturation and ascospore dispersal are inadequately studied. *Guignardia* ascospore trapping and concomitant weather data were obtained for three localities for three seasons (July through March from 2006 to 2009) in the Limpopo province of South Africa. Degree-days accumulated until first seasonal ascospore discharge (>10°C with 1 July as biofix; DDtemp), and DDtemp accumulated on rainy (rainfall >0.1 mm; DDrain) and moist days (vapour pressure deficit <5 hPa; DDvpd) were used in two Gompertz models to predict onset of ascospore dispersal: a temperature model [Event = exp(-exp(-(-2.725 + 0.004 × DDtemp)))] and a temperature/moisture model [Event = exp(-exp(-(-3.238 + 0.008 × DDvpd + 0.004 × DDtemp -0.009 × DDrain)))] (R^2 = 0.608 and 0.658, respectively). Both models predicted a delay in pseudothecium maturation in climates with colder winters and springs, while the temperature/moisture model predicted a further delay in drier seasons or climates. A Gompertz equation was also used to predict the proportion of *Guignardia* ascospores trapped (PAT) per season from DDtemp data accumulated on wet or moist days from the first seasonal ascospore discharge [PAT = exp(-4.096 × exp(-0.005 × DDwet2); R^2 = 0.908]. These models can be used to predict the onset and dynamics of ascospore dispersal in climatically diverse regions.

Geminiviral (PHYVV and PepGMV) and cucumoviral (CMV) co-infection in chili pepper fields: The *AC1* gene in PepGMV with a mutation with aminoacid change
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Phytopathology 101:S54

Emerging diseases in chili pepper (*Capsicum annuum* L.) are causing important agricultural crop losses in chili producing provinces of central part of Mexico. Symptoms exhibited by the affected plants consist of yellowing, dwarfing, or sprout death, scarce and small size fruits without commercial value. The object of this study is to identify the possible viral species as causal agents of these emerging diseases in chili pepper in producing areas in Zacatecas state, México. Protocols of nucleic acids hybridization (colony hybridization, Southern blot, tissue printing), rolling-circle amplification of nucleotide sequences, sequencing and sequence alignment in GeneBank and immunology assays (ELISA) where used. Results show the presence of the PHYVV and PepGMV (Begomovirus) with high homologies to isolates

previously reported in other latitudes in this country. The immunology assays with monoclonal antibodies indicate the presence also of the CMV (Cucumovirus). In the *AC1* gene of the PepGMV a nucleotide change of C instead of A was found. This mutation impact the central domain of the respective protein, where the aminoacid N (Asn) is changed by H (His). In conclusion, in chilli pepper crop with yellowing and dwarfing symptoms in this latitude, three species of virus are present, the PHYVV, the PepGMV and the CMV; in the PepGMV a single nucleotide mutation was found with impact in the central domain of the Rep protein.

Citrus cybrid response to biotic stress caused by *Xanthomonas citri* subsp. *citri*

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Phytopathology 101:S55

A cybrid is an asymmetric hybrid that contains the nucleus of one parent in combination with the mitochondrion and/or chloroplast of the cytoplasm donor parent. Twenty cybrids of highly susceptible Red grapefruit (RG, *Citrus paradisi*) and the more tolerant Valencia orange (VO, *Citrus sinensis*) as the cytoplasm donor, were screened for their susceptibility to *Xanthomonas citri* subsp. *citri* (Xcc). Tolerance inherited from VO appeared to be quantitative based on an intermediate lesion phenotype in selected cybrids. In contrast to the callus-like lesions typical for susceptible RG, lesions were more necrotic for VO and the cybrids. This lesion phenotype indicated cell death arrested the proliferation of Xcc. Populations of Xcc at 14 days post inoculation in cybrids (7.2 Log cfu), were similar to VO (7.6 Log cfu) and one log unit lower than RG (8.4 Log cfu). Expression of genes related to host pathogen interaction in VO and cybrids differed from RG. The contrasting pattern suggested a differential interaction of genes from the nucleus with the mitochondria and chloroplast genes from the cytoplasm donor. Mitochondria and chloroplasts have a central role in stress and programmed cell death signaling. The response of cybrids to Xcc may be expressed at different levels depending on whether mitochondrial and/or chloroplast genomes are transferred in the cybridization process.

Temperature and fungal isolate influence canker development in black walnut caused by *Geosmithia morbida*

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Phytopathology 101:S55

Thousand cankers disease of black walnut (*Juglans nigra*) is the result of aggressive feeding by the walnut twig beetle (*Pityophthorus juglandis*) and subsequent canker formation around galleries caused by *Geosmithia morbida*. We studied temperature effects and *G. morbida* isolate aggressiveness on canker development in black walnut. One-year-old trees were placed in growth chambers maintained at 20°C at night and either 25 or 30°C during the day. Each tree was inoculated with four different haplotype isolates (based on rDNA ITS sequence data) positioned on stems in a Latin square design. There was no effect ($P > 0.10$) of inoculation position on canker development but cankers were larger ($P < 0.05$) at 25°C compared to 30°C six weeks after inoculation. All isolates tested caused cankers, although in two experiments an isolate collected from Arizona walnut (*J. major*) in Arizona resulted in slightly smaller ($P < 0.05$) cankers than the other isolates collected from black walnut. Furthermore, all canker areas were smaller in experiments in which trees were entering dormancy or were fully dormant (i.e. they had defoliated by late fall in the greenhouse) prior to inoculation in growth chambers. These data suggest that black walnuts are more susceptible to canker development when they are actively growing.

Genetic based population analysis of the nucleocapsid protein of Tomato spotted wilt virus isolates in New Mexico

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Phytopathology 101:S55

Tomato Spotted Wilt Virus (genus Tospovirus; family Bunyaviridae) is an important pathogen of many ornamental, greenhouse and agronomic crops worldwide. Over one thousand different plant viruses have been characterized and Tomato Spotted Wilt Virus (TSWV) ranks in the top ten most economically destructive, with losses reported over one billion dollars worldwide. In New Mexico, TSWV causes sporadic problems in peppers, peanuts, tomatoes, cowpeas and a variety of landscape and ornamental plants. In an effort to better understand the population ecology of TSWV in New Mexico, a survey and genetic analysis of the viral nucleocapsid gene was performed. The protein sequence of the nucleocapsid gene of 295 isolates from 6 different host species:Capsicum annuum, Lycopersicon esculentum, Arachis hypogaea, Vigna unguiculata, Solanum tuberosum, and Cucurbita pepo in 12 different counties in New Mexico were compared. Each amino acid difference and the predicted steric implications to the secondary protein structure were examined. The viral population among the major host groups was relatively homogeneous and the population of all TSWV isolates from New Mexico was 98.8% similar. When the viral population in New Mexico was compared to other TSWV isolates from around the world, including known resistance breaking strains, a similar homogeneity resulted. Most of the amino acid substitutions observed were conserved changes that had little or no effect on the final protein product.

Diapause in northern corn rootworm (Coleoptera: Chrysomelidae)

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Phytopathology 101:S55

Diabroticite corn rootworms are prominent pests of maize and have adapted to both cultural and chemical management methods. In response to a widely used corn-soybean crop rotation in the U.S. Corn Belt over several years, northern corn rootworm (NCR) populations adapted by increasing the proportion of eggs that diapause over two winters instead of one. The frequency of eggs diapausing for two years increased with time and prominence of the maize-soybean rotation in the landscape. We investigated the pattern of inheritance of egg diapause duration in relation to male and female parent phenotypes for diapause duration. We collected NCR as pupae from a maize field that had been in a maize-soybean crop rotation for several years. We sexed the pupae and maintained them individually. We also collected pupae from a NCR lab colony that had been selected for one year diapause for several generations. We established reciprocal F1 families from the extended diapause (ED) and one year diapause (D) lines. Eggs obtained from the females were provided two overwintering periods, one each for five months at 8°C. Eggs were allowed to hatch at 25°C for 45 days after each overwintering period. Eggs obtained from ED females had a significantly higher proportion of eggs with the ED trait compared to eggs obtained from D females ($F \geq 4.13$ $P \leq 0.015$). With a strong genetic influence on diapause duration in NCR, we can begin selecting for a non-diapausing line to facilitate research on this important pest.

Generation and affinities with antigen of single chain variable fragment antibody against Odontoglossum ringspot virus from phage display library

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Phytopathology 101:S55

Odontoglossum ringspot virus (ORSV) is one of the commonest viral pathogens of the cultivated orchids. In this experiment a set of ORSV-specific oligonucleotide primers were designed from the region of the coat protein (CP) gene of ORSV. The ORSV CP gene was cloned into the protein expression bacterial plasmid vector of the gutathione S-transferase (GST) fusion protein expression system. The recombinant ORSV CP was injected into mouse to induce immune response of the animal. The cDNAs of VH and VL of ORSV antibody genes were obtained by using reverse transcription polymerase chain reaction from the total the RNAs that were extracted from the spleen cells of immunized mouse. ScFv (single-chain variable fragment) library of ORSV were constructed with gene splicing by overlap extension. Thirty seven scFvs were selected from ORSV-scFv library following three rounds of affinity selection with ORSV CP as an antigen that was expressed in bacteria. Four scFv antibody have specific binding reaction against ORSV CP was selected. Comparing the sensitivity between scFv antibodies and ORSV polyclonal antibody tisted in enzyme linked immunosorbent assay (ELISA) to detect ORSV in leaf extracts of diseased Phalaenopsis plants. Unfortunately, the affinity between scFv antibody and ORSV was weaker than that between polyclonal antibody to the same antigen. The results highlight the potential of applying the scFv antibodies in the diagnosis of virus diseases.

Monitoring behaviors of *Ralstonia solanacearum* cells by GFP labeling during infection process to plant cells

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Phytopathology 101:S55

Growth and movement of *Ralstonia solanacearum* harboring the phage-modified plasmid were monitored using tomato seedlings and tobacco cultured cells BY-2. The plasmid contains a gene for green fluorescent protein (GFP) and is stably maintained in *R. solanacearum* cells without selection pressure. Bacteria harboring the plasmid can be tracked in planta by visualizing GFP fluorescence. For real-time monitoring of bacteria in planta, tomato seedlings were grown on agar medium and bacterial suspension was applied to the root apex. Aseptic inoculation of plants grown on solid agar medium eliminates the effects of other bacteria in the soil. In susceptible tomato cultivars, strong GFP fluorescence was observed in hypocotyls and lateral roots as well as the taproot. In resistant cultivars, however, GFP

fluorescence was rarely observed on lateral roots. The difference may be due to gaps between the taproot xylem and the lateral root xylem. It appears that *R. solanacearum* cells require a long period of time to move between xylem gaps in resistant cultivars. We also observed that bacterial growth was suppressed in the hypocotyl and stem of the seedlings of resistant cultivar. Our results show that this monitoring system can be used to assess bacterial pathogenicity. For further study, we made strains of *R. solanacearum* lacking cell wall degrading enzymes (CWDE) and investigated the effects using BY-2 cells.

Phylogenetic background of Japanese *B. cinerea* isolates resistant to benzimidazoles, dicarboximides, and other fungicides

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Phytopathology 101:S56

B. cinerea, a causal agent of gray mold, is one of pathogens showing a high risk of development of fungicide resistance. We previously identified four types of benzimidazole-resistant mutations in beta-tubulin *BenA* gene, three types of dicarboximide-resistant mutations in histidine kinase *BcOS1* gene, and a QoI-resistant mutation in cytochrome b (*cytb*) gene. We analyzed genetic background to investigate the origins of fungicide-resistant strains in Janan. Eighty three isolates were divided into three groups by microsatellite primed-polymerase chain reaction (MP-PCR) and further divided into 21 groups by the PCR-restriction fragment length polymorphism (RFLP) at nitrate reductase, ADP/ATP translocase, and ATP synthase genes. Major dicarboximide-resistant *BcOS1*^{I365S} and benzimidazole-resistant *BenA*^{E198A} mutations were found in various strains suggesting that these mutations occurred in various strains independently during long term use of these fungicides. In contrast, dicarboximide-resistant isolates with *BcOS1*$^{V368F+N369H}$ mutation had low genetic diversity. Interestingly most of these isolates showed fenhexamid resistance. Although QoI-resistant isolates were present at low rate in fields, six QoI-resistant isolates were divided into three different genotypes. We will discuss why *B. cinerea* has high genotypic diversity and also how fungicide resistant strains raised and spread in population.

New *Phomopsis* species identified from wood cankers in eastern North American vineyards

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Phomopsis cane and leaf spot, caused by the Ascomycete fungus *Phomopsis viticola*, is a destructive fruit and foliar disease in eastern North American vineyards. The pathogen typically attacks green tissues, but can also cause wood cankers, presumably due to infection of pruning wounds, as is the case for most canker pathogens of grape (e.g., *Eutypa lata*). If pruning wounds are an infection court for *P. viticola*, then controls for preventing infection of green tissues may not prevent pruning-wound infection. Accordingly, we surveyed the *Phomopsis* community (teleomorph *Diaporthe*) from wood cankers in vineyards of the northeastern U.S. (CT, MA, MD, MI, NH, NJ, NY, OH, RI, VA, VT) and southeastern Canada (Ontario, Quebec), and evaluated the susceptibility of pruning wounds to infection by the *Phomopsis* species recovered. We used conidial dimensions, colony growth on potato dextrose agar, and phylogenetic analyses of nuclear loci (rDNA internal transcribed spacer region, elongation factor subunit 1-alpha, actin), to identify *P. viticola* from wood cankers and two new species not previously reported from grape: *Diaporthe eres* and a species with DNA sequences identical to isolates identified as *P. fukushii* in Japan. Pathogenicity tests on *Vitis labruscana* 'Concord' and *V. vinifera* 'Chardonnay' in Geneva, NY demonstrated that pruning wounds of both are susceptible to infection by strains of all three *Diaporthe/Phomopsis* species.

Assessment of prescription programs using Peanut Rx for management of peanut diseases

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Phytopathology 101:S56

Peanuts in the southeastern U.S. are affected by numerous fungal diseases and management programs often include 7 fungicide applications per season. Since 2007, a risk index, Peanut Rx, has been updated by researchers from the University of Georgia, the University of Florida, and Auburn University. From points assigned to production variables, disease risk is described as low, moderate or high. Prescription fungicide programs have been developed appropriate for risk (4, 5, or 7 applications/season). Studies were conducted at 3 sites in Georgia in 2010 to assess prescription programs that included flutolanil, propiconazole, tebuconazole + prothioconazole, tetraconazole, tebuconazole, azoxystrobin, thiophanate methyl, and chlorothalonil. Plots were planted to 'Georgia-06G' and maintained according to recommendations from Cooperative Extension. Fungicides were applied at timings appropriate for prescription programs. Severity of leaf spot diseases was reduced in all fungicide programs as compared to the untreated control; incidence of southern stem rot tended to be significantly lower in fungicide programs than in the untreated control. Yields in treated plots were numerically, often significantly, greater in treated versus untreated plots. Differences in control of stem rot and yields were not different within related prescription programs, i.e. azoxystrobin programs; however leaf spot severity was frequently greater in plots sprayed 4 times versus 7 times.

Detction of Phomopsis sclerotioides in commercial cucurbit field soil by a nested time-release PCR-based technique

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Phytopathology 101:S56

A PCR-based molecular technique to detect Phomopsis sclerotioides in soil was developed using a species-specific primer pair. Three PCR techniques were combined to improve detection sensitivity: nested PCR using the primer pair ITS1 and ITS4, time-release PCR with two different polymerases (rTaq and AmpliTaq Gold polymerases), and fluorescent PCR to obtain fluorescent-labeled PCR products that can be analyzed by capillary electrophoresis. Using these techniques, soil samples collected from 241 commercial cucumber or melon fields in Akita Prefecture in Honshu, Japan, were diagnosed to detect the pathogen. Disease incidence had not been observed in any of the fields. The pathogenic fungus was detected in soil samples collected from 30 fields but was not detected in samples from 207 fields. Samples from four fields remained inconclusive. In nine of the 30 fields showing a positive diagnosis, disease incidence has been confirmed or the pathogen has been isolated. The results demonstrate that the pathogen can be detected in cucurbit fields in which visible disease symptoms have not appeared. In order to prevent the invasion of the pathogen or delay its spread among fields in cucurbit-growing regions by periodic monitoring of the field soil, a highly sensitive detection technique is required. The technique developed here is practicable for this purpose.

Species-specific detection of *Mycosphaerella* spp. as classical biological control agents for *Fallopia japonica* (Japanese knotweed) by PCR assay

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Mycosphaerella polygoni-cuspidati has potential as a classical biological control agent for the invasive weed, *Fallopia japonica*. It also suggested that a novel species, *M. shimabarensis*, which has been isolated only from Shimabara, Nagasaki Pref., Japan, can be synergists with *M. polygoni-cuspidati* based on the results of promoting the disease severity. For direct, rapid and specific detection of *M. polygoni-cuspidati* and *M. shimabarensis* after introduction to the fields in the UK, specific primer sets were designed based on the sequences of rDNA-ITS region. PCR products of approximately 300 and 450 bp were obtained only when DNA extracted from mycelial fragments of *M. polygoni-cuspidati* and *M. shimabarensis* were used. No amplification was observed from other *Mycosphaerella* spp. and fungal endophytes isolated from *F. japonica*. Using the primer pairs, both isolates were specifically detected from naturally infected plants by *M. polygoni-cuspidati*. Therefore, the primer pairs can be useful for specific detections of both *M. polygoni-cuspidati* and *M. shimabarensis*. Foliar distribution of *M. shimabarensis* detected by specific primers designed in this study was also investigated. A PCR product specific to *M. shimabarensis* was amplified from the genomic DNA extracted from the lesions but not from healthy area of diseased leaves. These results indicated that *M. shimabarensis* strongly associated with *M. polygoni-cuspidati.*

Evaluation and popularization of integrated pest management module in onion

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Phytopathology 101:S56

The major cause for low productivity of onion is the severe incidence of pests and diseases leading to yield losses up to 30 per cent and in severe outbreak situations the total crop loss. Five different onion IPM modules were evalu-

ated in two seasons during 2008–09 to select the best module for popularization among onion growers. Of the five, the module comprising of bulb treatment and soil application of *Pseudomonas fluorescens* and *Trichoderma viride*, soil application of AMF, Azophos and neemcake, installation of yellow sticky traps and sex pheromone traps, foliar spray of *P. fluorescens*, *Beauveria bassiana* and azadirachtin and need based application of chemical pesticides registered the lowest pest and disease incidence coupled with higher bulb yield. The above IPM module was demonstrated in larger plots in farmers' holdings in two locations in Tamil Nadu during 2010–11 through IPM CRSP, USAID Project and compared with farmers' practice. The above biointensive IPM module registered reduced mean thrips population (6.45/plant), leaf miner damage (13.67%), cutworm damage (4.38%), basal rot (2.00%), purple blotch (27.75%) and pink root (10.40%) with higher bulb yield (13.74 t/ha) and cost benefit ratio of 1: 5.4 as compared to farmers' practice registering higher incidence of thrips population (13.69/plant), leaf miner damage (19.34%), cutworm damage (7.10%), basal rot (4.69%), purple blotch (52.25%) and pink root (49.70%) with reduced bulb yield (10.58 t/ha) and cost benefit ratio of 1: 4.5.

Two newly detected populations of *Fusarium graminearum* in the United States

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Detailed analysis of extensive U.S. *Fusarium graminearum* collections from the past ten years has revealed surprising substructuring of the species into several populations that are characterized by their genetic coherence, their geographic distributions and by distinct phenotypic characteristics. Here, we introduce two newly detected populations of *F. graminearum*, the Northland and the Arkansas population. The Northland population mainly has been detected in Minnesota, and to a lesser extent in North Dakota, Wisconsin and South Dakota. This population appears to be endemic to native grass populations in Minnesota and its current distribution indicates outward radiation into agricultural areas. A subset (30%) of this population does not produce the common trichothecene mycotoxins, but still maintains aggressiveness on wheat. Arkansas appears to be a melting pot. Four populations are present in the state, with the widespread MW15ADON population and the newly detected Arkansas population each constituting about 30% of the total population. While the Southern Louisiana and the Gulf Coast populations also were encountered, a large percentage (ca. 30%) could not be assigned with a high degree of certainty. Unaligned isolates may be the result of interbreeding among populations in this region. All three trichothecene types were identified in the MW15ADON and Arkansas populations, but the proportions were different with 15ADON at 95% and 50%, 3ADON at 4% and 50%, and NIV at 1% and 4%, respectively.

An unusual serological reactivity revealed in isolates of *Potato virus Y* from Brazil

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Phytopathology 101:S57

During characterization of a panel of *Potato virus Y* (PVY) isolates collected in Brazil between 1995 and 2008, several isolates were found displaying an unusual serological reactivity towards a series of PVY^{O} and PVY^{N}-specific monoclonal antibodies. Specifically, recombinant PVY isolates typed as PVY^{NTN} by RT-PCR assay were found non-reactive to a PVY^{N}-specific monoclonal antibody SASA-N (Scottish Agricultural Science Agency) while reacting normally to another PVY^{N}-specific antibody 1F5 (Agdia). All these unusual isolates were typed as PVY^{O}-negative with monoclonal antibodies MAb2 (Agdia) and SASA-O (SASA). Some of these isolates were identified as ordinary PVY^{NTN} isolates by whole genome analysis but some were found to have a novel type of PVY^{NTN} recombination pattern. At least two isolates with this unusual serological profile, PVY-AGA and PVY-AST, induced typical potato tuber necrotic ringspot disease in a susceptible potato cv Yukon Gold under greenhouse conditions. This serological profile, 1F5-positive and SASA-N-negative, has never been reported before and presents a significant challenge posed by these isolates to seed potato certification programs.

Molecular typing of *Potato virus Y* isolates from Brazil reveals a history of introduction of necrotic strains

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Phytopathology 101:S57

A panel of 31 *Potato virus Y* (PVY) isolates, collected in Brazil between 1985 and 2009 from potato, was subjected to molecular and serological typing using RT-PCR and a series of PVYO and PVYN-specific monoclonal antibodies. The data collected were combined with biological characterization of the same isolates in tobacco. Of the 31 isolates tested, two were typed as PVYO, eight PVYN:O, eighteen PVYNTN, and three mixed or inconclusive. When these data were compared to the original dates of collection of the isolates tested, several conclusions could be made. The only two PVYO isolates were identified in the oldest group, collected in late 1980s and early 1990s, and no PVYO isolate was collected since then. PVYN:O strain has long been known to enter Brazil in mid-1990s; this strain was responsible for drastic changes in PVY epidemiology in Brazil. Interestingly, of the most prolific PVYNTN group tested in this work, three recombinant PVYNTN isolates were initially collected as early as mid-1990s. Taken together, these data suggest that the switch from PVYO to recombinant strains of PVY occurred in Brazil in mid- to late-1990s, approximately at the same time as in Europe. Currently, the two recombinant strains PVYNTN and PVYN:O are apparently dominant in Brazilian potato crop.

A novel type of *Potato virus Y* recombinant genome

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Phytopathology 101:S57

During characterization of recombinant *Potato virus Y* (PVY) isolates collected in Brazil, two isolates, PVY-AGA and PVY-MON, were identified with a novel type of PVY^{NTN} recombination pattern. Whole genome sequence analysis revealed that PVY-AGA and PVY-MON represented recombinants between two novel parent genomes, PVY^{NTN} and PVY-NE11. Specifically, new recombinants had an ordinary PVY^{NTN} genome structure for approximately 6.7-kb from the 5′-end of the genome, while the 3′-terminal 3.0-kb segment had two fragments of NE-11 sequence separated by another small NTN fragment. Only PVY-AGA induced vein necrotic reaction in tobacco. Both PVY-AGA and PVY-MON isolates did not induce hypersensitive resistance (HR) in potato cultivars carrying *Ny*, *Nc*, or (putative) *Nz* genes. An ordinary PVY^{NTN} isolate PVY-AST induced systemic HR in cultivar Maris Bard carrying a putative *Nz* gene. All three isolates, PVY-AGA, PVY-MON, and PVY-AST, induced typical potato tuber necrotic ringspot disease in a susceptible potato cv Yukon Gold under greenhouse conditions. In a standard multiplex RT-PCR assay, PVY-AST, PVY-AGA, and PVY-MON were all typed as ordinary PVY^{NTN} isolates, consistent with the presence of two prominent recombinant junctions in their genome, characteristic of an ordinary PVYNTN strain. Ability of these new PVY recombinants to overcome resistance genes in potato producing mild or no foliar symptoms presents a significant threat posed by these isolates to seed potato production areas.

Temperature-dependent development and reproduction of the whitefly Trialeurodes vaporariorum Westwood (Hemiptera: Aleyrodidae)

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Phytopathology 101:S57

Trialeurodes vaporariorum is a serious pest in sub-tropical regions and vector of the potato yellow vein virus. A profound understanding of the temperature-dependent population growth potential is important for understanding pest population dynamics and to design effective pest management strategies. The development and mortality of immature life stages, and reproduction and longevity of T. vaporariorum was studied at constant temperatures of 10, 15, 18, 20, 25, 28 and 32°C. Optimum temperature for development was between 27–28°C for the immature developmental stages. No complete development was observed at 10°C and 32°C. Survival time of adults was shortest at 15°C and 28°C with highest fecundity at 20°C. The data was used to establish functions for temperature-dependent development, mortality and reproduction. Established functions were used to compile a temperature-driven phenology model for T. vaporariorum, and life table parameters were simulated over a range of temperatures. The model was validated by comparing simulated life table results with life tables constructed under controlled daily fluctuating temperature (between 5.81–35.27°C). The model will be used in pest risk assessments studies and for predicting within year population growth potentials. Moreover, the information on the pest age-stage structure and distribution under specific field conditions will be useful for adapting IPM strategies.

Studies on *Peanut bud necrosis virus* affecting tomato in India

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Phytopathology 101:S58

Peanut bud necrosis virus (PBNV; genus *Tospovirus*, family Bunyaviridae) is a significant constraint to production of tomato by subsistence farmers in India. The IPM-CRSP of the USAID has funded a project to implement ecologically-based IPM strategies for sustainable management of the virus. For this purpose, we have conducted farmer-participatory field trials in select locations of Tamil Nadu State in India and evaluated the performance of selected tomato cultivars and hybrids against natural infection of PBNV. Although none of the entries showed resistance, the data obtained from these trials indicated that some cultivars and hybrids exhibited field tolerance with higher fruit yield compared to susceptible materials. The chemical composition of tomatoes harvested from PBNV-infected plants indicated significantly less amounts of lycopene, β-carotene, vitamin A, zinc, total sugars and carbohydrates suggesting that virus infection affected nutritive quality of the fruit. Studies on PBNV spread in new plantings indicated that virus-infected seedlings from commercial nurseries serve as a source of inoculum for secondary spread of the virus in the field. Roguing of virus-infected tomato seedlings during and soon after transplanting significantly reduced disease incidence leading to higher income for farmers. A combination of growing tolerant cultivars and roguing of virus-infected seedlings are being validated in IPM packages for mitigating impacts of PBNV for the benefit of farmers.

Differences in responses and protein profiles of soybean near isogenic lines (NILs) to *Phakopsora pachyrhizi* inoculation

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Phytopathology 101:S58

Asian soybean rust, caused by *Phakopsora pachyrhizi*, was first discovered in continental U.S. in late 2004. This pathogen has the potential to cause severe yield losses as all U.S. commercial soybean varieties are susceptible. In this study, ten Near Isogenic Lines (NILs) of three different populations were evaluated for differences in resistance to infection by *P. pachyrhizi* (Louisiana isolates). These lines, which had previously been evaluated against Florida soybean rust isolates, were evaluated in both growth chambers using detached leaves and under greenhouse conditions. For each line, sixteen plants were evaluated at R1 stage through inoculation with 200 µl of spore suspension (3×10^4 spores/mL) per leaf on the upper surface. For detached leaf assay, soybean leaves at R1 stage were inoculated in the same manner. Fifteen days after inoculation, plants in greenhouse and detached leaves in growth chamber were evaluated for lesion appearance, pustule formation, and pustule eruption and density. There was a significant difference among NILs in response to *P. pachyrhizi* infection in growth chamber and greenhouse conditions. Some of these lines are currently being compared for protein profile differences with and without soybean rust inoculation to identify potential proteins involved in soybean resistance to rust infection.

Development of loop-mediated isothermal amplification (LAMP) assays for the detection of Plum pox virus

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Phytopathology 101:S58

Plum pox virus (PPV) is a devastating viral disease of stone-fruitin worldwide, and caused severe economic and production losses in many European countries. Molecular-based detection methods for PPV using conventional PCR, multipex PCR and real-time PCR had been developed and applied. However, such methods require expensive instruments and a long time for detection. A more rapid, efficient and practical method, the reverse transcription-loop-mediated isothermal amplification (RT-LAMP) method was expected for the on-site detection of Plum pox virus. The PPV-LAMP specific primers were designed according to the sequences for coat protein gene of PPV-D and PPV-M. Amplification products were detected by agarose gel electrophoresis, checked up with the naked eye and by UV irradiation using SYBRGreen I, lateral flow devices(LFD) and a real-time turbidimeter. Accordingly, a typical ladder-like pattern on the gel electrophoresis, a visible green after adding SYBR green I and two clear lines on LFD were observed in all positive samples. The results of real-time monitoring showed that the detection limit of the PPV-LAMP assay was 1.6×10^2copies /ul in less than 30 min, and was approximately 100 times higher sensitive than that of the conventional PCR. The results above suggested that the LAMP technique was fit for the on-site detection of plum pox virus. With the improvement of the method, it might be extended to the survey and inspection for the purpose of quarantine.

Identification of biochemical function of *Agrobacterium* T-complex recruiting protein VBP

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A. tumefaciens can transfer a section of DNA from Ti plasmid to the host plants, resulting in crown gall tumor disease. The DNA was transferred in the form of a single-stranded DNA-protein complex (referred to as T-complex) and through the type IV secretion system (T4SS) located at both ends of the *Agrobacterium* cells. A recently identified protein that can bind to VirD2 (VirD2-Binding Protein, VBP) is proved to play a role of recruiting T-complex to the T4SS, and defined as recruiting protein of *Agrobacterium* T-complex. *Agrobacterium* contains three homologous genes that can encode three varieties of VBP proteins, named VBP1, VBP2, and VBP3. Motif search showed that VBPs contain a HEPN domain and a nucleotidyltransferase domain. To identify the biochemical function of VBPs, VBP1 was expressed as His-tagged-VBP1 fusion protein and purified by affinity chromatography. The purified fusion protein was partially renatured at low temperature, and then was added to a solution that contains eight nucleotide triphosphates. After incubating for a certain period of time, the composition of nucleotides in the solution was analyzed by using HPLC to check whether any of these eight nucleotides was bound to VBP1 or hydrolyzed by VBP1. The results showed that His-tagged-VBP1 could hydrolyze all the eight nucleotides in different rate. Further quantitative calculations showed that the hydrolysis ability of VBP1 fusion protein to NTPs is significantly higher than that to the dNTPs.

RNA-seq analysis of potato tuber transcriptome dynamics in response to the late blight pathogen *Phytophthora infestans*

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Phytopathology 101:S58

Cultivated potato is the world's number one non-grain food commodity. The late blight pathogen *Phytophthora infestans* is a notorious plant destroyer with the capacity to attack both potato foliage and tubers. Importantly, foliar resistance against late blight does not guarantee tuber resistance. Our research documents that *RB*, a broad spectrum late blight resistance gene, has the potential to mediate both foliar and tuber resistance against late blight. To better understand *RB*-mediated tuber blight resistance, we conducted a high-throughput RNA-seq study to examine potato defense mechanisms. Eight potato tuber RNA samples from *+RB* and *–RB* lines at 0, 24, 48 hours post pathogen inoculation were sequenced using Illumina GAIIx technology. Over 215 million cDNA sequence reads were generated. These represented 99% of known potato unigenes, and enabled a detailed study of tuber blight transcriptome dynamics. Groups of genes were identified that are candidate components of tuber blight resistance. Importantly, this preliminary study suggests that the tuber and foliage transcriptomes may be largely non-overlapping. An additional 42 potato RNA samples including *P. infestans*-challenged foliage and tuber samples, were subsequently sequenced using Illumina High-seq 2000 technology. The resulting ~300-600 million sequence reads will enable a more robust and fine-scale analysis of tuber blight transcriptome dynamics and a more detailed comparison between potato tuber and foliage blight defense mechanisms.

Prophages of "*Candidatus* Liberibacter asiaticus" and their distribution in southern China

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Phytopathology 101:S58

"*Candidatus* Liberibacter asiaticus" is a putative pathogen of citrus Huanglongbing (HLB), a destructive citrus disease worldwide. Prophage is an important biological trait of bacteria including "*Ca.* L. asiaticus". The role of prophages in bacterial virulence, environmental adaptation, strain specification and genome evolution has recently drawn strong interests in the HLB research community. In this study, 12 consecutive open reading frames in a "*Ca.* L. asiaticus" prophage/phage reported in Florida were used as a reference to examine for the presence of homologs in three different "*Ca.* L. asiaticus" strains in China. PCR analyses showed the amplification rates of 83.3% (10/12) for strain YN835, 33.3%(4/12) for strain GDws231, and 8.3%

(1/12) for strain GDws217. All amplicons were sequenced and shared >98% similarity among correspondent homologs with one exception. At this exceptional locus, strains YN835 and GDws231 showed high level of sequence heterogeneous with a gap of 140 bp. However, at the amino acid level, the two sequences were 100% comparable with 79% similarity (69% identity). This locus was not detected in strain GDws217. Results from this study show that there are at least three related but different prophages of "*Ca.* L. asiaticus" in southern China. Prophage YN835 is predominately in Yunnan Province. Prophage GDws217 and prophage GDws231 are found in Guangdong Province.

Improving reproduction of the Idaho population of the pale cyst nematode, *Globodera pallida*, for use in studies of its control and/or eradication

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The pale cyst nematode (PCN), *Globodera pallida*, was first detected in early 2006 in Idaho. A research program was implemented at University of Idaho in 2007 to establish an Idaho-PCN population to provide cysts for control and eradication studies. Initially, the hatching and cyst production rates from field derived cysts were low, restricting research efforts. With each successive generation of cyst production, the Idaho-PCN population's reproduction rate improved. At the onset, the average egg hatching rates of PCN field cysts was 2.8% in 2008. In subsequent generations there was an increase in hatching rate from 14.5% for the F1 generation to 44.5% in the F2 generation. This increase is likely due to an improvement in the viability of the eggs in the cysts and improvement of the culturing of the Idaho-PCN population. It is also possible that selection is occurring in the population to the rearing environments. With the increased viability, hatching and cyst production of the Idaho-PCN population the UI-PCN Laboratory is now producing an adequate number of cysts from the Idaho population for research on control and eradication of this pest in Idaho.

Interaction of Rosellinia necatrix, Fusarium oxysporum and Ophyostoma stenoceras in white rot of Rosa sp.

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Phytopathology 101:S59

Rosa cultivation in greenhouse is important in Mexico (south of the State of Mexico) mainly for ornamental purposes; 663 ha are currently cultivated. In recent times a root rot and subsequent death problem of this plant has accentuated. By conducting causal agent isolates, Rosellinia necatrix Prill, Fusarium oxysporum Schlechtend and Ophyostoma stenoceras (Robak) Melin & Nannf. were found. Thus the objective of the study was to conduct pathogenicity tests. In 12-inch pots with disinfected substrate plants of Rosa sp. vacara variety on Manetti pattern were sown; eight treatments were established: T1 = Ophyostoma stenoceras; T2 = Fusarium oxysporum; T3 = Rosellinia necatrix; T4 = O. stenoceras + F. oxysporum; T5 = F. oxysporum + R. necatrix; T6 = O. stenoceras + R. necatrix; T7 = O. stenoceras + F. oxysporum + R. necatrix; T8 = Control. Inoculation was done by incorporating 20 g of wheat with Rosellinia necatrix, 125 mL of a suspension of 10X6 conidia/mL of Ophiostoma stenoceras and 125 mL of 10X6 conidia/mL of Fusarium oxysporum. The results showed that in treatments where R. necatrix was inoculated and there was interaction with O. stenoceras and F. oxysporum there was mortality of plants: in T3, 87.5%, 59 days after inoculation (dai); T6, T5 and T7 recorded 62.5, 56.3 and 66.7% mortality at 63.2, 59.4 and 60 (dai), respectively; T4 suggests antagonism between them; T1 and T2 recorded no mortality, but there was a reduction in plant height compared to the control.

Population structure and genetic diversity of *Sclerotinia minor* from peanut research plots in Oklahoma

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Phytopathology 101:S59

Sclerotinia minor is the causal agent of Sclerotinia blight, a disease that significantly reduces peanut (*Arachis hypogea*) productivity. This study analyzed the diversity and population structure of 164 *S. minor* isolates from Oklahoma. Isolates were obtained from infected stems of peanut plants from four lots at the Oklahoma Agricultural Experiment Station. Isolates used for Sclerotinia blight resistance screening in peanuts, collected between 1981 and 1993, were also included in the analysis. Inter Simple Sequence Repeat (ISSR) fingerprinting was used for evaluation of the population structure and genetic diversity of the *S. minor* sample. Of a total of 50 fragments amplified, 38 were polymorphic (76%). AMOVA identified significant genetic differences within the sample ($\Phi PT = 0{,}091$; $p = 0{,}001$), of which 9.1% of the genetic variation exist between lots and the other 90.9% is distributed within lots. Nei's genetic distance, GST, and PCO revealed close relationships between the populations from lots used for peanut breeding and isolates used for disease resistance screenings, with little although significant differentiation between plots. However, isolates collected from chemical testing plots were significantly different from all the others. Our results confirmed the validity of the current isolate panel for peanut germplasm Sclerotinia blight resistance screening. New *S. minor* genotypes were identified that could be of value for peanut breeding programs in the future.

***Erwinia tracheiphila* colonization of cantaloupe fruits through flower inoculation**

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Phytopathology 101:S59

Cantaloupe *(Cucumis melo* var. *cantalupensis*) is a nutritious fresh fruit. Bacterial wilt, caused by *Erwinia tracheiphila*, is the most devastating cantaloupe disease globally. The pathogen is transmitted in nature by xylem-feeding spotted and striped cucumber beetles; other modes of infection have not been reported. We hypothesized that *E. tracheiphila* can enter cantaloupe plants through flower nectar thodes and/or pollen tubes and thereby contaminate fruit interiors and move systemically in the plant. Newly opened hand pollinated hermaphrodite/female cantaloupe flowers were inoculated with 5 µl of either *E. tracheiphila* (10^7, 10^8, 10^9 or 10^{10} cfu/ml) or peptone buffer (controls) by placing inoculum drops onto stigma tops and onto the nectaries. Eight of 9 *E. tracheiphila* inoculated plants showed wilting of part or all of the vines. Fruits on three inoculated plants developed small-to-large water soaked spots on the rind at 18 or more days post-inoculation. Peduncles collapsed, and further rind netting and fruit development was impaired on these fruits. Off-white ooze was observed on spotted fruit, the bacterial slimy string test was positive and bacteria streamed from cut stems and peduncles immersed in water, symptoms characteristic of bacterial wilts. This is the first report of infection and symptom development on cantaloupe fruits following flower inoculation with *E. tracheiphila*. Whether flower invasion is a significant mechanism of infection in a field setting is not known.

Lettuce cultivar influences *Xanthomonas campestris* pv. *vitians* population levels

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Phytopathology 101:S59

Bacterial Leaf Spot, caused by *Xanthomonas campestris* pv. *vitians* (*Xcv*), is a widespread and economically important disease of lettuce. Cultivars with resistance to *Xcv* have been identified, but mechanisms for resistance in this pathosystem have not been investigated. We hypothesized that susceptible and resistant cultivars differ in terms of populations of *Xcv* that they support on leaves. A rifampicin resistant strain of *Xcv* was inoculated onto seedlings of susceptible (Vista Verde) and resistant (Little Gem) cultivars and four cultivars intermediate in resistance. Bacterial populations were estimated by spreading dilutions on media containing rifampicin. In general, susceptible cultivars, including Vista Verde, supported the highest populations of *Xcv*, while Little Gem had the lowest populations by 14 days after inoculation. In initial experiments, in which the presence or absence of lesions was not considered in sampling, high within-treatment variability was noted; subsequently, we determined that lesions had higher populations than non-symptomatic tissue. As a result, in subsequent experiments both the most severe lesions (once symptoms developed) and non-symptomatic tissue were sampled. Preliminary data indicated that an increase in populations of *Xcv* on Vista Verde coincided with lesion occurrence. These results indicated that factors influencing *Xcv* populations could be productive targets for research on resistance mechanisms.

Development of a user-friendly identification system for the native and invasive pest thrips and their parasitoids in East Africa

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Phytopathology 101:S59

The information on thrips and their natural enemy diversity in Africa is obscure. Hence identification and timely detection of the native and invasive

thrips and their parasitoids from Africa is difficult. Absence of identification tools has contributed negatively to the invasion and widespread distribution of thrips like Western Flower Thrips, chilli thrips, corn thrips etc. To facilitate early and effective diagnosis of the native and invasive thrips and their parasitoids a user-friendly LucID 3.5 thrips identification system is under development. Detailed surveys on the thrips and their parasitoids in the region were undertaken and a geo-referenced database has been established at icipe. Based on the above surveys and the published literatures, a list of over 90 thrips species including the predatory thrips as well as terebrantian and tubelliferan pest thrips are included in the key under development. A LucID sub-key to 8 eulophid thrips parasitoids belonging to the sub-family Entedoninae from Africa is under development. Biogeographic information, online links to occurrence maps and IPM options for the pest thrips are innovative features of the East African LucID key. Such a key could benefit quarantine authorities, extension functionaries and entomologists in the region for effective and timely identification of the thrips and their natural enemies. A snapshot view of the key and its features and future plans on the updates will be presented.

Development of a forecast model for the carpogenic germination of *Sclerotinia sclerotiorum* sclerotia

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Phytopathology 101:S60

Sclerotinia sclerotiorum causes annual white mold epidemics in several Brazilian regions. The aim of this study was to develop a forecasting model for the carpogenic germination of *S. sclerotiorum* sclerotia, in order to use it as part of a system for disease prediction and in the development of disease risk maps. For this purpose, an experiment was carried out to determine the effect of temperature and soil moisture on the carpogenic germination of the pathogen's sclerotia. Sclerotia were submitted in the laboratory at different temperatures (10, 13, 15, 18, 20, 23, 25 and 30 degrees C) and soil moisture (61, 68, 75, 82, 89, 95, 100, 105, 110, 115 and 120% of field capacity, FC). The experimental units were composed of 20 sclerotia placed on 150 g of soil in 500 g plastic containers. The experiment was carried out in a randomized block design in a 8 × 11 factorial arrangement with four replications. Germination was initiated after 32 days of incubation at temperatures between 13° and 20°C, with humidity exceeding 68% of FC. The germination percentage was higher at 15°C and 40 days of incubation. A logistic regression model was used to estimate the probability of germination of sclerotia as a function of moisture and soil temperature. According to the model, temperatures between 15° and 17°C and humidity close to 100% of DC are more favorable for carpogenic germination of S. sclerotiorum sclerotia.

Biological control of fire blight disease *Erwinia amylovora* under field condition of Karaj, Iran

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Phytopathology 101:S60

Fire blight (*Erwinia amylovora*) annually causes devastating damages on pear and quince trees in Iran. The disease has been reported from several provinces and is in progress to the east. Due to efficiency of chemical controls and limitation of antibacterial usage, biological control could be an important method to reduce the disease damages. This research aimed at biological control of *E. amylovora* by using epiphytic bacteria, *Pseudomonas fluorescens* strain E10; *Pantoea agglomerans* strain Abp_2; *Pseudomonas putida* strain E_{11} and *Serratia marcescens* strain Kgh_1. The antagonists were isolated from pome and stone fruit trees and identified by morphological, biochemical, and physiological tests, as well as nucleotide sequence analysis of 16S-rRNA gene. The selected antagonist isolates were applied twice at 20% and 80% full bloom on a semi-susceptible pear cultivar 'Shah-Mieveh' in Karaj region in Iran. The results showed 46.87% disease severity in control plants, while the antagonistic bacteria reduced the disease symptoms between 23 to 50.2%. The most disease inhibition was belonging to *P. agglomerans* strain Abp*2* and least disease inhibition was showed in *S. marcescens* strain Kgh1 in orchard trials.

Evaluating the spread of potato powdery scab in storage

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Phytopathology 101:S60

Powdery scab, caused by the plasmodiophorid pathogen *Spongospora subterranean*, is a potato disease that has become a great concern in potato-growing regions of North America. Persistent cystosori can survive in soil for more than 6 years and their ability to cause disease is promoted by cool, moist weather and poorly drained soil conditions at tuberization. Cystosori are created in scab lesions that erupt through periderm and can cause infection at and post-harvest. In this 2-year study we investigated the spread of powdery scab from field-infected tubers to asymptomatic tubers in storage at the University of Wisconsin Hancock Storage Research Facility. In 2009, after 82 days in storage, 8 treatments composed of serial distributions of symptomatic and asymptomatic tubers resulted in 76–100% infected tubers. All treatments resulted in correlation between powdery scab infection and tuber desiccation. In 2010, the disease-spread experiment at 45 days resulted in low disease severity and slight desiccation. Additional treatments in 2010 included ambient ozone and phosphorous acid salts for limiting spread of infection in storage; no significant differences in control were observed at 45 days. Variable results between years may be resolved with extended ratings out to 90 days in storage. Due to the longevity of this pathogen in soil and limitations in management, it is critical that we better understand the role and risk of powdery scab in production and storage.

Evaluating the efficacy of fungicide programs for the control of potato early blight in the central sands of Wisconsin

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Potato early blight is a perennial and potentially destructive disease caused by the fungus *Alternaria solani*. Appropriately-timed, effective fungicides are necessary to limit yield and quality loss. In 2010, we evaluated 38 fungicide programs for early blight control at the University of Wisconsin Hancock Agricultural Research Station on 'Russet Burbank.' Programs included an untreated control, conventional and organic grower standard programs, and newer chemistries, all replicated 4X and arranged in a randomized complete block design. Programs were initiated on 16 Jun and all other production inputs were commercial standard. Plots were treated every 7 days and evaluated for disease bi-weekly using a modified Horsfall-Barratt scale. Plots were machine-harvested on 22 Sep and tubers were graded for size and yield. No tuber early blight was observed and the specific gravities of tubers from top yielding programs were not significantly different. Programs that had the lowest Area Under the Disease Progress Curve values were the highest yielding. The highest yielding program was the Wisconsin conventional grower standard. Organic treatments were ineffective. Several newer chemistries and modified standard programs were effective. At this time, and in the registration pipeline, there are excellent fungicides for the control of potato early blight that will contribute to good fungicide resistance management practices.

The ectomycorrhizal fungus, *Sebacina vermifera*, imparts drought tolerance to the bioenergy crop switchgrass (*Panicum virgatum* L.)

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Drought is one of the most significant abiotic constraints limiting crop production worldwide. Mycorrhizal fungi have been shown to provide various fitness benefits to their host plants, yet their role in drought tolerance has been largely overlooked. This study investigates the drought tolerance imparted by an ectomycorrhizal fungus, *Sebacina vermifera*, to the important bioenergy crop switchgrass. An in vitro experiment revealed that switchgrass seedlings co-cultivated with *S. vermifera* performed well under mild drought stress, producing up to 63% higher biomass than mock-inoculated seedlings. Greenhouse experiment revealed that co-cultivated plants produced significantly taller plants (84%) with higher shoot (219%) and root biomass (162%) than mock-inoculated controls ($P \leq 0.01$). Most significantly, co-cultivated plants under drought produced 120% and 156% higher shoot and root biomass, respectively, than mock-inoculated well-watered plants ($P \leq 0.01$). The total acquisition of primary and macronutrients (magnesium and sulfur) was significantly higher in co-cultivated plants compared to mock inoculated controls however, latter had equal or higher concentrations of these nutrients. The implications of these findings on the sustainable production of this important bioenergy crop switchgrass will be discussed.

Toward the development of integrated pest management (IPM) packages for tomatoes and other vegetable crops in West Africa

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Phytopathology 101:S60

Vegetable crop production is West Africa is typically done by smallholder farmers, many of which are women. Yield potential is limited by numerous

diseases, insect pests and weeds, as well as lack of access to improved varieties and technologies. The USAID-funded IPM-CRSP project in West Africa has made considerable progress in identifying disease and insect pest constraints on vegetable production. Through the plant virus global theme project, viruses causing diseases of a number of vegetable crops have been identified, including whitefly-transmitted viruses that limited tomato production in Mali and other West African countries. The international plant diagnostic network global theme has conducted a series of regional workshops on pest detection and diagnosis, and established an internet-based distance diagnostic network that allows for photographs of suspects pest problems to be sent to experts located throughout the world. This information together with results of surveys conducted about small farmers vegetable crop production practices are now being used to develop comprehensive IPM packages for key vegetable crops, including cabbage, potato and tomato. The package for tomato produced in the dry season that is presently being evaluated in Mali and Ghana will be presented. These IPM packages will hopefully be adopted by selected farmers and serve as models for other farmers and crops.

Identification of phytopathogenic fungi associated with giant miscanthus in Mississippi

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Phytopathology 101:S61

Giant miscanthus (*Miscanthus* x *giganteus*) is a tall, perennial C4 grass that is commercially produced as a bioenergy crop. In 2010, foliar diseases were observed in ten-year-old research plots in Starkville, Mississippi. A study was initiated to identify selected fungi associated with foliar symptoms of giant miscanthus clones 'Freedom' and 'Illinois' through classical techniques. Mature leaves showing symptoms of small, localized, elliptical lesions with straw-colored centers and reddish-brown margins were selected for fungal isolation. Lesions were excised from mature leaves and surface disinfested with NaOCl prior to being plated onto water agar. Fungal hyphal tips were transferred and cultured on water agar to facilitate identification based on reproductive structures. The predominant fungi isolated from foliar lesions of giant miscanthus were *Alternaria*, *Bipolaris*, *Curvularia*, *Nigrospora* and *Phoma*-like species. The presence of these fungi indicates possible pathogenic interactions capable of inciting disease on giant miscanthus. Confirmation of identity through DNA sequencing and pathogenicity of the fungi are being conducted. Further investigation is warranted to better understand the relationship between these fungi and giant miscanthus.

Foliar diseases identified on switchgrass in Mississippi

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Phytopathology 101:S61

Switchgrass (*Panicum virgatum*) is a warm season perennial grass native to areas in North America including the Black Belt of Alabama and Mississippi. Switchgrass is beneficial for wildlife habitat and is considered a primary renewable bioenergy source. Collections of foliar diseases that included leaf rust, anthracnose, and leaf spot from 'Alamo' switchgrass research plots at Mississippi State University were made in late summer 2009 and 2010. Symptomatic tissues were surface disinfested, plated onto water agar and incubated five days. Hyphal tips of fungi colonizing infected tissues were transferred to water agar. Light microscopy was used to identify fungi based on reproductive structures. *Colletotrichum* sp. was isolated from broad, elliptical lesions with reddish borders and light gray centers with acervuli and setae present. *Bipolaris oryzae* was identified from short, narrow, elliptical lesions with black borders and dark brown centers. Leaf rust, caused by *Puccinia emaculata*, was diagnosed on switchgrass leaves observed under light microscopy based on pustules filled with urediniospores and was co-colonized by a biotrophic mycoparasite, *Darluca* sp. Further studies will be conducted to confirm the pathogenicity of these fungi associated with foliar diseases of switchgrass in Mississippi.

Monitoring sugarcane rust spore concentrations by real-time qPCR and passive spore trapping

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Phytopathology 101:S61

Two rusts are recognized in sugarcane, brown rust caused by Puccinia melanocephala and orange rust caused by P. kuehnii. Both are economically important and limit production in many sugarcane industries. Sugarcane rust epidemics vary greatly in timing and severity, thus they remain difficult to predict. This hampers efficient control using chemical or cultural methods. Real-time qPCR assays for both sugarcane rusts were applied to quantify the number of spores captured on passive spore traps in the 2010 growing seasons in Florida and Louisiana. The limit of detection was found to be a single spore on each trap. Large fluctuations in the number of orange rust spores were observed throughout the Florida growing season with spikes evident in June, September and October. These coincided with favorable temperatures and rain events and preceded increases in the severity of orange rust symptoms on susceptible cultivars. The greatest number of orange rust spores detected in Florida occurred in October and coincided with the detection of spores in Louisiana. Symptoms of orange rust have not been reported in Louisiana and this is the first evidence that the pathogen is present in the state and suggests it was transmitted from Florida. These results show the value of combined real-time qPCR and passive spore traps for monitoring temporal and spatial differences in sugarcane rust spore concentrations and provide an important tool for predicting disease epidemics in the future.

Engineering resistance in cotton by RNAi mediated silencing of parasitism genes of *Meloidogyne incognita*

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Phytopathology 101:S61

In cotton the yield losses due to root-knot nematode are estimated to be in the range of 10–27%. A recent survey in cotton growing areas of north India has revealed widespread infestation of root-knot nematode in Bt cotton. The current battery of candidate nematode specific parasitism proteins secreted by nematodes which can be used for RNAi-mediated nematode management include CAzymes, cellulases, xylases, expansins, chorismate mutase, proteases, galactouronase, pectate lyase, *nodl* like and pioneer genes(27) unique to *Meloidogyne sp.* At CICR Nagpur, work has been initiated on RNAi-mediated protection of cotton against root-knot nematode. Ten sets of primers complementary to the conserved regions of 10 key parasitism genes were synthesized and used for amplification of specific sized amplicons. Evaluation of dsRNA for ten parasitism genes viz. Chitin binding protein, Cysteine protease, Chitin synthase, Integrase, Pectate lyases protein 40, aminopeptidase, polygalactouronase, 16D19, calreticulin was done against rooknot nematode penetration. dsRNA for polyglactouronase reduced rootknot nematode penetration by 69% compared to control. For pectate lyase the reduction in penetration was in range of 58–74%. However, this reduction in nematode penetration did not translate in proportional reduction in final population build up. Concomitant use of dsRNA of two parasitism genes resulted in significant reduction in penetration as well as final nematode buildup and can be used as potential management option.

Vitis californica* and *Vitis californica x Vitis vinifera* are hosts for *Grapevine leafroll-associated virus-2* and *-3*, and *Grapevine virus A* and *B

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The objective of this research was to determine if native *Vitis* are alternate *Grapevine Leafroll associated Virus* (GLRaV) hosts that might serve as reservoirs important in the continued spread of grapevine leafroll disease. One hundred fifty two *Vitis* samples surrounding nine Napa Valley vineyards were collected and tested for GLRaV-1 to 5 and -9, *Grapevine virus A* (GVA), *Grapevine virus B* (GVB), and *Grapevine virus D* (GVD) using both conventional and realtime RT-PCR. Twenty four *Vitis* samples from three riparian areas not near vineyards were also included. DNA fingerprinting indicated that the *Vitis* samples consisted primarily of *V. californica* followed by *V. californica* × *V. vinifera* hybrids. GVA and/or GLRaV-3 were detected in 53% to 80% of the *V. californica* and *V. californica x V. vinifera* hybrids adjacent to three of the nine vineyards. In two riparian areas not near vineyards, three of the 21 *V. californica* samples were positive for GLRaV-2, GLRaV-3, GVA, and GVB. At the third riparian site, all three *V. californica x V. vinifera* samples were positive for GLRaV-2 and GVB. Phylogenetic analysis of GLRaV-2 and -3 partial coat protein gene nucleotide sequences indicated the isolates from *V. californica* and *V. californica x V. vinifera* hybrids were closely related to *V. vinifera* isolates. Although we cannot conclude anything about GLRaV-3 transmission between *V. vinifera* and native *Vitis*, we did identify a GLRaV-3 reservoir within a 2 km region of Napa County.

Evidence of root graft transmission of two rose mosaic viruses, *Prunus necrotic ringspot virus* and *Apple mosaic virus* in rose rootstocks

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Phytopathology 101:S62

Rose mosaic disease is often caused by *Prunus necrotic ringspot virus* (PNRSV) and *Apple mosaic virus* (ApMV). It is primarily spread by propagation; observations indicate a means of natural spread. Cuttings from two rootstocks, *Rosa hybrida* 'Dr. Huey' and *R. multiflora* 'Burr', with and without virus were rooted and transplanted to pots. Viruses were: ApMV, PNRSV and a natural infection of ApMV + PNRSV. Treatment pots contained one virus-positive and one virus-negative plant in the same pot to permit root grafting. Control pots contained one virus-positive or one virus-negative plant; pots were arranged to allow stem contact. All virus-negative plants had a possibility of becoming infected by pollen or insect transmission. Dr. Huey included 60 virus-negative and 60 virus-positive control pots; and 120 treatment pots. *R. multiflora* included 20 virus-negative and 20 virus-positive control pots; and 20 treatment pots with ApMV + PNRSV. All plants were ELISA tested for 5 years. All virus-negative plants in control pots tested negative all 5 years. The percent of initially virus-negative plants in treatment pots that tested positive was 0, 5.3, 10.5, 10.5, 10.5 in Dr. Huey and 0, 12.5, 33.3, 46.7 and 46.7 in *R. multiflora* for years 1 to 5 respectively. All plants that became infected were potted with ApMV + PNRSV plants. Rose mosaic symptoms were observed only in plants with ApMV + PNRSV. This indicates that root grafting plays a role in spread of rose mosaic disease.

Report of chlorotic ringspot disease on peanuts caused by Tomato yellow fruit ring virus in Iran

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Phytopathology 101:S62

Tomato yellow fruit ring virus (TYFRV; genus *Tospovirus*, family *Bunyaviridae*) is considered as new emerging tospovirus in the mid-Eurasian region of Iran. In the present work, a tospovirus was isolated by mechanical sap transmission from peanut plants showing chlorotic ringspot symptoms and identified as TYFRV based on biological, serological and molecular studies. In host range studies, a wide range of indicator plants, including members of Amaranthaceae, Chenopodiaceae, Cucurbitaceae, Fabaceae and Solanaceae, closely similar to that of the N-biotype isolates of the virus, was infected. The isolates under study strongly reacted with TYFRV antibodies, but not with the specific antibodies of the other tospoviruses tested. The nucleotide sequences of nucleoprotein (N) genes of three isolates also were determined and compared with the sequences previously reported in the GenBank. BLAST search results confirmed that these sequences corresponded to the TYFRV N gene. Computer analysis of theses sequences revealed 97.0–99.5% and 98.2–99.3% identities at nucleotide and amino acid sequence levels, respectively. Neither of the TYFRV isolates seemed to be recombinant. Phylogenetic analysis of the nucleotide sequences using maximum-likelihood and neighbor-joining algorithms revealed that the Iranian isolates fell into the IRN-2 genogroup of the virus. To our knowledge, this study reports for the first time the biological and molecular properties of TYFRV isolates from peanut in the mid-Eurasia of Iran.

Fungal and bacterial diversity differ in their responses to fallow period in the Bolivian highlands

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Traditional fallow periods in the Bolivian highlands are being shortened in an effort to increase short-term crop yields, with potential long-term impacts on soil communities. Using 454-pyrosequencing, we characterized fungal and bacterial to responses to (1) the length of fallow period and (2) the presence of plants *Parasthrephia* sp. or *Baccharis* sp. (both locally known as 'Thola'), locally considered beneficial to soil health. The two study regions, Umala and Ancoraimes, differ in their soil characteristics, which may be a fundamental reason for the inherent differences in regional management practices. Soils in Ancoraimes have higher levels of organic matter, nitrogen and other macronutrients. These soils supported more diverse fungal communities, whereas Umala had more diverse bacterial communities. Unexpectedly, the longer fallow periods were associated with lower fungal and bacterial richness and diversity. Fungi such as *Bionectria* and *Chaetomidium* and bacteria such as *Thermofilum* decreased in abundance with longer fallow period. The presence of Thola after ten years of fallow had a positive effect on soil fungal diversity, but did not change the bacterial diversity. Our results suggest that fallow period has a wide range of effects on microbial communities, and that plant cover may be important in conserving some microbial communities.

Biocontrol potential and plant growth promotional activity of actinomycetes isolated from various herbal vermicomposts

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There is a growing interest in the use of secondary metabolites, such as toxins, proteins, hormones, vitamins, amino acids and antibiotics, from microorganisms, particularly from actinomycetes, for the control of plant pathogens as these are readily degradable, highly specific and less toxic to nature. It is a well-known fact that actinomycetes are found most common in compost and plays an important role not only in the decomposition of organic materials but also in their ability to produce secondary metabolites of commercial interest. Hence, in the present investigation, several herbal vermicomposts were screened for actinomycetes that contain antagonistic potential against *Fusarium* wilt and collar rot of chickpea (caused by *Fusarium oxysporum* f. sp. *ciceri* [FOC] and *Sclerotium rolfsii*, respectively) and charcoal rot of sorghum (caused by *Macrophomina phaseolina*). Fourteen most promising antagonistic actinomycetes were characterized for their biocontrol and plant growth promoting traits and further evaluated for their ability to suppress FOC, *S. rolfsii* and *M. phaseolina* under both green house and field conditions. The present study was successful in selecting effective actinomycetes that can be the potential candidates for discovery of novel secondary metabolites for various biological applications.

Emergence of a plant pathogen via hybridization of the Irish famine pathogen, *Phytophthora infestans*, and an unknown related species

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The global movement of plant pathogens threatens natural ecosystems, food security, and commercial interests. Introduction of a plant pathogen to new geographic regions has been the primary mechanism by which new pathogens have emerged. Another documented mechanism for the emergence of plant pathogens is hybridization between individuals of different species or subspecies. We investigated the genetic origin of *Phytophthora andina*, an increasingly common pathogen of Andean crops *Solanum betaceum*, *S. muricatum*, *S. quitoense*, and several wild *Solanum* spp. in Colombia, Peru, and Ecuador. We cloned four nuclear loci to obtain haplotypes and using these loci inferred the phylogenetic relationships of *P. andina* to the potato late blight pathogen *P. infestans* and other related species. Sequencing of cloned PCR products revealed two distinct haplotypes for each locus in *P. andina*, such that each isolate had one allele derived from a *P. infestans* parent and a second divergent allele derived from an unknown species that is closely related but distinct from *P. infestans*, *P. mirabilis*, and *P. ipomoeae*. To the best of our knowledge, the unknown parent has not yet been collected. We also observed sequence polymorphism among *P. andina* isolates at three of the four loci, many of which segregate between previously described *P. andina* clonal lineages. These results provide strong support that *P. andina* emerged via hybridization between *P. infestans* and another unknown *Phytophthora* species.

Development of a PCR based assay for detection of resistance to QoI fungicides in *Asochyta rabiei*

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Ascochyta blight of chickpea (*Cicer arietinum*) caused by the fungal pathogen *Ascochyta rabiei*, is an economically important disease in chickpea. In United States and Canada, management of this disease is dependent on fungicide applications. Azoxystrobin and pyraclostrobin are two quinone-outside inhibitors (QoI) that work by blocking the cytochrome (cyt) bc1 complex (complex III) in the mitochondrial respiration chain and can be used to manage this disease. Resistance to QoI can develop due to single point mutations in the *cyt b* gene. In the U.S., QoI resistance was first detected *A.*

rabiei populations in North Dakota in 2005, and has since been reported in other states. Currently, resistance monitoring of *A. rabiei* isolates involves an *in-vitro* spore germination assay which can be very laborious and time-consuming. Our goal was to identify the mutation associated with QoI resistance in this region and to develop a PCR based assay for identification of resistant isolates. Cloning, sequencing and multiple sequence alignment of a fragment of the *cyt b* gene from QoI sensitive and resistant isolates of *A. rabiei*, revealed that a point mutation in the codon 143 (G143A) was responsible for resistance. A diagnostic test was developed based on this mutation using a mismatch amplification mutation assay (MAMA) with allele-specific reverse primers for screening QoI sensitive and resistant isolates of *A. rabiei* and is being used for evaluating field isolates.

Engineering *Grapevine fanleaf virus* into a plant expression vector

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Functional genomics studies in grapevine are hindered by the lack of a suitable viral vector for virus-induced gene silencing or protein overexpression. *Grapevine fanleaf virus* (GFLV) has attributes that could make it a worthy grapevine vector including a small bipartite RNA genome, no phloem restriction, the availability of functional cDNA clones, and known coat protein residues that abolish nematode-mediated transmission. cDNA fragments corresponding to full-length GFLV RNA1 and RNA2 were cloned into a *Cauliflower mosaic virus* 35S expression cassette and into a binary vector suitable for *Agrobacterium tumefaciens*-mediated delivery. Agroinfiltration of these constructs into *Nicotiana benthamiana* initiated GFLV systemic infection. Reassorting two GFLV isolates' (GHu and F13) bipartite genomes has yielded different frequencies of systemic infection and the concomitant application of heterologous viral RNA silencing suppressors has provided new insights into the infection process. Fluorophor-tagged GFLV clones are being tested as a proof of concept for the stable expression of foreign genes in plants. Wild-type and recombinant GFLV are currently being delivered to grapevine.

Spatial dynamics of *Plum pox virus* in *Prunus* spp. in Ontario and Pennsylvania

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Phytopathology 101:S63

Understanding the spatial dynamics of a disease epidemic can provide important information concerning the development of appropriate sampling designs to optimize detection efficiency. The objective of this project is to quantify the spatial dynamics of *Plum pox virus* epidemics in *Prunus spp*. in Ontario, Canada and Pennsylvania. Spatial dependence was measured using a modified form of Ripley's K function. In Pennsylvania, spatial dependence among PPV-positive *Prunus spp*. blocks ranged from 0.7 to 4.3 km, whereas in Ontario, spatial dependence ranged from 1 to 25 km. Spatial analyses also revealed that PPV-positive blocks were clustered around PPV-positive blocks that had tested positive for PPV the previous year. Within *Prunus* blocks, PPV-positive trees had a random spatial pattern in most blocks (9 of 12 blocks), while the pattern was clustered in some blocks (3 of 12). Because PPV-positive trees are sometimes clustered within blocks, a systematic sampling design with multiple sampling arms should be used because this sampling design can accommodate both random and clustered spatial patterns.

Identifying resistance to white mold in annual bedding plants

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Phytopathology 101:S63

White mold, caused by *Sclerotinia sclerotiorum*, is a serious disease of annual bedding plants resulting in stem rot, wilt and death. Ornamental beds infested with this pathogen often lose significant numbers of plants year after year. Fourteen genera of annual bedding plants were identified as having no reported susceptibility to white mold. One to three varieties per genus were inoculated with a mycelial plug of *S. sclerotiorum*, incubated in a dew chamber for 48 hrs, and then maintained at 18C for 4 weeks. Disease severity was rated as percent stem infection for plants with a single primary stem and percent plant infected for bunch grasses. Percent of rot in bulbs, rhizomes or corms was recorded where appropriate. Disease severity varied greatly among genera. *Portulaca*, *Pentas*, and *Scaevola* had greater than 50% stem infection by day 14. *Impatiens* became infected but abscised the diseased stem and recovered. *Acorus* developed a slow rhizome rot. *Penistetum* did not exhibit symptoms of white mold, but sclerotia and mycelia were detected between the stem and leaf sheath. *Caladium* developed soft rot in the corm, and entire plants wilted and died. *Colocasia* bulbs and *Canna* rhizomes were free of rot, and leaf necrosis was usually restricted to the inoculation site. *Juncus*, *Carex*, *Cyperus*, *Setaria* and *Scirpus* developed no disease symptoms. Eight of the fourteen genera were identified as potentially resistant to white mold and will be field tested.

Global phenotypic variation in *Phytophthora capsici*

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Phytopathology 101:S63

To determine global phenotypic variation, 124 *P. capsici* isolates from 12 countries were characterized for sporangial length and width, pedicle length, oospore diameter, sporangial and chlamydospore production, and growth at 32, 35, and 38°C. Sporangia were 23 to 35 µm wide and 38 to 60 µm long; differences in width and length were noted when isolates were grouped by genetic cluster and continent of origin. Length:breadth ratio (1.34 to 2.07) and pedicle length (20 to 260 µm long) varied widely among isolates; differences were apparent by continent and host family of origin. Oospore diameters varied among isolates (22 to 37 µm), but no differences were noted by isolate genetic cluster, host family of origin, continent of origin, mating type, or sensitivity to mefenoxam. Differences in sporangial production were observed among isolates grouped by continent, and tropical isolates produced fewer sporangia than isolates from vegetable hosts. When cultures were incubated in liquid medium, 35 P. capsici isolates formed chlamydospores. Growth at high temperatures did not reliably separate *P. capsic*i from *P. tropicalis* in this study. The results of this study indicate that separation of *P. capsici* from closely related *Phytophthora* species based on morphological and physiological characters alone could be misleading.

Differences in virulence of *Phytophthora capsici* isolates from a global collection

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*Phytophthora capsic*i causes root, crown, and fruit rot of vegetable and tropical hosts. Cucumber, zucchini, tomato, and pepper fruits were inoculated using 6-mm-diameter agar plugs of *P. capsici*, incubated in clear plastic boxes at room temperature (~26°C and 100% relative humidity), and virulence was estimated by measuring the lesion diameter three (cucumber, zucchini) or four (tomato, pepper) days later. When isolates were grouped by genetic cluster, differences in virulence were observed for cucumber and zucchini. On tomato, no significant differences were observed for isolates grouped by genetic cluster, but isolates from vegetable crops were generally more virulent than isolates from tropical hosts. No significant differences in lesion diameter were noted on pepper when isolates were grouped by host family of origin or genetic cluster membership. Our findings suggest that isolate characteristics such as host family of origin and genetic cluster membership may be used to guide initial isolate selection for cucurbit fruit resistance screening. Final isolate selection should incorporate the phenotypic and genetic diversity of *P. capsici*, including isolates with differing virulence to the host organ of interest.

Exploring the insect vector-virus interactome using co-immunoprecipitation coupled to mass spectrometry

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Phytopathology 101:S63

Aphid transmission of Potato Leaf Roll Virus (PLRV) requires virions to be internalized into aphid midgut and accessory salivary gland cells, and to survive in the hemolymph suggesting multiple aphid proteins interact with PLRV. We used co-immunoprecipitation to isolate aphid-virion protein complexes, separate by 1-D SDS-PAGE, and then analyze by nanoscale reverse phase chromatography and tandem mass spectrometry. Over 52 aphid proteins were identified with high statistical confidence ($P < 0.01$) that interact either directly, or in complex, with purified virions with high affinity. Twelve proteins were enriched more than 10-fold and another 12 proteins were specifically co-immunoprecipitated by purified virus. Ppreviously, we reported that some of these 52 proteins were differentially expressed in vector and non-vector aphids. Using orthogonal approaches we identified all aphid proteins reported by others to interact with luteoviruses including actin, GAPDH, and RACK-1, but we did not identify the bacterial endosymbiont protein symbionin (GroEL). Additional proteins identified in this study include virus receptors, cytoskeletal proteins, vesicle trafficking proteins, chaperones, signaling proteins, proteins that modify insect feeding behavior, and enzymes involved in aphid metabolism. These proteins may function at various steps in the circulative transmission pathway to promote virion internalization, translocation in aphid cells, and transmission to new hosts.

De novo generated eIF4E resistance genes protect potato from infection by Potato virus Y

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Natural mutations in translation initiation factor eIF4E confer resistance to potyviruses in many plant species, but no known eIF4E-mediated resistance genes have been identified in potato. We have converted a susceptible potato ortholog of an eIF4E virus resistance gene from pepper, known to confer resistance to Potato virus Y (PVY), into a de novo allele for resistance to PVY using site-directed mutagenesis. Potatoes were transformed to over-express the mutated potato alleles and tested for resistance to PVY. One of the de novo alleles conferred resistance to all strains of PVY when plants were mechanically inoculated in the greenhouse. Two years of field trials were completed and several lines expressing the de novo allele remained virus free following extreme natural virus inoculum pressure that resulted in >80% of the control plants becoming infected. The resistance was stable through three generations, the plant growth and yield characteristics were similar to untransformed controls, and none of the tubers contained virus. The use of natural or modified eIF4 resistance genes to disrupt a key step in the potyvirus infection process could potentially be used to engineer virus resistance in a number of economically important plant-viral pathosystems. Furthermore, the "intragenic" nature of this approach, whereby the transferred coding region is modified from a gene in the target crop, may be advantageous with respect to consumer acceptance.

The iron responsive sigma factor, AcsS, responsible for regulation of achromobactin biosynthesis in *Pseudomonas syringae* pv. *syringae* B728a

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Phytopathology 101:S64

Available iron is extremely limited in many bacterial environments, but is essential for growth and synthesis of many virulence associated factors. Therefore, the study of iron acquisition systems, such as iron-chelating siderophores, is critical to understanding bacterial pathogenesis. The genome of *P.s.s.* B728a encodes two siderophore systems, the fluorescent siderophore, pyoverdine, and the recently defined citrate siderophore, achromobactin (ACR). Directly upstream of the 18.4 kb ACR biosynthesis and secretion gene cluster is an extracytoplasmic function (ECF) sigma factor gene, *acsS*. Genetic and phenotypic analyses were performed using an *acsS* deletion mutant strain of *P.s.s.* B728a in low iron conditions, due to the iron responsiveness of this sigma factor. Illumina RNA-Seq analysis of the *acsS* mutant strain revealed several hundred differentially expressed gene targets, with the ACR biosynthesis gene cluster showing the largest fold change when compared to the wild type B728a strain. Additional studies confirmed that ACR biosynthesis is regulated by the AcsS sigma factor. The deletion of *acsS* also negatively impacted the transcription of numerous flagellar genes and the predicted non-ribosomally synthesized antimetabolite toxin, mangotoxin. Characterization of the regulatory network controlled by AcsS will contribute to understanding the ACR siderophore system and the role it plays in the *P.s.s.* B728a lifecycle.

European nanoviruses: Identification of three new species and new DNA components

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Phytopathology 101:S64

The genome of viruses of the genus *Nanovirus* (family *Nanoviridae*) is typically formed by a set of eight circular single-stranded DNAs, each of which is ~1 kb in size and individually encapsidated in unusually small virions (~18 nm). When characterizing viruses of pea crops in Germany in 2009, we identified a hitherto undescribed nanovirus. Its eight DNA components were sequenced, and clones thereof served to reconstitute an infectious and aphid-transmissible nanovirus. Since this virus differed from known nanoviruses by ~40% in nucleotide sequences we named this new nanovirus species pea necrotic yellow dwarf virus (PNYDV). Analysis of about 100 symptomatic pea plants collected in Austria, Hungary, Serbia and Sweden in 2010 revealed that >50% of these plants were infected by PNYDVs. Moreover, we identified two further nanovirus species in Europe, which differ in CP amino acid sequences from other nanoviruses by >40%. When sequencing the genomes of these nanoviruses, two strikingly distinct variants of DNA-U2 were encountered in each isolate. In addition to a genetically diverse range of hitherto undescribed paraRep-encoding DNAs ('alphasatellites') that we found associated with European nanovirus isolates, we identified a small (503 nts) DNA component as a satellite DNA from 9 of 16 PNYDV isolates from Austria. Our data suggest that nanoviruses are more numerous andwidespread in the Old World than originally thought and show new features in genome organization and association with satellite DNAs.

New race of *Phytophthora sojae* in southern Buenos Aires province (Argentina)

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Phytopathology 101:S64

Phytophthora root rot of soybean caused by *Phytophthora sojae* is one of the most important diseases in Argentina. Physiological race 1 was identified in the country in 1991 and six years later pathogen variability was detected. Two plants of one supposedly resistant cultivar with typical symptoms of the disease were collected from soybean fields in Tandil (Buenos Aires province) The objective of this study was to report the presence of *P. sojae* and to determine whether a new race had broken the resistant genes. The isolation was made from the margin of stem lesions, plated on V8 juice medium with antibiotics and fungicides. Cultural characteristics showed identical patterns to those described for *P. sojae*. The ITS rDNA region was amplified (ITS5/ITS4), sequenced and BLAST aligned with the NCBI and a high similarity with *P. sojae* strains was verified. In addition, so as to identify the physiological race, the isolate was inoculated by the hypocotyl technique in eight differential soybean isolines: HARO (1-7)1 (rpsrps), HARO 1272 (Rps1a, Rps7), HARO 13 (Rps1b), HARO 14 (Rps1d), HARO 15 (Rps1k), HARO 3272 (Rps3a, Rps7), HARO 6272 (Rps6, Rps7) and Corsoy 79 (Rps1c). Race evaluation was recorded 5 days after inoculation. The virulence/avirulence reaction was: 7,1a, 1c, 1d, 1k and 3a/6, 1b, corresponding to a new race of the pathogen, capable of defeating Rps1-k and Rps1-c, the major genes used for control of this disease in Argentina. This is the first report of *P. sojae* in the southern soybean area of Argentina.

Top rot form of red strip caused by *Aciovirax avenae* subsp. *avenae* in Louisiana sugarcane

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Phytopathology 101:S64

Red stripe of sugarcane caused by *Acidovirax avenae* subsp. *avenae* consists of two forms – leaf stripe and top rot. Symptoms of red stripe in Louisiana over the past 25 years have been limited to the leaf stripe form which causes no apparent yield loss. During 2010, the more severe top rot form was observed in several commercial sugarcane fields. Both forms were found, either separately or together. Two fields of cultivar HoCP 00-950, one plant-cane (PC) crop and one first-ratoon (FR) crop, affected by top rot were subdivided into 113 and 84 plots, respectively. In the PC test, plots with >20% affected stalks averaged a 5%, 10%, and 14% loss of tonnes cane/hectare, kg sugar/tonne, and kg sugar/hectare, respectively. In the FR test, the infection level was lower and a 10% loss threshold was utilized, resulting in a 1%, 4% and 4% loss of tonnes cane/hectare, kg sugar/tonne, and kg sugar/hectare, respectively. A disease incidence, nitrogen fertility rate, and soil texture interaction was noted in plots of nitrogen fertility rate experiment. Incidence was higher among plots in heavy clay soils verses lighter, more silty soils. Disease incidence increased with increasing rates of added nitrogen in the heavy clay soil compared to the control, no nitrogen added plots. In the lighter soil, disease incidence was higher among treatments with added nitrogen compared to the control, but incidence did not differ among the different rates of added nitrogen fertilizer.

The effects of swathing versus straight-cut combining on FHB DON accumulation in barley

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Phytopathology 101:S64

Fusarium head blight (FHB) frequently reduces barley quality, due to the occurrence of deoxynivalenol (DON) mycotoxin. Barley is susceptible to *Fusarium graminearum* infection and DON formation from head emergence until harvest. The fungus may colonize the barley kernel if dew periods favor fungal growth. Because barley often matures unevenly, it maybe swathed (windrowed) to accelerate crop maturity and drying. However, barley in a swath may have additional high humidity if rainfall occurs, favoring fungal growth and DON production. To test this, additional mist irrigation was applied after Feekes11.3 to swathed plots and plots left for straight cutting

over a five year period. Plots were arranged in a split plot design and treatments were applied to Conlon and Robust barley cultivars. The data indicated that for individual years barley type had no significant affect on DON accumulation and DON levels were only significantly different in one year for swathed verse straight-cut plots. Misted plots had significant difference in DON in three out of five years. When years were combined the data indicated swathed and straight-cut plots with added mist irrigation had significantly higher DON than those without mist. Barley type and combined type treatments had no effect on DON. Our results indicated that post-dough (Feekes11.3) occurrence of rainfall may have more significant influence on DON accumulation than swathing or straight-cut practice or barley type.

Cytological alterations in *Gibberella zeae* germlings induced by combinatorially selected defense peptides

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Phytopathology 101:S65

Head blight of wheat, caused by the fungus *Gibberella zeae*, results in reduced grain yield and mycotoxin accumulation. Germplasm with partial resistance to blight is available but efforts are focused on the development of wheat with engineered defense. Previously, we identified peptides from combinatorial phage-display libraries that disrupt ascospore growth and development. If expressed in wheat, these peptides could complement partial resistance. We have initiated studies on the effects of defense peptides on endocytosis, a process cells use for uptake of molecules from the surrounding environment. Endocytosis is central to proper apical growth of fungi. We used the membrane-selective dye, FM4-64, to stain components of the endocytic machinery in *G. zeae*. In germlings derived from ascospores germinated overnight, FM4-64 rapidly stained in succession the plasma membrane, early endosomes, vesicles, and vacuoles. Cells within ascospores and basal hyphae typically contained a single vacuole, comprising most of the cell space, while apical cells contained smaller vacuoles. In germlings grown similarly but in the presence of a defense peptide, FgF3A, FM4-64 staining revealed much smaller and many more vesicles throughout germlings and non-germinated cells of ascospores. Endocytic alterations induced by defense peptides are being compared to the abnormal germling morphologies that they induce, including isotropic swelling of non-germinated ascospore cells, and irregularly shaped germtubes and hyphae.

***Phytophthora obscura* sp. nov. defines a novel *Phytophthora* subclade 8d**

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Phytopathology 101:S65

We describe a new *Phytophthora* species detected in the U.S.A. infecting foliage of *Kalmia latifolia* and in substrate underneath *Pieris* and in Germany in soil samples underneath *Aesculus hippocastanum*. The species *P. obscura* sp. nov. is named based on phylogenetic analysis, host range, Koch's postulates and morphological examination. *P. obscura* is homothallic with paragynous antheridia and semipapillate sporangia. *P. obscura* is genetically closely related to *P. austrocedrae* and *P. syringae*. Furthermore, *P. obscura*, *P. austrocedrae* and *P. syringae* define a new subclade 8d with significant support for all genetic loci analyzed. Horse chestnut, kalmia, pieris and rhododendron could all be infected with this pathogen. Koch's postulates were confirmed for kalmia.

Management of onion bacterial diseases using alternative mulches and plant spacing

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Phytopathology 101:S65

Onions are plagued by a number of bacterial pathogens that cause bulb rots and leaf decay. During the past five years, fresh market sweet onion growers in Pennsylvania and New York have lost $3,500 to $7,000 per acre annually as a result of reduced onion quantity and quality due to diseases caused by the center rot pathogens *Pantoea ananatis*, *P. agglomerans*, the soft rot pathogens, *Pectobacterium caratovora* and *Pseudomonas marginalis* and sour skin caused by *Burkholderia cepacia*. In on-farm trials, reducing onion plant spacing from the grower standard of 91-cm^2 (4 rows per bed with 15-cm plant spacing in-row) in PA and 122-cm^2 in NY, to 81-cm^2 or 61-cm^2, in general, reduced the total number of leaves per plant and onion neck diameter, increased percent lodging and reduced bacterial disease incidence at harvest between 52 and 66% when conditions were favorable for disease. However, in some trials this came at the expense of an increased proportion of less marketable small to medium sized bulbs. Growing onions on alternative mulches compared to the grower standard black plastic mulch reduced soil temperatures on average by 1.4 to 5.7°C and reduced bacterial bulb decay between 59 and 75% for the metallic silver, black biodegradable and bare soil/no mulch treatments. Multi-factorial trials combining alternative mulches and plant spacing to reduce bacterial disease losses are planned for 2011.

Inducing of the systemic resistance against Fusarium crown and root rot of tomato (*Fusarium oxysporum* f. sp. *radicis-lycopersici*) by rhizobacteria

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Phytopathology 101:S65

Crown and root rot of tomato caused by *Fusarium oxysporum* f. sp. *radicis-lycopersici* (FORL) is a common disease in commercial greenhouses in Turkey. The aim of this study is to investigate the effect of rhizobacteria strains isolated from greenhouses on plant growth and biocontrol against to FORL by in vivo tests and molecular analysis. As plant material, one resistant (Bandita F_1) and one sensitive (Kardelen F_1) tomato varieties against FORL were used. In the first step, out of 30 rhizobacteria strains were tested for the effects of plant growth-promoting and biocontrol against FORL under in vitro conditions. According to results of in vivo tests, the most effective strains of rhizobacteria, which were effectively inhibited the growth of FORL (TR21/1, *Pseudomonas putida*) and showed growth-promoting effect (TR2/1, *Pseudomonas fluorescens bv3*) were selected for molecular analysis in order to explain their mode of action. For this purpose, we determined ACO1 (LEACO1; regulated by ethylene) gene expression profiles by RT-PCR method. According to the result of molecular tests, TR21/1+FORL application increased the ACO1 gene expression compared to control and only PGPR or FORL inoculated plants. The results of the study showed that PGPR and FORL applications have positive effects on ethylene biosynthesis mediation with ACO1 gene expression and this effect could be important for inducing the systemic resistance of tomato plants against FORL.

Plant and food biosecurity: A European Union Network of Excellence

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Phytopathology 101:S65

A European Union Network of Excellence (NoE) was recently established involving thirteen partner institutions from eight countries: Italy, France, Germany, United Kingdom, Hungary, Turkey, Israel and United States. The consortium will focus on biological threats which have the capacity to infect plants, damage agriculture production and ultimately have impact on food and feed at any stage in the supply chain. Funded by the European Commission's Programme Security, the NoE includes the following areas of emphasis (Work Packages): Epidemiology and crop biosecurity; Food biosecurity; Analysis of risks to European food systems and society from the intentional introduction of new pest and disease agents; Development and deployment of diagnostic and detection systems; Responder systems for eradication and containment; Training for plant and food biosecurity; Dissemination, awareness and communication. Funded for five years, the Network of Excellence will renew and reinforce a previously established European partnership on crop biosecurity. New countries, institutions and topics have been included with the aim of establishing a virtual Centre of Competence in plant and food biosecurity that will enhance preparedness and response capabilities. The ultimate goal is to develop the capability and capacity to prevent, respond and recover from a biological incident or deliberate criminal act threatening the European agrifood system.

Molecular characterization through IGS sequencing of *formae speciales* of *Fusarium oxysporum* pathogenic on lamb's lettuce and rocket

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Phytopathology 101:S65

Twenty-nine isolates of *Fusarium oxysporum* collected from wilted lamb's lettuce plants (*Valerianella olitoria*) and thirty-six *Fusarium oxysporum* isolates collected from wilted rocket plants (*Eruca vesicaria* L., syn. *E. sativa*, cv. 'Rucola coltivata'), including ATCC strains, were examined for differences in the nucleotide sequences of the ribosomal DNA (rDNA) intergenic spacer (IGS) region, about 2.5 kb long in the isolates analyzed. The isolates were tested for pathogenicity on lamb's lettuce or rocket in glasshouse. The results showed that the isolates were slightly, moderately and highly pathogenic except for four non-pathogenic isolates from lamb's lettuce. Most of the isolates from wilted rocket and lamb's lettuce plants collected from Italy were very similar to F. *oxysporum* f. sp. *raphani*. In conclusion, the analysis of the IGS sequences revealed that the isolates studied had different origins and that phylogeny and pathogenicity were related; non-pathogenic isolates differed genetically from those with low, moderate and high level of virulence. To our knowledge, this is the first report of differentiation of *formae speciales* of F. *oxysporum* on rocket and lamb's lettuce by IGS sequence analysis.

Cloning glucanase and chitinase genes from antagonistic yeasts for postharvest disease control

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Phytopathology 101:S66

Different yeast – including strains of *Pichia guilliermondii*, *Metschnikowia pulcherrima*, and *Metschnikowia fructicola* – have been isolated from the carposphere of fruit, selected for their antagonistic properties against different postharvest pathogens of apples and peaches, and studied for their biocontrol mechanism. Production of hydrolases is one of the components of the mechanism of action. An exo-1,3-beta-glucanase gene (PgExg1 gene) was amplified from the genomic DNA of the antagonistic yeast P. *guilliermondii* strain M8 and confirmed by Smart-RACE on the cDNA. Sequencing and nucleotide analysis showed that there were no introns inside the gene. An open reading frame (ORF) of 1,224 bp encoding a 408 amido acid protein with a calculated molecular weight of 46.9 kDa was characterized. Protein BLAST revealed that the gene belongs to the cellulose superfamily, and prediction of the deduced amino sequences suggested that the protein has a signal peptide. Similarly, the chitinase genes (*MpChi1* and *MfChi1* genes) were amplified from the genomic DNA of M. *pulcherrima* strain MACH1 and M. *fructicola* strain AP47, respectively. Nucleotide analysis showed lack of introns inside both genes. For *MpChi1*, an ORF of 1,080 bp encoding a 359 amido acid protein was characterized, while for *MfChi1*, an ORF of 1,098 bp encoding a 365 amino acid protein was characterized. Protein BLAST revealed that both genes belong to GH18-chitinase-like superfamily.

Selection of antagonistic yeasts for the control of *Salmonella enterica* serovar *typhimurium* on fresh cut lettuce

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Phytopathology 101:S66

Fresh-cut products represent a promising and innovative sector, responding to the customer needs and adapting to his lifestyle. Therefore, it is necessary to ensure the microbiological safety of fresh-cut products, because they go through different steps of processing and manipulation (cutting, washing and packing), for this reason they are more prone to contamination than whole products. *Salmonella enterica* is the most frequently isolated species in cases of foodborne infections. The complete removal or killing of this pathogen on fresh or fresh-cut products is currently a difficult task. In recent years, several chemical, physical and biological control methods have been developed to control *Enterobacteriaceae* on plant products. The aim of the present study was to evaluate and select some potential antagonist yeasts against *Salmonella enterica* serovar *typhimurium*. The antagonist yeasts tested were *Pichia guilliermondii* strain M8, *Metschnikowia pulcherrima* strains GS35, MACH1, BIO126, GS9, 3008 and 3345, *Hanseniaspora uvarum* strain SAL3, *Rhodotorula glutinis* strain PW8, *Rhodotorula mucilaginosa* strain PW34 and *Debaryomyces hansenii* strain AR37. Among the antagonists tested, the strain M8 showed a potential biocontrol potential against *S. typhimurium* at room and low temperature and after 17 or 24 hours of incubation.

Control of soil-borne plant pathogens by microorganisms isolated from suppressive composts

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Composts showed to be suppressive against several plant pathogens in various cropping systems. Microbiological composition is one of the most important aspect for compost suppressiveness. The objective of the present work was to isolate microorganisms from a suppressive compost and to test them for their activity against soil-borne pathogens. A compost originated from green wastes, organic domestic wastes and urban sludges that showed a good suppressive activity in previous trials was used as source of microorganisms. Serial diluted suspensions of compost samples were plated on five different media: selective for *Fusarium* sp., selective for *Trichoderma* sp., selective for oomycetes, potato dextrose agar (PDA) for isolation of fungi, lysogeny broth (LB) for isolation of bacteria. Colonies were isolated from plates and tested under laboratory conditions on tomato seedlings growing on perlite medium in Petri plates infected with *Fusarium oxysporum* f. sp. *radicis-lycopersici* and compared to a commercial antagonist (*Streptomyces griserovidis*, Mycostop, Bioplanet). Among them, those microorganisms showing a biocontrol activity were assessed also under greenhouse condition on three pathosystems: *Fusarium oxysporum* f. sp. *basilici*/basil, *Phytophthora nicotianae*/tomato and *Rhizoctonia solani*/bean. None of the microorganisms was able to control the three soil-borne pathogens and only a few to control *R. solani*.

Linkage analysis of soybean Phytophthora root rot resistance loci on chromosome 13

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Phytopathology 101:S66

Phytophthora root rot, caused by the plant pathogen *Phytophthora sojae*, is a limiting factor in the production of soybean worldwide. Genes that contributeto the expression of *Rps*-mediated and partial resistance toward *P. sojae* provide valuable breeding resources. *Rps3* and *Rps8* are two distinct soybean loci that mediate hypersensitive-response (HR) resistance against *P. sojae*. Both resistance genes have been previously mapped within the resistancegene-rich soybean chromosome 13. *Rps3* locus is believed to have three known alleles (*Rps3-a*, *Rps3-b*, and *Rps3-c*) each from a different source; PI 86972-1, PI 82.312N, and PI 340046 respectively. *Rps8* and each of the *Rps3* alleles mediate resistance to different isolates of *P. sojae*. Two $F_{2:3}$ mapping populations were recently generated to elucidate the genetic relationship and segregation pattern between *Rps3* alleles and *Rps8*. The first population originates from a cross between L83-570 (Containing *Rps3-a*) and PI 399073 (Source of *Rps8*), and the second originates from a cross between L92-7857 (Containing*Rps3-c*) and PI 399073. Linkage analysis within chromosome 13 was conducted through combining data from simple-sequence-repeat (SSR) marker polymorphisms, and soybean hypocotyl inoculation tests. Preliminary data from these two populations suggest that this region is segregating for both markers and resistance in a skewed manner, similar to previous studies on these loci.

The genome of Arachis hypogaea: Genetic linkage map will aid the whole genome sequence assembly

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Phytopathology 101:S66

The allotetraploid peanut genome assembly will be a valuable resource to researchers studying polyploidy species, in addition to peanut genome evolution and domestication other than facilitating QTL analysis and the tools for marker-assisted breeding. Therefore, a peanut linkage map will aid genome assembly, acting as an independent resource against which contig assembly can be validated. The objective of this study was to develop a comparative integrated map from two recombinant inbred line populations. A total of 4576 SSR markers from three sources: published SSR markers, newly developed SSR markers from ESTs and from BAC end-sequences were used for screening polymorphisms. Two CAP markers were also included to differentiate ahFAD2A alleles and ahFAD2B alleles. A total of 324 markers were anchored on this integrated map covering 1,352.1 cM with 21 linkage groups (LGs). Combining information from duplicated loci between LGs and comparing with published diploid maps, 7 homoeologous groups were defined and 17 LGs (A1 to A10, B1 to B4, B7, B8, and B9) were aligned to corresponding A-subgenome or B-subgenome of diploid progenitors. One reciprocal translocation was confirmed in the tetraploid cultivated peanut genome. Several chromosomal rearrangements were observed. This genetic

linkage map and others could provide a framework for QTL analysis and a scaffold for integration of the physical map and genome sequence assembly.

Recent advance of plant protection science in China

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Phytopathology 101:S67

Plant protection science in China is a comprehensive discipline involved in the study on biological characteristics of plant diseases, insect pests, weeds and rodents, and their interactions with the environmental factors, and development of IPM theory and technology. Chinese plant protectionists have been focusing on the strategic needs of modern agricultural development, the food safety, ecological safety, and income increase of farmers, and carrying on tradition, exploring and innovating, and tackling key problems collaboratively. Through crossover and mergence of different disciplines and ceaseless innovation of research technology and measures, the plant protection discipline has achieved the rapid development in research, discipline construction, personnel training, and establishment of scientific research bases. A series of important research achievements and breakthroughs in the branch of plant pathology, agricultural entomology, weed science, rodent control science, biological control, pesticide science, invasion biology and bio-safety on GMO supported by the national basic and applied basic research program, high-tech R&D, and applied technology research, have been made during the past five years. A systematic and comprehensive study on mechanisms of important agricultural pest outbreak, and the theory and technology of their prevention and integrated control would be highlighted in the future.

Characterization of the DSF-mediated quorum sensing regulon of *Xanthomonas citri* ssp. *citri*

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Phytopathology 101:S67

Xanthomonas citri ssp. *citri* (Xcc) is the causal agent of citrus canker disease. A diffusible signal factor (DSF), characterized as cis-11-methyl-2-dodecenoic acid, serves as a signaling molecule for the communication among Xcc cells. Three genes, *rpfF*, *rpfC* and *rpfG*, are involved in the DSF production, detection and signal transduction. Xcc cells use DSF-mediated quorum sensing to coordinate their gene expression and biofilm dispersal according to the local density of their population. To investigate the role of quorum sensing in citrus canker disease development and characterize quorum sensing regulon, deletion mutants of *rpfF*, *rpfC* and *rpfG* were constructed, and a time-course microarray was applied to analyze the differential gene expression profiles of those mutants and wild type strains in XVM2 medium mimicking the plant extracellular environment. Four hundred and forty-one genes showed differential expression with fold-change greater than 2 in at least one of the three mutants compared to wild type strain. These genes encode proteins and enzymes belonging to 19 functional categories such as production of extracellular enzymes and extracellular polysaccharides, chemotaxis, flagellum biosynthesis, transport and binding proteins. Quantitative real-time PCR is being utilized to further confirm the microarray results.

Effects of grapevine leafroll disease on berry anthocyanins and other flavonoids in a wine grape cultivar

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Phytopathology 101:S67

Red-berried grapevines (*Vitis vinifera*) affected with grapevine leafroll disease (GLRD) produce grapes with uneven ripening and poor color. We conducted molecular and biochemical studies on grapes harvested at different developmental stages from healthy and GLRD-affected (and tested positive for Grapevine leafroll-associated virus 3) wine grape cultivar (cv. Merlot) to gain a better understanding of events leading to these phenotypic changes. Total RNA extracted from berry skin was used in reverse transcription-quantitative real time polymerase chain reaction (RT-qPCR) assay, based on SYBR green detection, to study expression of flavonoid biosynthetic pathway genes. A set of reference genes were used for normalization of gene expression data obtained from gene-specific RT-qPCR assays. The overall results showed down-regulation of the pathway genes in berries from GLRD-affected grapevines during véraison and post-véraison stages. Estimation of total anthocyanins, flavonols and proanthocyanidins, and HPLC profiling of different classes of anthocyanins and flavonols further supported the molecular data that metabolism of different classes of flavonoids is altered during the development of berries produced by grapevines infected with GLRD.

Association mapping of stem rot resistance in a world collection of Brassica napus

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Phytopathology 101:S67

Sclerotinia sclerotiorum is a necrotroph fungus with a board host range. Genetic resistance to this pathogen is difficult to identify due to the quantitative nature of resistance and lack of appropriate screening methods. We have developed a reliable stem inoculation protocol for screening of Brassica napus. A world wide collection of ~400 B. napus accessions were screened by inoculating stems of flowering plants with mycelium of an aggressive isolate (# 321). Disease severity index (DI) consisted of lesion length and depth of stem penetration. DI ranged from resistant (DI = 7) to highly susceptible (DI = 250). A sub set of 200 lines were selected comprising resistant and susceptible lines from China, Pakistan, Korea, Japan, and Europe. In order to determine population structure within the lines and to relate the SNP data to existing molecular linkage maps, these lines are being screened with microsatellite markers most of which are publicly available. In future they will be used for association mapping using a newly developed B. napus Illumina Goldengate SNP array able to query 1,536 loci simultaneously. In addition, eight susceptible and eight resistant lines were selected for gene expression studies using deep RNA-seq data. The objective is to associate transcripts and/or markers with the resistant response and to determine the allelic relationship of QTLs or specific genes among the resistant B. napus lines we have identified so far.

Rapid field-deployable detection of *Ralstonia solanacearum* race 3 biovar 2 in environmental samples using magnetic bead separation and real-time PCR

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Phytopathology 101:S67

Ralstonia solanacearum race 3 biovar 2 (R3bv2) is a quarantine pathogen that causes wilting of potato, tomato, geranium and many other crops. Standard real-time PCR (RT-PCR) using R3bv2-specific primers is a sensitive, rapid, and reliable technique that detects as few as 1000 cells ml^{-1}. However, it cannot be directly implemented for detection of R3bv2 in complex environmental samples, such as plant tissues or soil, due to the presence of PCR-inhibitory compounds. Immunomagnetic separation (IMS) and magnetic capture hybridization (MCH) methods were developed to purify and concentrate *R. solanacearum* cells or DNA free from PCR inhibitors and nontarget cells or DNA. These methods utilize paramagnetic beads conjugated to *R. solanacearum*-specific rabbit antiserum (IMS) or a single-stranded oligonucleotide capture probe that specifically hybridizes to R3bv2 DNA (MCH). After the conjugated beads bind to target cells or DNA, they are magnetically retained while undesired materials are rinsed away. At concentrations $>10^3$ cells ml^{-1}, IMS and MCH increased the sensitivity of subsequent RT-PCR by ~40 and 10 fold, respectively. At lower cell concentrations where direct RT-PCR was not possible, both IMS and MCH allowed detection of R3bv2. Moreover, RT-PCR of plant and soil samples pre-treated by IMS or MCH permitted detection of only R3bv2 strains at >500 cells ml^{-1} in ~5 h, whereas detection by direct RT-PCR of these samples was blocked.

Agent-based model of plant virus-host-vector interactions

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Phytopathology 101:S67

In agent-based model, a system comprises of a collection of autonomous decision-making entities called agents. Each entity makes decision based on a pre-defined heuristic rules. In agent-based modeling, the overall behavior of a system emerges out of the interactions of the agents. In this way, agent-based models mimic biological system rather closely. In the last decade, agent-based modeling technique has been used to study epidemiological systems such as smallpox and influenza in human population, yet its utilization in plant disease is rather sparse. This paper describes an agent-based model of insect-borne plant virus system built using NETLOGO programming language. There has been a steady accumulation of knowledge on the interactions between plant viruses, plant hosts and the insect vectors' behavior and reproduction. Experimental systems allow researchers to identify individual interactions, for example, between a plant virus and its vectors host search behavior as mediated by the host. Yet, picturing the epidemiological scale implication of such interactions can be proven daunting. Through this model, the effects of virus infection on individual vector host search, feeding preference, and reproductive variables are simulated and their resultant impact on the virus epidemiology can be assessed. Through this model we hope to show that

agent-based modeling is a powerful simulation technique available for theoretical study of plant disease epidemiology.

Non-host plant defense against multiple genera of fungal pathogens - initiated with DNase signals released by the pathogen
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Phytopathology 101:S68

Non-host disease resistance is a dynamic, seldom broken, resistance against most of the plant pathogenic fungi. Surprisingly, the fungus itself is a source of DNase signals that can activate the plant's defense. When not sufficiently activated, a plant can be susceptible to specific pathogens. The cloning of fungal DNase reveals that N-terminal amino acid sequences possess signal peptides that enable secretion, entrance to the pea plant cell and the single strand nicking of nuclear DNA. Proposedly, this action promotes the transcription of genes in sensitive regions of the chromosome. DNases have not been previously touted as major signals. We recently reported the differential depletion of histone H2A and HMG A protein from the DNA sequence in the vicinity of a PR gene (DRR206) 4 h after inoculation with *Fusarium solani* f. sp. *phaseoli (*Fsph) or *pisi* (Fspi) (Plant Sci. 177:439). Currently, we discovered defense-inducing DNases from several major genera of plant pathogenicc fungi: *Puccinia*, *Colletotrichum*, *Verticillium*, *Phytophthora*, *and Rhizoctonia*. The DNase genes from *Fusarium solani* and *Verticillium dahliae* have been cloned and specific concs. of the purified enzyme used both to induce resistance in pea to Fspi and to break resistance against Fsph.

Production practices and cultivar selection impacts the occurrence of diseases and the yield of peanut
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Phytopathology 101:S68

In 2009 and 2010, impact of tillage, planting date, cultivar, and row pattern on the occurrence of tomato spotted wilt virus (TSWV), leaf spot, and stem rot along with peanut yield was evaluated. Plot design, with four replications, had conventional or row tillage as the whole plot with late April, mid-May, and early June planting dates as the split plot, Georgia Green and Tifguard cultivars as the split-split plot, and single or twin row patterns as the split-split-split plot, which had four 30-ft rows. While row pattern had little impact on disease, significant 2- or 3-way interactions between tillage, planting date, and cultivar on the occurrence of TSWV, leaf spot, stem rot, and yield were noted. Overall, Tifguard often had lower disease ratings and higher yield than Georgia Green. Under conventional tillage, TSWV, leaf spot and stem rot ratings trended higher on Georgia Green than Tifguard. While stem rot incidence was often highest with the mid-April planting date, higher leaf spot ratings were seen with the early June planting date. Impact of tillage on leaf spot and stem rot varied by year. Higher yields were obtained with the twin compared with single row peanuts in 2009, while significant yield gains with the twin row pattern were noted for conventional but not row tilled peanuts in 2010. Results suggest that lowest disease and highest yields would be obtained by planting Tifguard on twin rows using conventional tillage.

Impact of nitrogen rate and variety selection on disease severity and yield of rainfed forage and sweet sorghum grown for biofuel
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Phytopathology 101:S68

Impact of nitrogen rate on the biomass and sugar yield, as well as disease severity on selected sweet and forage sorghum varieties was assessed in 2009 and 2010 on a site in continuous sorghum production in southwest Alabama. Study design was a split plot with nitrogen rates of 20, 40, 60, and 80 lb actual N/A as the main plot and sorghum variety, which in both years included Dale and M81-E sweet sorghum and SS1515 forage sorghum, as the subplot treatments. The sweet sorghum Sugar Drip was replaced with the forage sorghum SS405 in 2010. The study was not irrigted. No nitrogen rate × sorghum variety interaction for any yield or disease variable were noted. Variety selection had a significant impact in both years on fresh (wet), dry, and bagasse yield as well as brix values and sugar yield, and overall disease severity. While variety selection also significantly impacted sugar cane borer (*Diatraea saccharalis*) damage in 2009, insect activity was minimal in 2010. Primary disease was anthracnose (*Colletotrichum graminicola*) on M81-E and SS405, and rough leaf spot (*Ascochyta sorghi*) and/or zonate leaf spot (*Gloeocercospora sorghi*) on Sugar Drip, Dale, and SS1515. While M81-E had the highest biomass yield in 2009, that variety and SS405 had equally high biomass yields in 2010. Highest brix values and sugar yield were obtained in both years with M81-E and Dale. Disease severity was not impacted by nitrogen rate. Equally high biomass and sugar yields were obtained across all nitrogen rates.

Drench and foliar fungicides compared for control of Entomosporium leaf spot on photinia
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Efficacy of the soil fungicide drench All-In-One Rose and Flower Care Concentrate was compared with the foliar fungicides Daconil Weather Stik 6F, Immunox Multipurpose Fungicide, Disease Control for Roses, Flowers & Shrub Concentrate and RosePride Disease Control Concentrate for the control of Entomosporium leaf spot on field-grown red-tip photinia (*Photinia* x *fraseri* 'Birmingham'). While the All-In-One drench was poured over the root zone monthly from January 4 to July 5, 2006; January 12 to July 11, 2007; and January 17 to June 23, 2008, the above foliar fungicides were applied at 2-wk intervals during this same time period. Disease severity was periodically rated using the 1 to 10 Florida peanut leaf spot scoring system of during the winter and spring of each study year. When Entomosporium leaf spot data were pooled over years, All-In-One drench and the non-fungicide treated-photinia had similarly high AUDPC values, in contrast to the significantly lower values recorded for all the foliar fungicides. In May, defoliation levels on the non-fungicide treated controls, which ranged from 25 to nearly 75% in 2006 and 2007, respectively, reached similar levels in all 3-years on the All-In-One-treated plants. In contrast, little leaf spotting and no defoliation was seen on the photinia treated with Daconil Weather Stik 6F, Immunox, Disease Control and RosePride. Poor Entomosporium leaf spot efficacy of the All-In-One was attributed to an inadequate rate of the tebuconazole component.

Pathogenicity test of four potential fungal biocontrol agents on Setose Cephalanoplos weed and their safety on agricultural crops
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Setose Cephalanoplos weed, *Cephalanoplos setosum* (willd) Kitam, is a economically significant weed in the fields. To develop biocontrol agents as alternative management method to herbicide, the pathogenicity to *C. setosum* (willd) Kitam was tested by four fungal pathogens *Sclerotinia sclerotiorum*, *Alternaria* sp., *Epicoccum nigium* and *Fusarium tricinctum* which were isolated from diseased plant of *C. setosum* (willd) Kitam. The results of plant virulence assay indicated that *S. sclerotiorum* is a highly virulent pathogen that could cause foliar and stem necrosis. *Alternaria* sp. and *E. nigium* could cause symptoms on the leaves of *C. setosum* (willd) Kitam, However, *F. tricinctum* only showed a low level of virulence toward the weed. The pathogenicity test of different combination among the four pathogens to *C. setosum* (willd) Kitam was also conducted, the mixture of 3 pathogens (*S. sclerotiorum*+*Alternaria* sp.+*E. nigium*) in 1:1:1 ratio showed higher virulence than individual inoculums. All the leaves blighted in 5 days after inoculation, and the whole plants died after 10 days. The safety of these potential biocontrol agents to major crops in Qinghai was tested by bioassay. The test showed that combination of 3 pathogens were virulent to wheat and highland barley, but safe to broad bean and pea. The results suggested that the combination of 3 strains have a high potential to be developed as fungal herbicides to the weeds *C. setosum* (willd) Kitam in the fileds of broad bean and pea.

Expression of hemolysin (exotoxin) of '*Candidatus* Liberibacter asiaticus' in citrus using Citrus tristeza virus vector
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Citrus Huanglongbing (HLB) also known as citrus greening is one of the most destructive diseases of citrus worldwide. The causative agent Candidatus Liberibacter asiaticus (CLas) is a fastidious, Gram-negative, phloem-limited, alpha-proteobacterium. To understand how CLas induce disease in citrus, it is important to express the CLas effectors directly inside the phloem. Using bioinformatics tools, we have identified a number of putative effector genes based on the genome sequence of CLas. One of the important effectors or virulence factors is hemolysin, a 50 kDa protein secreted by type I secretion system. The hypothesis is that the hemolysin might interfere with metal ion transportation leading to host metabolic imbalance potentially resulting in disease symptoms. By the use of citrus tristeza virus (CTV) vector, we could express hemolysin effectors of the CLas bacterium directly inside the phloem of citrus. Hemolysisn gene from CLas was amplified, engineered into a binary vector based CTV vector and agro-inoculated to Nicotiana benthamiana seedlings. CTV virions containing hemolysin effector were purified and inoculated to citrus plants by bark-flap inoculation. The resulting systemic

spread and expression of the putative effectors throughout citrus trees will enable us to understand the role of the putative effector in disease induction.

Study of *Citrus exocortis viroid* replication in citrus protoplasts

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Phytopathology 101:S69

Citrus exocortis viroid (CEVd) is single-stranded, circular, non-encapsidated, non-coding RNA that infects citrus and other hosts. CEVd replicates in the cell nucleus using host polymerase. Citrus protoplast systems coupled with a quantitative (q) PCR assay was developed to study and measure viroid replication at the cellular level. We were able to obtain up to 40 million protoplasts reliably from a 40 ml of suspension culture of *Citrus amblycarpa*. The level of CEVd progeny RNA increased by 2.5 fold at 2 days post-transfection (dpt) compared to 1 dpt. The level of accumulation of progeny CEVd RNA increased to 80, 100 and 130 fold by 3, 4, and 5 dpt, respectively. Asymmetry in the ratio of the replicative intermediates and progeny molecules was also observed (analogous to the negative-strand in positive-stranded RNA viruses). The ratio of positive-strand to negative-strand varied with time of incubation and peaked at 3 dpt, with a ratio of 15:1. The protoplast system developed in this present study should allow in-depth investigation of the structural elements of CEVd essential for replication, as well as potential host factors for replication.

Presence of the potato late blight resistance gene *Rpi-blb1* does not promote adaptive parasitism of *Phytophthora infestans*

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Phytopathology 101:S69

The gene *Rpi-blb1*, from the wild potato species *S. bulbocastanum*, confers partial resistance to late blight, caused by the oomycete pathogen *Phytophthora infestans*. In order to determine whether a single strain of *P. infestans* can adapt to overcome this partial resistance source, we subjected *Rpi-blb1* containing plants to multiple rounds of infection with *P. infestans*, with sporangia from a late blight lesion used to infect the next leaflet. A parallel line of inoculations was done using susceptible leaflets. At the end of the experiment, sporangia passaged through resistant or susceptible leaflets were compared for their ability to cause disease. Variants of the corresponding *P. infestans* effector IPI-O, which is recognized by the Rpi-blb1 protein to elicit resistance, were also cloned and sequenced to determine whether variation occurred after selection on the partially resistant host. After 20 rounds of selection, no breakdown in *Rpi-blb1* resistance was observed. In fact, the strain that was continually passaged through the partially resistant host produced smaller lesions on susceptible leaflets and had a lower infection frequency than the strain passaged through susceptible cultivar Katahdin. Our results indicate that continual exposure to the *Rpi-blb1* gene can reduce *P. infestans* virulence.

Effect of temperature on survival of *Phytophthora* and bacterial species in irrigation water

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Phytopathology 101:S69

Plant pathogens, especially *Phytophthora* species, in re-circulating irrigation system present a significant health risk to floral crops. One of current technologies for water disinfection is heat treatment, which is highly effective and has minimal human health and environmental hazards. The primary objective of this study was to examine whether water temperature required to inactivate major pathogens in re-circulated irrigation water can be lowered from 95°C, the recommended temperature in the current protocols. Specifically, we investigated the effect of water temperature on zoospore survival and infectivity of *P. nicotianae* on annual vinca (*Catharanthus roseus* cv. Little Bright Eye) under greenhouse conditions, and on survival of six common plant and one human bacterial species. Inoculation of annual vinca with zoospore suspensions of *P. nicotianae* treated at 42°C for 12 h or at 48°C for 6 h did not result in any disease. None of the seven bacterial species survived 48°C for 24 h. Comparatively, *P. syringae* was the most heat-sensitive, while *E. coli*, *E. carotovora*, and *R. solanacearum* were more heat-resistant. In conclusion, along our previous data on oospores and chlamydospores of *Phytophthora* spp., the results suggest that required water temperature for heat treatment may be lowered substantially from 95°C without sacrificing efficacy. This research is being expanded to understand the underlying mechanisms of pathogen killing at lower than physically lethal temperatures.

Fungicidal efficacy of oxysilver nitrate and sodium diperiodatoargentate (III) for control of seed-borne and foliar diseases

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Silver and oxidized silver compounds have recently been shown to be effective plant disease management tools. For example, some non-oxidized silver compounds are effective fungicides when applied to inoculated foliage. Oxidized forms of silver (e.g. oxysilver nitrate) also have significant potential in crop protection, particularly as seed treatments for eradicating seed-borne bacteria and fungi on pulses and other crops. We report here that oxidized silver compounds, oxysilver nitrate and diperiodatoargentate (III), are potential disease management tools for field and horticultural crops as seed treatments and foliar-applied fungicides. In replicated, small-plot field trials, oxysilver nitrate was shown to reduce seed piece decay (*Fusarium sambucinum*) on potato. Furthermore, both oxysilver nitrate and sodium diperiodatoargentate (III) reduced foliar diseases on field crops including Ascochyta blight (*Ascochyta rabei*) on chickpea and white mould (*Sclerotinia sclerotiorum*) on dry bean. The ability of oxidized silver compounds to reduce disease symptoms was especially evident when tank mixed with other commercially available fungicides such as Bravo®500 and Allegro®500F. In tank-mixed treatments enhanced, and perhaps synergistic, efficacy was observed.

***In vitro* sensitivity of the Pythium blight pathogens of snap bean to various fungicides**

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Phytopathology 101:S69

Sensitivity of the pathogens causing Pythium blight on snap bean was determined *in vitro* against the active ingredients azoxystrobin, cyazofamid, mefenoxam, copper hydroxide, and potassium phosphite, which are fungicides commonly used in snap bean production. Twenty-two *Pythium* isolates were collected from symptomatic plants in Virginia, Georgia, and New Jersey, representing *P. aphanidermatum*, *P. deliense*, *P. ultimum*, and *P. myriotylum*. Isolates were placed on media amended with each active ingredient at 0, 100 µg/ml, the concentration equivalent to the labeled rate if applied on succulent beans at 187 L/ha, and the equivalent if applied at 374 L/ha. All isolates were completely sensitive (100% growth reduction, or GR) to all active ingredients at the labeled rates, except azoxystrobin. GR due to azoxystrobin showed a wide range across the isolates. At 100 µg/ml azoxystrobin, one *P. deliense* isolate demonstrated 8.9% GR. All isolates had 100% GR to copper hydroxide at 100 µg/ml, and the lowest GR on mefenoxam-amended medium was 91.9%. *P. aphanidermatum* isolates varied in sensitivity at 100 µg/ml cyazofamid, ranging from 69.2 to 100%, and all *P. deliense* isolates were completely sensitive. At 100 µg/ml potassium phosphite, significant GR similarities were recorded within isolates of the same species, and less than 50% GR was observed in all *P. deliense* isolates. No relationship was observed between collection location and isolate sensitivity.

Evaluation of Raspberry (*Rubus* sp.) cultivars for postharvest quality and resistance to *Botrytis cinerea*

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Phytopathology 101:S69

Raspberries (*Rubus sp.)* are a delicate high value specialty crop with an extremely short shelf life. This is exacerbated by their susceptibility to postharvest decay caused by *Botrytis cinerea*. Of the three commercially available species, red raspberry (*Rubus idaeus* subsp. Strigosus) is the most widely grown. Yellow (*R. idaeus*), black (*R. occidentalis* L.) and purple raspberries (*R. neglectus* Peck. or *R. occidentalis* × R. *idaeus* hybrids), are mainly available at local markets and U-pick farms. There are no recent studies examining post-harvest quality differences between these raspberry types and the role of host genotype in decay resistance. Therefore, the post-harvest quality of 13 cultivars of red, yellow, purple and black raspberries was examined twice weekly from June to September in 2010. Storage life was assessed at 4°C and 20°C for 6 days by recording decay incidence and percentage bleed. Firmness, color, respiration and ethylene emission rates were measured in select harvests. Additional raspberries were inoculated with 3 different B. cinerea isolates to examine their levels of decay resistance. Preliminary results show that black and purple raspberries outperformed the red and yellow varieties in storage. Black raspberries had the lowest ethylene emission rates and incidence of decay when inoculated with B. cinerea. Future studies will focus on confirmation of 2010 decay data and the relationship between B. cinerea resistance and cultivar physiology.

Diversity of TonB-dependent outer-membrane proteins in plant-associated strains of *Pseudomonas fluorescens*

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Genomic sequences of ten strains of plant-associated *Pseudomonas* spp. were surveyed for the presence of TonB-dependent outer-membrane proteins (TBDPs), which function in the uptake of substrates from the environment by many Gram-negative bacteria. The ten strains represent *P. fluorescens*, *P. chlororaphis*, and *P. synxantha* isolated from the phyllosphere, rhizosphere or soil. 14 to 45 TBDPs were identified in each strain, and phylogenetic analysis of the TBDPs identified five that are conserved across all ten genomes. Comparisons to proteins with known functions allowed the assignment of putative roles in uptake of heme, vitamin B12, copper, and the siderophore ferrichrome to the conserved TBDPs. Each strain also has multiple TBDPs with predicted functions in the uptake of pyoverdines, a structurally diverse class of siderophores produced by the fluorescent pseudomonads. For example, strain Pf-5 has six such TBDPs. Using crossfeeding assays, we found that Pf-5 utilized pyoverdines having 17 distinct structures. Mutants of Pf-5 lacking each of the six putative pyoverdine receptors were constructed and tested in crossfeeding assays, which linked the uptake of specific pyoverdines to individual TBDPs. The identification of the core TBDPs present in all genomes as well as the TBDPs unique to each genome highlights functions conserved across the species as well as those specific to the distinctive lifestyles of each strain.

Evolution of the '*Ca.* Liberibacter asiaticus' genome for and intracellular lifestyle

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Phytopathology 101:S70

'*Ca*. Liberibacter asiaticus' (CaLas) is a member of the Rhizobiales. Intracellular pathogens, Calas and *B. henselae* have genomes with many characteristics reduced genomes adapted to their lifestyle. This includes drastically reduced gene content and genome size as well as a much lower content of guanine and cytosine. Codon and amino acid preferences that emphasize low guanosine and cytosine usage are employed globally in these genomes. The length of orthologous proteins is generally conserved, but not their isoelectric points, consistent with extensive amino acid substitutions to accommodate selection for a low GC genome. Massive amino acid substitution requires an equally massive accumulation of mutations to the genome. This remarkable process could be facilitated by impaired DNA replication and repair capabilities. Detailed analysis of the repertoires of enzymes required for DNA replication and repair in *CaLas* and *S. meliloti* suggests that the ability of CaLas to repair mutations in its genome may be impaired. The AT rich genome of intracellular pathogens is likely selected for by energy savings for both the pathogen and the host. This includes a lower metabolic cost for AT vs GC base pairs. We hypothesize that replication and transcription of an A+T rich genome is energetically favored by a lower ATP cost for strand separation by DNA helicase. The reduced genome of CaLas enables an expanded host range, including diverse plant hosts and the citrus psyllid.

Determining the prevalence and distribution of bacterial diseases in Nebraska dry bean production fields

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Phytopathology 101:S70

Dry beans in Nebraska may be affected by a complex of different bacterial diseases, including common blight, halo blight, brown spot, and wilt. Control measures are more readily available for some of these diseases than others, therefore correct identification of the various bacterial diseases and determining their incidence and distribution across the different growing regions of Nebraska becomes important for choosing between the various options for disease management. A comprehensive survey was conducted between early June and mid-September, over two seasons (2009–2010) to establish the presence of the most important diseases and whether any are predominant in some production areas but not others. The survey included 519 samples from multiple market classes of dry beans representing 222 fields from 11 counties in western Nebraska. Wilt was found to be the most commonly occurring disease (23.5%). The other three diseases were found readily in fields: halo blight (9.5%), common blight (17.5%), but the presence of brown spot (15.5%) was much higher than expected over the two year project. Based on the results of this study, resistance evaluations are currently being conducted for wilt and brown spot in the effort to produce new highly resistant cultivars for use in Nebraska.

Genetic and biological variability of *Pepino mosaic virus* isolates infecting tomato plants

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Phytopathology 101:S70

Pepino mosaic virus (PepMV) is considered as one of the most dangerous pathogen infecting tomato worldwide. The virus is highly diverse; four distinct genotypes have been described so far. The aim of our work was to find correlation between different PepMV isolates and the severity of symptoms expression in infected tomato plants. Several Polish isolates, (Chilean 2 genotype-CH2) displaying wide range of symptoms from mild mosaic to severe necrosis on tomato plants, were used in this study. The coat protein, triple gene block and part of polymerase genes were amplified using PepMV specific primers, cloned and sequenced. The sequences comparison were performed and single nucleotide polymorphism sites were identified. Analysis of symptoms and their correlation with specific amino acid positions was also performed. Sequence comparison showed up to 99% identity between CH2-mild and CH2-necrotic isolates. Mutations affected on amino acid changes were randomly distributed however unique nucleotide substitutions in isolates causing leaf necrosis and yellowing were observed. Results of this study show that different symptoms induced by PepMV isolates on tomato plants may be related to minor differences at the nucleotide level between them. These results might help in future identification of genome regions involved in the expression of PepMV symptoms in tomato.

Characterization of a novel Emaravirus infecting blackberry

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Phytopathology 101:S70

Several new viruses have been identified in blackberry in the last decade. These viruses are usually found in mixed infections and are associated with blackberry yellow vein disease (BYVD), a disorder caused by virus complexes. In order to investigate the epidemiology of the viruses involved in the disease, virus-tested sentinel blackberry plants were placed in a field with high BYVD incidence and several developed viral-like symptoms after only a month exposure in the field. Symptoms included leaf mottling, chlorotic ringspots and curved midribs. Some of the symptomatic plants were subjected to deep sequencing using the Illumina platform and regions of an apparently new virus were obtained. The genome organization of the new virus is similar to that of emaraviruses and phylogenetic analysis showed that it is closely related to Fig mosaic and Rose rosette viruses. RT-PCR detection protocols have been developed and successfully used to detect the virus in BYVD plants. These tests showed that the new virus is prevalent in blackberry fields and may be involved in the etiology of BYVD. Mite transmission experiments are being conducted to identify the vector that accounts for the rapid movement of the virus.

Editing in Wikipedia to learn concepts in plant pathology

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Phytopathology 101:S70

Wikipedia is a collaboratively written online encyclopedia. Wide use by the public and the fact that anyone can edit its articles makes it an accessible format for students to share information they have learned. This study examines the value of editing in Wikipedia as an assignment for a college plant pathology course. It was hypothesized that the assignment would generate student enthusiasm and therefore lead to better learning outcomes and a higher quality product than a standard written paper. Success was evaluated with a student survey and an instructor evaluation of the final product. We found that student enthusiasm was indeed a positive outcome of this assignment and that we did generally achieve our learning goals. However, we did not find that the quality of the final products were any better. We think this is valuable assignment and we will continue to use it with a few revisions aimed at increasing the connection to lecture concepts and an emphasis on writing clarity and use of citations.

Accessing phosphoglucose isomerase: A gene with potential links to fitness and invasibility of the leafroller *Epiphyas postvittana* (Lepidoptera)

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Phytopathology 101:S70

Light brown apple moth, *Epiphyas postvittana*, is a significant horticultural pest native to Australia, and currently with a limited global distribution. However it can tolerate very heterogeneous climatic and vegetation conditions and has recently invaded California with considerable consequences for U.S. international and domestic trade. A genetic factor that may contribute to its environmental adaptability, and consequently invasive capability, is the phosphoglucose isomerase gene (*pgi*). This gene codes for a key enzyme in the second step of glycolysis and for which the isozyme composition has been associated with the fitness and dispersal capacity of other moths. As a first step, to determine if this locus is variable within *E. postvittana*, novel primers were designed enabling access to 957 bp of the coding region across exons 4 to 11 of *pgi*. Exon-primed intron-crossing (EPIC) primers were then designed to compare sequences of 17 specimens across one laboratory and three wild New Zealand populations from a laditudinal range of ~39-45° S. A total of 70 segregating sites in the exons were found, including 61 synonymous and nine nonsynonymous. Introns 3 to 11 (excluding intron 10) were also sequenced for 13 individuals revealing significant length variation within and between introns and populations. The level of variation revealed here indicates that this could be a useful target gene to assess fitness factors associated with invasibility of *E. postvittana*.

Ascospore viability and dispersal from pruned branches infected with *Anisogramma anomala*

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Phytopathology 101:S71

Viability and dispersal of ascospores of *Anisogramma anomala*, cause of Eastern Filbert Blight on European hazelnut, from diseased branches pruned from trees was measured. Branches bearing stromata of *A. anomala* were cut in mid-Dec and compared to branches cut near budbreak in Mar, when trees are susceptible to infection. The experiment was replicated three times at separated locations. At each location, 125 diseased branches (the source) were piled loosely in a 1 × 1 m area. From Mar to Jun, spore traps (rain sampling-type) were placed next to piled branches, 6.4 m upwind and downwind, and 20 m downwind. During four significant rain events, hazelnut seedlings (3-month-old) were placed adjacent to the traps. At the source, significantly higher ($P < 0.01$) spore counts were obtained for branches cut near budbreak compared to those pruned in Dec. At least one infected seedlings was obtained for 3 of 4 rain events regardless of pruning time. Significantly higher ($P < 0.05$) spore counts were obtained at 6.4 m downwind compared to 6.4 m upwind or 20 m downwind. Spore viability, as assessed by trypan blue, was similar for both pruning times at all distances, and averaged 50%. Based on these results, spores from diseased branches pruned from trees in both treatments remained viable and were dispersed downwind of each treatment.

Importance of vector movement on the epidemiology of a complex of mite-transmitted wheat viruses

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Phytopathology 101:S71

The wheat curl mite (WCM), *Aceria tosichella* Keifer, transmits three viruses to winter wheat: wheat streak mosaic virus, High Plains virus, and Triticum mosaic virus. WCMs disperse by wind, and the extent of this dispersal determines the risk of serious virus infections in winter wheat. Estimates of the extent of this movement have never been made because the minute size of the mites makes direct measurement of mite movement very difficult. The symptoms of this virus complex can be detected via remote sensing, thus remote sensing was used to establish its potential to track virus spread and consequently, mite movement. Field plots of simulated volunteer wheat were established and the spatial pattern of virus spread was measured by using remote sensing. Virus symptoms occurred in an oval pattern displaced to the southeast or the direction of prevailing winds. Data from the spatial spread in these small plots were used to estimate the potential sphere of influence for volunteer wheat fields.

Transgenic rice with inducible overproduction of ethylene exhibits broad-spectrum disease resistance

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Phytopathology 101:S71

Rice blast (*Magnaporthe oryzae*) and sheath blight (*Rhizoctonia solani)* are the two most devastating rice diseases in the U.S. and around the world. Recent studies suggest that ethylene may play a positive role in host resistance to rice blast and sheath blight diseases. In this study, we attempt to genetically manipulate endogenous ethylene levels in rice for enhanced disease resistance. To generate transgenic rice with inducible overproduction of ethylene, a pathogen-responsive rice ACS2 (1-aminocyclopropane-1-carboxylic acid synthase, a key enzyme for ethylene biosynthesis) cDNA was placed under a strong pathogen-inducible promoter and introduced into rice by *Agrobacterium*-mediated transformation. In comparison with control plants, the transgenic rice lines show significantly increased levels of the *ACS2* transcripts, endogenous ethylene and defense gene expression, especially in response to pathogen infection. Importantly, these transgenic rice with inducible overproduction of ethylene exhibit enhanced resistance to *R. solani* as well as different races of *M. oryzae*. These results suggest that genetic manipulation of endogenous ethylene levels can significantly increase host resistance to a broad-spectrum of races.

Sporulation dynamics of *Spilocaea oleagina* and timing of olive leaf spot infection in the orchard

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Phytopathology 101:S71

Fall infections of olive leaf spot, incited by *Spilocaea oleagina*, have a major impact in defoliation of olive trees. Control measures are based upon the spray of fungicides starting with the first fall rains. Sporulation dynamics were studied washing 20 individual lesions every 2 weeks since October 2008 to May 2010, in 2 Chilean orchards located in the coastal and central regions. Sporulation was expressed as the number of conidia in one centimeter of spot. Conidia were produced continuously in the coastal orchard with the highest amounts produced by mid springs and lowest amounts during the summers. A discontinuous production was observed in the central orchard with initial high sporulation in the spring 2008 that ended in early summer due to defoliation, reassuming at the end of winter 2009. Timing of fall infections was studied in 3 orchards where individual branches were covered with paper bags, to avoid infections. 50 branches were unwrapped at monthly intervals from April 22th until October 28th 2010, allowing 7 exposition periods for infections to occur. Infections were found in all exposition periods with higher incidence during the months of April, May and June. Differences among orchards were related to the different climatic conditions, with higher incidence in the coastal orchard. These results indicated that disease onset started before the first rains and are the result of free water coming mainly from morning dew. Therefore, fungicides should be sprayed earlier.

Inhibitory effects of *Bacillus amyloliquefasciens* and *Paenibacillus polymyxa* on *Botrytis cinerea* causing gray rot of grapes

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A collection of seven strains of bacteria isolated from leaves of table grapes and an apple fruit were screened *in vitro* for inhibition of *Botrytis cinerea*. For the *in vitro* inhibition assay, the bacteria were directly transferred from a pure culture 0, 24, 48 and 72 hours before pathogen inoculation on potato dextrose agar (PDA) media, using a 5 mm *B. cinerea* PDA plug. Plates were incubated at 22°C for 5 days. Two of the eight strains inhibited the mycelial growth of the pathogen by 22 to 25% when bacteria were present on the plate both at 0 or 24 hours prior to *B. cinerea*. Inhibition varied from 37 to 48% when bacteria were incubated 48 hours before pathogen inoculation, but no further inhibition increase was observed at 72 hours of incubation. The bacterial strains were identified to be *Bacillus amyloliquefasciens* and *Paenibacillus polymyxa* based on 16S DNA sequence analysis. Also, cyprodinil and two citric extract fungicides significantly inhibited the *in vitro* growth of *B. amyloliquefasciens*, while *P. polymyxa* was unaffected. Finally, the *in vivo* effect on gray rot development was evaluated on wound inoculated Thompson Seedless berries, where 10 µL of a 10^8 CFU/ml bacterial suspension was applied and incubated at 22°C for 24 hours before inoculation with 10 µL of a conidial suspension (10^5) of *B. cinerea*. *B. amyloliquefasciens* and *P. polymyxa* reduced the lesion size by 29.2 and 67.1% respectively, while disease was completely reduced by fenhexamide (chemical control).

Functional biodiversity: Study of the raspberry bush - Rubus idaeus (rosaceae)

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Phytopathology 101:S71

Natural ecosystems are usually self-balancing. Interactions between plants and animals are regulated by various mechanisms, without the need for human intervention. In protected raspberry culture, we have researched many cases of failure of integrated pest management. The analysis of causes for these failures was found to be extremely complex, due to the numerous interactions between organisms. The aim of this work is to offer a simplified assessment scheme for the interactions present across phytosociological and entomological data. We observed that: 8 direct auxiliairies of R. ideaus are also

present on associated plants; The enormous biomass, mainly made up of 217 neutral arthropods for R idaeus, is composed of indirect auxiliairies because they act as additional food to the 8 direct auxiliairies; 13 associated plants contribute to maintaining the auxiliary populations.

Influence of *Maize mosaic virus* on the fitness and wing morphology of *Peregrinus maidis* (Hemiptera: Delphacidae)
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Phytopathology 101:S72

Maize mosaic virus (MMV; Rhabdoviridae) is a corn virus transmitted propagatively by the corn planthopper, *Peregrinus maidis* (Hemiptera: Delphacidae). We examined the fitness of *P. maidis* developing on MMV-infected or healthy corn leaves; however, our results showed no significant differences in mean developmental time, mean fecundity, and mean longevity. Delphacid planthoppers can differentiate wing dimorphic forms called either macropters (long wing forms) or brachypters (short wing forms). Since the abundance of these forms may vary in response to the physiological status of the host plant, we examined the effect of MMV on the wing morphology of *P. maidis*. Our results showed that planthoppers that developed on young (21–28 days old) infected plants produced 17% more of brachypters than planthoppers that developed on healthy plants of the same age. Conversely, planthoppers that developed on old (42–49 days old) infected plants produced 16% more of macropters than planthoppers that developed on healthy plants of the same age. Our results suggest that MMV infection may modulate the density and dispersal of planthoppers according to the stage of plant infection. At early stage, the virus may increase the vector population by producing more brachypters; however, at late stages of plant infection the virus may promote vector dispersal by triggering larger production of macropters.

Nuclear magnetic resonance for non-destructive imaging of belowground damage caused by *Heterodera schachtii* and *Rhizoctonia solani* on sugar beet
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Phytopathology 101:S72

Belowground symptoms of sugar beet caused by the beet cyst nematode (BCN) *Heterodera schachtii* include the development of compensatory secondary roots and beet deformity that can be assessed only following destructive removal of entire root systems from the soil. Infections by the soil-borne basidiomycete *Rhizoctonia solani* cause brown or black decay on beet and roots (*Rhizoctonia* crown and root rot, RCRR). Nuclear magnetic resonance imaging was applied for the detection of belowground symptoms caused by BCN and/or RCRR on sugar beet. Excessive lateral root development and beet deformation of plants infected by BCN was obvious 28 days after inoculation (dai) on resonance images when compared to non-infected plants. Three dimensional resonance images recorded 56 dai gave insight on BCN cysts attached to the roots in the soil. *Rhizoctonia* crown and root rot was visualized by lower intensity of the NMR signal at sites where rotting occurred. The disease complex of both organisms together resulted in RCRR development on the site of nematode penetration. Analysis of damage of sugar beet plants indicated to synergistic activity of both pathogens in combination which may result from direct and indirect interactions. Nuclear magnetic resonance imaging of plants can give new insights into the development of pathogens infecting belowground (and aboveground) plant parts because of the non-destructive nature and high spatial resolution of the method and may be also valuable in plant breeding.

Effect of soil-incorporated cover crops and Actinovate biocontrol on suppression of Fusarium wilt of watermelon
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Phytopathology 101:S72

Fusarium wilt, caused by *Fusarium oxysporum* f. sp. *niveum* (FON) has resurfaced as an economically important watermelon disease due to the loss of the use of methyl bromide fumigant, and the increase in production of triploid cultivars that lack resistance. Cover crops and biocontrol products have shown promise in reducing Fusarium wilt. For example the soil-incorporated cover crop *Vicia villosa* has suppressed Fusarium wilt of watermelon, but the mechanism of suppression is not known. We measured soil respiration following incorporation of a *V. villosa* cover crop to determine if it conferred general suppression. We also evaluated cover crops *V. villosa*, *Trifolium incarnatum*, *Secale cereale*, and *Brassica juncea*, and no cover, alone and in combination with Actinovate biocontrol (*Streptomyces lydicus*) for induction of suppression. In 2009 Actinovate significantly increased marketable fruit yield in plots inoculated with FON compared to plots without Actinovate, or plots with no inoculation; however in 2010 this effect was not seen. Fusarium wilt incidence was not significantly different among treatments in 2009 or 2010. Measurements in both years indicated that incorporation of *T. incarnatum* residue significantly increased the rate of soil respiration at the beginning of the field season compared to *V. villosa* and other cover crops. Because wilt suppression has been reported for *V. villosa* but not *T. incarnatum*, this may imply that disease suppression is not general.

The identification and characterization of genes involved in foliar infection of maize by *Cercospora zeae-maydis*
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Phytopathology 101:S72

Gray leaf spot, caused by *Cercospora zeae-maydis*, is one of the world's most devastating foliar diseases of maize. Despite the impact of this disease, little is known about the interaction between *C. zeae-maydis* and maize at the molecular level. The recent discovery and characterization of *CRP1*, a putative ortholog of the White collar-1 family of fungal blue-light photoreceptors, established a linkage between the perception of light and the infection of the pathogen through stomata. To further dissect pathogenesis at the molecular level, laser-capture microdissection is being utilized to isolate fungal tissue during infection, specifically hyphae approaching stomata, nascent appressoria, and mature appressoria. From these samples, the transcriptome of the pathogen is being obtained with next-generation sequencing technologies (RNA-seq) and analyzed to identify genes associated with specific stages of the infection process, such as stomatal tropism and appressorium formation. Candidate genes will be disrupted through targeted mutagenesis to determine their specific roles in pathogenesis. Regulatory genes characterized in this study will further elucidate the genetic mechanisms underlying foliar infection in *C. zeae-maydis* and related filamentous fungi.

Incursion of Myrtle rust in Australia caused by *Uredo rangelii*
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On April 25 2010 a new rust of Myrtaceae was detected in a nursery on the central east coast of New South Wales, Australia. The pathogen was subsequently identified as *Uredo rangelii* belonging to the 'guava rust complex'. Given the wide host range of pathogens in this complex and the large number of Eucalyptus and other native Myrtaceae in Australia, an extensive campaign was established to slow down the spread of the disease. However, it has been found on many properties and in the bush in NSW and Queensland to the point that it is now not technically feasible to maintain this campaign. Governments and industry are now in the process of transitioning to long term disease management to mitigate the impact on the natural environment, including endangered species and industries that rely on Myrtaceae. The knowledge of this disease and its potential impact in Australia is very limited. Taxonomic studies are underway to determine the exact relationship of this pathogen within the guava rust comples. It has now been identified on 55 species of Myrtaceae but further seedling testing suggest that most Myrtaceae might be susceptible to the disease although there are some indications of useful sources of host plant resistance. Other studies focus on modelling environmental impact based on current data. We are seeking international science collaborations to broaden our understanding of the ecology and behavior of this disease relative to other members of the guava rust complex.

Biocontrol of bacterial wilt of tobacco via induced resistance by endophytic bacteria
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Phytopathology 101:S72

Plant growth-promoting endophytic bacteria, *Pseudomonas* spp., significantly reduced the disease incidence and severity of bacterial wilt of tobacco caused by *Ralstonia solanacearum*. By means of real-time quantitative polymerase chain reaction (qPCR) using specific primers targeted 16S rDNA region of *Pseudomonas* spp., the bacterial population in tobacco plants was quantified. *Pseudomonas* spp. colonized the plant root up to 10^6 cfu per gram fresh weight. Real-time quantitative reverse transcription-PCR (qRT-PCR) analysis on the expression of defense-regulatory genes *Coi1*, *NPR1* and *EREBPs* and on the down-stream defense genes *PR1* (*PR-1a* and *PR-1b*) and *PDF1.2* in the tobacco leaves was carried out to determine the nature of the resistance induced by *Pseudomonas* spp. The expression of *PR1* gene, related to the salicylic acid (SA) dependent pathway, was highly up-regulated in the leaves after dipping the roots in the suspension of *Pseudomonas* spp. at the concentration of 10^8 cfu per ml for 24 h. However, no significant change in the expression of the *PDF1.2* gene, related to the SA independent pathway

and the selected defense-regulatory genes was found. Furthermore, *Pseudomonas* spp. was not able to reduce the wilt incidence in the NahG transgenic line (defective in SA accumulation). Our results indicate that resistance in tobacco against *R. solanacearum* induced by *Pseudomonas* spp. is associated with the systemic induction of PR proteins in the SA dependent pathway.

Improving the detection of new and emerging pests and diseases through the Plantwise Initiative

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Phytopathology 101:S73

The high percentage of the world's crops lost to pests and diseases seriously impacts on global food security. CABI is working with a network of international partners, including those from the CGIAR, regional plant protection organisations and governments, to create a new system that can help detect, monitor and control emerging pests more effectively, particularly in the developing world. This Plantwise Initiative contains a central, open access Knowledge Bank to collate information on the distribution, identification and effective control of thousands of pests and diseases. Plantwise also delivers appropriate scientific knowledge to farmers in developing countries through plant health clinics held in local markets. Here farmers get face-to-face advice from Plantwise trained 'plant doctors' who can also collect local pest information to pass back to the Knowledge Bank. Researchers can then easily access on-the-ground data to improve pest modelling systems while the 'doctors' can retrieve relevant fact sheets for the pests encountered. This process helped identify, and provide advice on, the first incidence of Citrus Black Spot caused by *Guignardia citricarpa* in Uganda and the planned increase in Plantwise clinics, from 80 to 400 over the next five years, will create much improved reporting of emerging problems. The Knowledge Bank, updated with these records and all new pest distribution data extracted from the scientific literature, will be a core resource on emerging pests.

The National Plant Diagnostic Network: First detector training and education

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Invasive arthropods, plant diseases, and weeds cost U.S. agriculture billions of dollars annually through direct pest damage and indirectly via eradication and management programs. Each year, integrated pest management (IPM) programs are disrupted by the threat of new, unwanted invaders. In order to raise awareness concerning the threat of invasive species and appropriate sampling as well as communication protocols, the NPDN launched an extensive First Detector training program in 2003. First Detector training occurs through traditional, face-to-face training (2003–2011), interactive, content management-based E-Learning modules (2008–2011), and a wiki platform-based series of pest information pages (2008–2011). First Detectors completing training receive certificates of completion, and the national First Detector newsletter. Nationwide to date, 10,736 First Detectors have been trained in 821 training sessions conducted by First Detector Educators, and 799 have participated in the E-Learning FD training.

Is the striped mealybug, *Ferrisia virgata*, a vector of huanglongbing bacterium *Candidatus* Liberibacter asiaticus?

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Candidatus Liberibacter asiaticus (Las) is the prevalent species of three different Liberibacter species that cause huanglongbing (HLB), the globally most devastating disease of citrus. Two psyllid species, *Diaphorina citri* and *Trioza erytreae* are currently known to transmit this endemic disease. Striped mealybugs, *Ferrisia virgata* (Cockerell) (Pseudococcidae; Hemiptera), were recently collected from Las-infected periwinkle plants in a USHRL greenhouse and tested for the presence of Las bacterium. Positive Las results were found in 46 of 73 (63%) mealybugs sampled using HLBasp primers and probe, and the Las populations were estimated to be 3.11×10^3 to 2.32×10^5 cells per insect. Additional confirmation was made using conventional PCR with six alternative primer sets targeting different Las loci and by 100% sequence similarity of all seven PCR amplicons. Using qPCR LJ900 primers that target the prophage genes of the Las genome it was found that the range of Ct values were 15.9 to 29.9 for the infected mealybugs. Infected insects reared on healthy plants for 30d continued to maintain Las infection with an average LJ900 Ct value of 21.6. Twenty five mealybugs, collected from non-infected plants, tested negative for Las using all detection methods. The striped mealybug has a wide host range with over 68 plant families and 264 plant species reported. To our knowledge, this is the first report detecting a high titer of *Ca*. L. asiaticus in the *F. virgata* mealybug.

Assessment of seed treatments to protect against biological winterkill in winter wheat

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To assess the efficacy of 11 seed treatments against pathogens associated with winterkill, four non-inoculated trials were planted in north-central Montana and four inoculated trials were planted in Bozeman, MT. Plots in non-inoculated trials were six-rows wide, 4.5-m long, and treatments were replicated six times. Inoculated plots were four-rows wide, 2.5-m long, and treatments were replicated four times. All trials were planted with the cv. Genou, a solid-stem, hard red winter wheat. Trials at Bozeman were inoculated in furrow at planting with oat inoculum infested either with *Fusarium culmorum* (Fusarium crown rot), *Bipolaris sorokiniana* (common root rot), *Typhula ishikariensis* (speckled snow mold), or *Microdochium nivale* (pink snow mold). For all trials, plant emergence (percent stand), green-up vigor, plant height and yield were measured. Additionally, plant crown samples were collected in the springtime from non-inoculated plots for fungal isolations. Pink snow mold caused the greatest winterkill in inoculated trials with non-treated inoculated controls having a 98% stand reduction. Only one treatment, BASF's Charter/Acquire/Axcess/Stamina had a significantly higher percent stand and yield compared to the non-treated inoculated control. This product also yielded the most across inoculated trials. Fungi isolated from non-inoculated trials were dominated by two *Fusarium* species, *F. acuminatum* and *F. tricinctum*, and a common soil saprophyte *Mortierella elongate*.

Fungal community analysis in wheat residues infested with *Fusarium pseudograminearum* through internal transcribed spacer region (ITS) sequencing

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Phytopathology 101:S73

Fusarium pseudograminearum is dependent on infested wheat crown residues for survival between cropping cycles. It is hypothesized that *F. pseudograminearum*'s ability to protect this resource base from fungal saprophytes is crucial to its survival. To test whether *F. pseudograminearum* actively manipulates fungal communities within wheat crown residues, twelve plots each of two spring wheat cultivars, Choteau and Outlook, were planted in Huntley, MT. Six plots of each cultivar were inoculated in furrow at planting with *F. pseudograminearum* oat inoculums and six plots were left non-inoculated. Crowns were collected eight months post senesce and their DNA extracted. *Fusarium pseudograminearum* populations were quantified for these samples using qPCR. Additionally, fungal internal transcribed spacer region (ITS) sequences were amplified from each DNA sample using the primers ITS-1F and ITS-4, bulked together based on treatment and cultivar, and then cloned into *E. coli* TOP10 cells. A hundred clones per treatment were sequenced and representative sequences for each operational taxonomic unit (OTU) were identified through BLAST-n analysis. Fungal communities from inoculated plots were significantly different from those in non-inoculated plots (Unifrac analysis, $p \leq 0.001$), and more diverse (Shannon diversity index = 3.15 inoculated versus 2.21 un-inoculated). Further exploration of this hypothesis is ongoing using samples from additional locations analyzed with 454 sequencing.

The biofumigation potential of *Brassica juncea* against black shank of tobacco

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Phytopathology 101:S73

Kentucky is the nation's leading producer of burley tobacco and the crop's most economically important disease is black shank, caused by *Phytophthora nicotianae* (Pn). Current management is effective, however, problems with expense and pathogen persistence are issues. A potential alternative control method, biofumigation, involves incorporating fresh brassica biomass into the

soil where brassica-produced glucosinolates are converted into volatile antimicrobial compounds. To evaluate the potential of biofumigation as a management tool for black shank in the field, a mustard (*Brassica juncea*) cover crop was incorporated into soil versus bare ground in 2008 and wheat cover in 2009–10. Results demonstrated that populations of Pn in soil were lower for mustard than bare ground in 2008, whereas no significant differences were found between mustard and wheat in 2009 and 2010. Significant differences in disease progression were not seen for any treatment in 2008–10. Greenhouse experiments compared mustard and wheat biomass incorporation at varying levels (0, 10, 50, 100, 200, and 490 g per 0.03 m^2 of soil). The higher mustard rates (200 and 490 g) reduced populations of Pn in soil, while rates comparable to our field study (100 g) did not reduce survival of Pn propagules compared to controls. Our results will be useful for Kentucky burley growers who are interested in utilizing biofumigation as an alternative to current practices to manage black shank.

Control of powdery mildew and Phytophthora blight of red-pepper by microbial and chemical fungicides

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Effect of combination application of five microbial fungicides and twenty five chemical fungicides for control of Phytophthora blight and powdery mildew were examined *in vitro* and under greenhouse condition. Five chemical fungicides significantly suppressed three microbial agents in microbial fungicides used for the control of powdery mildew. Also, two microbial agents for the control of Phytophthora blight were affected by 6 chemical fungicides *in vitro*. In the greenhouse test, the control values were in the range of 71.8% to 87.9% when microbial fungicides (*B. subtilis* DBB1501, *B. subtilis* QST-713) and chemical fungicide (trifloxystrobin) for the control of powdery mildew were mixed-sprayed four times at 7 days interval whereas the control value were 58.6%–81.9% when each of the three chemical fungicides (myclobutanil, trifloxystrobin, hexaconazole) were sprayed four times at 7 days interval. In Phytophthora blight, the mixed application of *B. pumilus* QST2808 and dimethomorph+ethaboxam among four mixed applications of microbial fungicides and chemical showed the highest control effect against Phytophthora blight. Also, control effect of mixed application of *B. pumilus* QST2808 and dimethomorph+ethaboxam was similar to that of single application of dimethomorph+ethaboxam. Therefore, we concluded that the mixed application of the microbial fungicides and chemical fungicides could reduce effectively the amount of chemical fungicides.

Occurrence of early blight on black nightshade caused by *Alternaria tomatophila* in Korea

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Phytopathology 101:S74

Early blights occurred on leaves of the black nightshade grown in Bongwha, Korea. The symptoms initially appeared as small and irregular spots on leaves and then enlarged to large necrotic lesions with concentric rings. The lesions were effuse, medium to dark brown, irregular, somewhat sunken and often surrounded by chlorotic haloes. A total of four *Alternaria* isolates were obtained from the diseased leaves of black nightshade. Morphological and cultural characteristics of present isolates were similar to those of *Alternaria tomatophila* Simmons described previously. To confirm the result of conventional identification, the major allergen *Alt a1* from these isolates were sequenced and analyzed. A phylogenetic tree showed that present isolates could be distinguished from other *Alternaria* species and form an independent clade together with *A. tomatophila* in GenBank. Pathogenicity tests were made on black nightshade, tomato and potato leaves spray-inoculated with conidial suspension (5×10^5 conidia mL^{-1}) with three replicates. Necrotic lesions were produced on the leaves of three plants 7 days after inoculation. However, no symptoms developed on control plants inoculated with sterilized distilled water. The pathogen was reisolated from inoculated leaves. This study shows that *A. tomatophila* is a pathogen causing early blight on black nightshade in Korea and could serve as a source of inoculums for spread of early blight to neighboring tomato and potato fields.

Diversity of *Phytophthora* species identified in a nursery irrigation runoff water containment basin of eastern Virginia

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Capture and use of agricultural runoff water in containment basins for irrigation is of strategic importance to the ornamental horticultural industry in the light of growing global water scarcity. However, this practice may recycle destructive plant pathogens. The primary objective of this study was to determine the diversity of *Phytophthora* species present in a containment basin at an eastern Virginia nursery. Whole leaves of *Rhododendron catawbiense* cv. 'Boursault' were used as baits and placed mostly in surface water at different locations for 7 days. Baits were retrieved, rinsed and transported in a cooler to the lab. Recovered leaves were surface-sterilized in 0.525% hypochlorite for 30 seconds, rinsed in deionized water twice then plated in 10-cm Petri dishes with PARP-V8 and PARPH-V8 agar. Emerging colonies were identified to species level using colony PCR-SSCP, morphology and DNA sequencing. The baiting was performed from 2005 to 2008 at monthly intervals for the first year and quarterly thereafter. A total of 21 *Phytophthora* species were identified. These include *P. aquimorbida*, *P. cactorum*, *P. citrophthora*, *P. cryptogea*, *P. gonapodyides*, *P. hydropathica*, *P. insolita*, *P. irrigata*, *P. inundata*, *P. megasperma*, *P. nicotianae*, *P. pini*, *P. polonica*, *P. pseudosyringae*, *P. sansomeana*, *P. syringae*, *P. tropicalis* and several new taxa. The implications of finding such diverse *Phytophthora* species in a single basin is discussed.

***Phytophthora* species identified from streams in Virginia**

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The genus *Phytophthora* was added to the list of 'water molds' in 1944 and this notion has since been generally accepted. However, little is known about its aquatic biology. Here we report the species of *Phytophthora* recovered during a statewide survey of streams for *Phytophthora ramorum* from 2006 to 2010. Rhododendron leaves were used as baits and placed in streams for 1 to 2 weeks, depending on the temperature. A total of 56 streams were baited each for five to six times from April to October. Leaves retrieved from water were wrapped in moist paper towels and shipped overnight to the lab, rinsed in deionized water then plated in 10-cm Petri dishes containing PARPH V8 agar. Emerging colonies were identified to species level by colony PCR-SSCP, morphology and DNA sequence analysis. A total of 1372 isolates were examined and 415 subcultured. Seven species of *Phytophthora* were recovered from 7 streams in 2006, six from 10 streams in 2007, 15 from 20 streams in 2008, 13 from 19 streams in 2009 and nine from 10 streams in 2010, respectively. By stream, the minimum number of species isolated was 1 and the maximum was 9. Fifteen species and several new taxa were recovered across all streams. These include *P.* taxon '*aquatilis*', *P. cambivora*, *P. citrophthora*, *P. cryptogea*, *P. gonapodyides*, *P. hydropathica*, *P. insolita*, *P. irrigata*, *P. megasperma*, *P. parsiana*, *P. pini*, *P. plurivora*, *P. pseudosyringae*, *P. sansomeana*, *and P. tropicalis*.

Microsatellites and microsatellite associate loci confirms diversity of *Ralstonia solanacearum* strains isolated from the southeastern United States

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Bacterial wilt, caused by *Ralstonia solanacearum*, is problematic in the southeastern U.S. *R. solanacearum* strains isolated from this region were collected and characterized based on biovar, pathogenicity tests, a hypersensitive reaction test, and phylogenetic analyses of the *egl* sequence. Microsatellites and their flanking regions (MALs) were identified in the GMI1000 genome. The MALs of representative strains were sequenced and phylogenetically analyzed. The groups formed using the MALs were identical to the sequevars identified in *egl* trees. Based on phylogentic analyses it was that strains isolated from Florida were more diverse than strains from Georgia and the Carolinas. These unique Floridian strains grouped with strains originating from the Caribbean and Central America. It was suspected that one of these exotic strains could be pathogenic on *Musa* plants due to a unique banding pattern upon using the *Musa* multiplex PCR. Upon inoculation of this strain into three different *Musa* genotypes, no wilt symptoms were observed; however, the bacterium was recovered in the roots and stems indicating systemic movement. The root mass of *Musa* plants infected with this strain was significantly smaller than the root masses of the control plants. This is a first report for using microsatellites for typing *R. solanacearum* strains and for isolating a *R. solanacearum* strain in the U.S. that is deleterious to the growth of *Musa* plants.

South American Leaf Blight of rubber tree: Dynamics of pathogen inoculum, progress and damages, in three topographical strata

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Phytopathology 101:S75

The South American Leaf Blight of rubber tree (SALB), caused by *Microcyclus ulei*, is the major limiting factor for rubber production in Brazil. Between June 2005 and December 2008 we studied the: i. dynamics of ascospores and conidia, ii. host phenology and disease progress and iii. effects of height, disease severity, and leaf density on growth and yield of rubber trees. Experiments were set in commercial rubber plantations, in three topographical strata: top, hillside, and lowland. In each stratum, boxes were placed to collect leaves. Disease severity; stroma occurrence and incidence on fallen leaflets; leaf density; phenology; and prevalence of leaflets in stages B, C, and D were evaluated weekly. Starting July 2008, a Burkard spore trap was installed in each stratum and weather variables were registered. Ascospores and conidia were trapped throughout the experimental period. Higher number of hours with leaf wetness and minimum relative humidity were registered in the lowland, and based on path analysis, these variables influenced SALB severity. Height affected directly and positively, and severity directly and negatively, both rubber production and growth in all strata. Under favorable weather conditions both ascospores and conidia were produced throughout the year suggesting that a review of the life cycle of the pathogen should be conducted. Planting of clones with horizontal resistance is anticipated to be the most effective control measure.

Development of a simple and practical detection method of seed-borne bacterial pathogens from potato tubers

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Phytopathology 101:S75

Of the seed-borne bacterial diseases of potato, black leg disease caused by *Pectobacterium atrosepticum* (Pa), *P. carotovorum* ssp. *carotovorum* (Pcc), and *Dickeya* spp. (Ds), ring rot caused by *Clavibacter michiganensis* ssp. *sepedonicus* (Cms), and brown rot (bacterial wilt) caused by *Ralstonia solanacearum* phylotypes I and IV (Rs) have been important problems for seed potato production in Japan. In this study, we tried to develop a simple and practical detection method of these bacterial pathogens from seed potato tubers at the same time. To enhance the detection sensitivity of the pathogens from potato tubers, incubation of bacterial cells in King'B liquid medium with shaking at 25°C more than two days (for Pa, Pcc, Ds, and Rs) or six days (for Cms) was most effective. Next, we prepared polyclonal antibodies (IgG) against each bacterial pathogen and used them to detect each bacterial species from artificially infected potato tubers. In combination with enrichment (pre-incubation in King'B) and DAS-ELISA method, we could detect each black leg pathogens (Pa, Pcc and Ds) and Rs with high level of sensitivity ($>10^1$ cfu/tuber). Similarly, we tried to detect these pathogens from the potato tubers in combination with enrichment and PCR method (Bio-PCR). Consequently, each species-specific DNA band for Pa, Pcc, Ds and Rs could be amplified from the potato samples infected at lower concentration (10^1~10^2 cfu/tuber). A Cms-specific band also could be amplified, but its detection limit was $>10^5$ cfu/tuber.

Microplate assay for copper resistance in *Xanthomonas* spp.

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Phytopathology 101:S75

Copper-containing compounds are the primary means of control for bacterial diseases of tomato; however, field populations of copper-tolerant bacterial strains may increase substantially after repeated sprays of copper. The objectives of this study were to develop a high throughput screening method for monitoring copper resistance in field populations of bacteria, and to isolate bacterial strains that are resistant to high levels of copper (1.2 mM) for further experimentation. Isolates of *Xanthomonas* spp. were collected from symptomatic plants during a disease survey of tomato fields in Tennessee and used in the assay. The assay utilized conversion of resazurin to resorufin, a reaction that occurs in the presence of respiring cells, as an indication of cell viability. Bacteria and a liquid growth medium containing resazurin were added to each well of a 96-well microtiter plate. The effect of copper sulfate, at final concentrations ranging from 0.2 to 1.2 mM, on bacterial growth was tested. Microtiter plates were incubated at room temperature for 24 h. Samples were removed from selected wells and cultured on solid media with and without copper. Absorbances (595 nm and 490 nm) were measured for wells not sampled for bacteria. Absorbance values correlated well with bacterial population counts made from the microtiter plate wells. Isolates capable of growth on solid medium with 1.2 mM copper were recovered for future studies.

Distribution and population density of tea root lesion nematode (*Pratylenchus loosi*) in Iran

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Tea (*Camellia sinensis*) is an edible and evergreen plant which has a medicinal and calmative characteristic. At present tea root lesion nematode (*Pratylenchus loosi*) is one of the most important crop loss agents in Iran, which cause loss in quantity and quality of tea. In this study, distribution and population density of this pathogen was carried out. To investigate these aims, during 2010 to 2011 about 170 complex sample (soil & feeder roots) were collected from all of tea plantation regions in Iran. Sampling was done based on international acceptable generic soil sampling and satellite maps (Google Earth) and GPS. Nematodes were extracted from root samples with Coolen & d'Herd techniques (1972) and were counted by using of counting slide. Total result showed that 86.7% of samples were infested by *Pratylenchus loosi*. Range of population density was 0.6 to 884 nematode in one gram of feeder roots. Among of the infested samples, 94.2% population density were over than 1 nematode/each gr of root and 5.8% less 1 nematode/1gr of root. As though the average population in each gram of infested feeder roots was 97.04 nematode.

Evolutionary and epidemiological consequences of using host resistance genes for controlling *tomato spotted wilt virus*

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The rapid evolution of resistance-breaking in plant viruses threatens the durability of host resistance genes in crops. To understand the impact of resistance genes on the epidemiology and evolution of resistance-breaking, we conducted an experimental evolution study to determine if pathogenicity and virulence increase during sequential passages in hosts carrying a resistance gene. Sequential passages through hosts without a resistance gene were expected to result in the loss of resistance-breaking. Resistance-breaking isolates of *tomato spotted wilt virus* (TSWV) were sequentially transferred through pepper (*Capsicum annuum*) host lines of TSWV-resistant and susceptible varieties. The change in pathogenicity and virulence was measured in resistant and susceptible varieties across multiple passages in a single host type (resistant or susceptible). Pathogenicity and virulence of resistance-breaking isolates increased with the number of passages in hosts carrying the resistance gene and was rapidly lost in hosts without the resistance gene. Consistent exposure to the TSWV resistance gene can drive the evolution of resistance-breaking virus isolates in a population. If resistant varieties are absent, resistance-breaking should rapidly decline in the population. IPM strategies should consider the epidemiological consequences of using resistant varieties in areas where resistance-breaking is present.

Characterization of the roles of the putative secreted protein-encoding XAC1496 in the growth and pathogenesis of *Xanthomonas citri* subsp. *citri*

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Phytopathology 101:S75

Xanthomonas citri subsp. *citri*, a causal agent of citrus canker, is one of the most economically important pathogens in citrus production. A hypothetical gene XAC1496 encoding a secreted protein of *Xanthomonas citri* subsp. *citri* was characterized. Mutation of the gene XAC1496 with EZ-Tn5 transposon resulted in a loss of pathogenicity and HR reaction in plants of Duncan grapefruit and Meiwa Kumquat, respectively. The growth of the mutant was 10–1000 times decreased in citrus compare to wild-type strain 306 during 20 d post inoculation, however, no difference was observed on the growth *in vitro* (NA and XVM2 medium). The resistance of the mutant against H2O2 was much less than that of strain 306, which might be one of the reasons affecting its growth *in planta*. The XAC1496 gene was found to be required for biosynthesis of extracellular polysaccharides (EPS) and biofilm formation. The mutation of the gene had no effect on swimming and swarming, and activities of cellulase, amylase and proteinase. The effect of mutation of XAC1496 on virulence genes will be presented. Further study is being carried out to investigate the exact roles of XAC1496 in growth, biofilm production and pathogenesis.

Monilinia species in China--Surprising facts

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Phytopathology 101:S76

Between 2008 to 2010, brown rot samples from peaches or nectarines were collected from peach growing areas in Beijing, Shandong, Zhejiang, Fujian, Shanxi, Gansu, Hubei and Yunnan provinces in China. Cultural, morphological and molecular characteristics of Chinese *Monilinia* isolates and European or North American isolates were recorded and analyzed. One Chinese species was morphologically most similar to *M. fructigena* from Europe, however, most of the isolates started to develop stroma after 10 days of incubation on PDA at 22°C while no stroma was observed in any of the *M. fructigena* isolates from Europe. Another Chinese species most closely resembling *M. laxa* often developed more than two germ tubes per germinating conidium while Western *M. laxa* isolates only developed one germ tube per conidium. In pathogenicity tests, lesion growth rates, sporulation, and symptoms on peach fruit were different between Chinese and Western species. Blast analysis of the ITS sequences exhibited eleven, eight nucleotide differences between the corresponding Chinese species and *M. fructigena*, *M. laxa*, respectively. Phylogenetic analysis based on sequences of two taxnomically informative genes G3PDH and TUB2 indicates that the Chinese species were individually grouped into different lineages from *M. fructigena* and *M. laxa*. Our data suggest that the Chinese species may be new *Monilinia* species, and a new molecular tool was developed to distinguish Chinese from European and American *Monilinia* species.

Duplex qPCR assay to detect and quantify pathogenic _Guignardia citricarpa_ and non-pathogenic _G. mangiferae_ in plant samples

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Phytopathology 101:S76

The fungal citrus pathogen *Guignardia citricarpa* causes the potentially devastating disease, black spot, that primarily affects fruit. The disease is found in many humid sub-tropical citrus production regions. It was discovered in Florida in 2010 and quarantine measures are in place to prevent further spread. A nonpathogenic species, *G. mangiferae*, is an endophytic fungus with a wide host range, including citrus, and is found worldwide. These two *Guignardia* species are frequently isolated from citrus and have been confused with each other for many years, especially the indistinguishable ascospores. A duplex TaqMan™ qPCR assay was developed to simultaneously detect and quantify these two *Guignardia* spp. Specific primers and probes labeled with Hex (*G. citricarpa*) and FAM (*G. mangiferae*) were designed from polymorphic regions of the internal transcribed spacer (ITS) and actin gene, respectively. The qPCR assay was specific and did not amplify DNA from 6 common fungal citrus pathogens. The detection limits for the two *Guignardia* spp. was 2 copies of the target sequence inserted into the pDrive cloning vector and 100 fg of genomic DNA, respectively. The two primer pairs had high efficiencies and were not affected by the presence of citrus DNA extracts. Cycle threshold (Ct) values were linearly correlated with the concentration of the target DNA. The duplex qPCR assay described in this study will be a useful tool for quarantine measures and epidemiological research.

Genetic diversity of antagonistic _Bacillus subtilis_ against citrus canker bacteria

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Phytopathology 101:S76

Bacterial canker of citrus caused by *Xanthomonas axonopodis* pv. *citri* resulted in great yield loss of various citrus cultivars worldwide. No known biocontrol agent has been recommended for this disease. To explore the potential of bacilli native in Taiwan for control of this disease, *Bacillus* species with broad spectrum of antagonistic activity against various phytopathogens were isolated from culture substrate and soils. Seven strains including TKS1-1, OF3-16, SP4-17, HSP1, WG6-14, TLB7-7 and WP8-12 which showed superior antagonistic activity were chosen for biofungicide development. The genetic identity based on 16S rDNA sequences indicated that seven native strains were close relatives of the *B. subtilis* group which appeared to be discrete from the *B. cereus* group. The DNA polymorphisms of strains WG6-14, SP4-17, TKS1-1, and WP8-12 as revealed by repetitive sequence based PCR with BOXA1R primer were similar while different from those of respective type strains. However, molecular typing of the strains with either tDNA-intergenic spacer region or 16S-23S intergenic transcribed spacer region was unable to differentiate the strains at species level. For strain WG6-14, the effectiveness of controlling citrus canker infection has been demonstrated and an endospore formulation has been officially recommended for controlling bakanae disease of rice in Taiwan. Information obtained from molecular typing would provide DNA fingerprints valuable for patenting or commercializing these bacilli strains.

Search for the volatiles of _Bacillus cereus_ C1L involved in the induction of systemic disease resistance and plant growth promotion

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Phytopathology 101:S76

Bacillus cereus C1L was isolated from the rhizosphere of Formosa lily (*Lilium formosanum* Wall.) in Taiwan. Application of *B. cer*eus C1L effectively decreased disease severity of Botrytis leaf blight in different kinds of lilies, southern corn leaf blight, and the gray mold disease of tobacco; in addition, *B. cereus* C1L significantly promoted the growth of maize and tobacco. The growth promotion effects were also observed in *L. formosanum* and *Arabidopsis*. To find the acting compounds for plant growth promotion and the induction of systemic disease resistance, effects of the volatiles of *B. cereus* C1L on the growth of tobacco and *Arabidopsis* were analyzed and positive effects were observed. Dimethyl disulfide was identified to be one of the volatile compounds produced by *B. cereus* C1L, and effective to reduce severity of diseases caused by *Botrytis cinerea* in tobacco and *Cochliobolus heterostrophus* in maize. However, significant inhibition of dimethyl disulfide on the mycelial growth of *B. cinerea* and *C. heterostrophus* were not observed. Thus, we presumed that dimethyl disulfide could be an active compound of *B. cereus* C1L to induce systemic disease resistance of plants, such as tobacco and maize. In addition, dimethyl disulfide was also active to promote plant growth as demonstrated in tobacco.

The rice blast fungus, _Magnaporthe oryzae_, copes with plant-generated reactive oxygen species through the virulence factor _MoHYR1_

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Phytopathology 101:S76

Reactive oxygen species (ROS) are antimicrobial compounds and appear to be active in the critical zone where pathogens and plants come in contact. During plant-pathogen interactions, the plant may mount several types of defense responses to either block the pathogen completely or ameliorate the amount of disease. A successful pathogen will likely have its own ROS detoxification mechanisms to cope with this inhospitable situation. We focus on one potential fungal virulence factor *MoHYR1* from the rice blast fungus *Magnaporthe oryzae*, and its role in ameliorating effects of plant-produced ROS. *MoHYR1* contains a glutathione peroxidase domain and its yeast homologue was reported to be a thioredoxin-dependent phospholipid peroxidase that detoxifies phospholipid peroxides by forming an inter-molecular disulfide bond with YAP1. We observed that the fungal mutants lacking this gene had a decreased ability to tolerate ROS generated by a susceptible plant, including ROS found associated with cell wall appositions (CWAs). Moreover, deletion of this gene caused a virulence defect in M. oryzae, which we believe is associated with the mutant's inability to detoxify plant-generated ROS. Together, our data suggest that HYR1 is a virulence factor in the rice blast pathogen, and its role in virulence is directly related to sensing and managing plant-generated ROS during early infection events.

The amplification culture of endospore formulation of _Bacillus subtilis_ biofungicide and its use in disease management

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Phytopathology 101:S76

The antagonistic *Bacillus subtilis* has long been used as probiotic biofungicide for the control of various fungal and bacterial diseases. The field application of this biofungicide for disease management in Taiwan, however, was far from extensive mainly due to the cost effectiveness of the commercially available products. In order to reduce the cost of biofungicide production and to strengthen the effectiveness of disease control, a grower self-assisted culture amplification system was developed. With the use of traditional fermentor produced *B. subtilis* biofungicide preparation as a seed inoculum, and the use of common agricultural waste as major constituent of growth medium, the amplification culture was performed with a self-constructed open tank system where in a timer controlled stirrer and a temperature controlling device was equipped. In a non-sterilized condition, the developed system amplify the seed inoculum by thirty times. The yield of endospores reached approximately 10^{10} cfu/mL 10 days after inoculation. For disease control

application, the efficacy of the amplified preparation on the control of citrus canker (*Xanthomonas axonopodis* pv. *citr*i) was shown comparable to that by fermentor produced biofungicide. The success of the amplified preparation for disease control warrants the extensive and repetitive application of the attempted biofungicide in the routinely practiced cultural management.

Development of genome-based diagnostic markers to detect and differentiate strains of *Xylella fastidiosa*
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Phytopathology 101:S77

Xylella fastidiosa causes many economically important diseases including pierce's disease (PD) of grapevine, citrus variegated chlorosis (CVC) and almond leaf scorch (ALS). It also causes bacterial leaf scorch (BLS) of forest and/or landscape trees and shrubs such as oak, elm, mulberry, sycamore and oleander. At present, complete or draft genomes of seven strains of *X. fastidiosa* are available in public databases, including the CVC strain 9a5c, PD strains Temecula 1 and GB514, ALS strains Dixon, M12 and M23, and oleander BLS strain Ann 1. By using comparative genomics, these genome sequences were exploited to reveal unique and distinguishing features for identification of highly specific diagnostic markers for target strains of *X. fastidiosa*. Unique sequences were identified by comparing blastN alignments of defined segments of each respective genome against the other genomes. PCR primers were designed and tested for amplification of DNA sequences from targeted and related strains of *X. fastidiosa*, with the goal of developing highly specific, easy to use, genome-based approaches to detect and differentiate strains of *X. fasitidiosa* for use in epidemiological studies and in diagnosis and management of diseases caused by *X. fastidiosa*.

"Peak", a nutritional formulation to suppress bacterial plant diseases
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Phytopathology 101:S77

There are limited options available to control bacterial plant pathogens. Bacteria require certain nutrients for pathogenesis, and plants are dependent on micronutrients as the regulators of defense reactions. "Peak" is a special formulation of beneficial and essential nutrients that is inhibitory to many copper and antibiotic plant pathogenic bacteria. Field trials of "Peak" have demonstrated its efficacy as a plant nutrient and to suppress plant diseases caused by species of *Erwinia* (fire blight of apple and pear), *Clavibacter* (Goss' wilt of corn), *Pseudomonas* (angular leaf spot of strawberry), *Xanthomonas* (citrus canker), and *Candidatus* Liberibacter (HLB, greening of citrus). "Peak" is absorbed through roots, stems, and foliage to provide systemic control of bacterial pathogens within plant tissues. Application timing, rate, and water volume are important considerations to maximize 'Peak' efficacy for bacterial disease suppression. A single application of 100 gm ai/a provides effective control of most extracellular bacterial pathogens infecting parenchyma tissues. Intracelular bacteria, such as *Ca.* Liberibacter spp., require several applications for suppression of this phloem-limited bacterium. A single application at mid-bloom has provided control of fire blight of apple comparable to or better than pre- and mid-bloom applications of Mycoshield (oxytetracycline), Agri-strep (streptomycin sulfate) or Bloomtime FD TME325 (*Pantoea aggomerans* E325).

Genetic diversity of environmental and clinical strains of the *Enterobacter cloacae* complex determined by multilocus phylogenetic and genome analyses
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Phytopathology 101:S77

Enterobacter cloacae is the causal agent of plant disease on a variety of hosts, as well as the cause of opportunistic infections in immunocompromised humans. However, the genetic relationships between environmental and clinical *E. cloacae* strains have not been determined. We used multi-locus sequence analysis (MLSA) of nine housekeeping genes, to determine phylogenetic relationships among 30 *E. cloacae* strains from onion, other plants, and humans. Preliminary analyses with a subset of strains and three genes demonstrated that *E. cloacae* isolates from plants form a clade which is distinct from clinical isolates. In order to further study the relatedness of these strains, we have sequenced the genome of *E. cloacae* EcWSU1, which causes Enterobacter bulb decay on storage onions. EcWSU1 has a circular chromosome of 4.8 Mb and a megaplasmid of 0.6 Mb for a total genome size of 5.4 Mb, which is similar in size to previously sequenced *E. cloacae* strains. The genomic sequence of EcWSU1 is on average about 85% similar to the sequence of *E. cloacae* ATCC 13047, a clinical isolate from spinal fluid, and about 98% similar to *E. cloacae* P101, an endophyte of switchgrass. This information and the preliminary multilocus phylogenetic data has led to a genome comparison study of EcWSU1 and P101 with other *E. cloacae* strains to determine exactly how closely related the environmental *E. cloacae* are to the clinical strains.

Fluorescence spectra and lifetime of relevant weed species as impacted by selected herbicides
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Phytopathology 101:S77

In the present study we evaluated the impact of four herbicides of different modes of action (glyphosate, bromoxynil, mesotrione, and amitrole) on the physiological response of four weed species (STEME, SETVIR, CHEAL, and VIOAR), as measured non-destructively by means of pigment fluorescence. As main outcome, we show for the first time the suitability of the laser-induced fluorescence technique to evaluate in vivo herbicide-induced physiological changes as revealed by modifications of both fluorescence spectra and lifetime. Alterations of the fluorescence signature depend on the interaction agrochemical-plant species, as well as the time after herbicide application. Measurements in the red and far-red spectral region indicate disturbance in the functionality of the photosynthetic apparatus and chlorophyll concentration, e.g. after application of bromoxynil or mesotrione. Measurements in the blue and green spectral regions reveal changes of both amount and composition of specific fluorophores, i.e. after application of glyphosate and amitrole. The fluorescence lifetime, expressed as LTmean or differentiated in lifetime 1 (short-duration, < 1 ns) and lifetime 2 (long-duration, 5-6 ns) fractions, provided additional information to the spectrally resolved data. In summary, this technique opens new perspectives for explorative studies on the action mode of a.i.s in screening programs, dose-response studies, as well as impairment and recovery of weeds and main crops.

Multiplex PCR assay for the simultaneous detection of *E. coli* O157:H7 and *Salmonella* spp. from fresh produce
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Phytopathology 101:S77

Pathogenic strains of *Escherichia coli* and *Salmonella* cause foodborne diseases if consumed in contaminated produce. In the present study we describe a one step multiplex PCR method for specific detection of *E. coli* O157:H7 and *Salmonella* spp. from fresh produce. Three primers targeting different pathogen related genes were prepared and applied for the simultaneous detection of target pathogens. The primers were able to specifically detect the target pathogens from complex culture in the presence of other bacteria as well from the food matrix. The sensitivity of the primers was up to 50 cells per reaction of bacterial culture and 5 pg of genomic DNA. This multiplex PCR offers an efficient microbiological tool for detection of *E. coli* O157:H7 and *Salmonella* in fresh produce.

Impact of clubroot resistance on root hair infection, disease severity, and growth of canola in soil inoculated with *Plasmodiophora brassicae*
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Phytopathology 101:S77

Clubroot, caused by *Plasmodiophora brassicae* Woronin, has become a serious threat to canola (*Brassica napus* L.) production in western Canada. Several clubroot-resistant canola cultivars have recently been released onto the Canadian market, but resistance needs to be managed carefully because some sources of resistance have broken down quickly in other regions. To improve our understanding of resistance management, root hair infection, clubroot severity, and plant growth parameters were compared in resistant and susceptible canola hybrids grown in inoculated versus noninoculated soil. Primary plasmodia were visible at 4 days after seeding in both cultivars, but root hair colonization, secondary infection, and clubroot severity were always highest in the susceptible cultivar. Plants were shorter when grown in inoculated soils, but the resistant cultivar was taller than the susceptible cultivar in infested soil. The effect of repeated cultivation of resistant and susceptible cultivars on subsequent infection levels was studied by growing the cultivars in clubroot-infested soil for six weeks, macerating the galls and putting them back into the soil, and planting the same cultivars in the same soil for two more cycles. At the end of the third cycle, a susceptible cultivar was sown in both soils. Seedling emergence and plant height were lower and clubroot severity was higher, in soil in which the susceptible canola had been grown.

A new model for races of *Xanthomonas campestris* pv. *campestris*

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The race structure of 165 strains of *Xanthomonas campestris pv. campestris* (Xcc), including 67 strains from known collections and 98 strains from weedy crucifers from coastal California (Ignatov et al, 2008) was determined using a set of seven crucifer differential cultivars, including Just Right F1, Tokyo Cross F1, Seven Top Green, Wirosa F1, Miracle F1 PI199947, and Florida Broad Leaf. Five cultivars of white cabbage- Bejo 2660, Invento F1, Bejo 2659, Jubilee F1, Bronco (Bejo Seeds, NLD), and two breeding lines, PEB1-764 Bi21Pi3 (Russian. St. Agric. University, Moscow), with known resistance to black rot were included. Twenty one Xcc strains were either avirulent or failed to cause systemic symptoms of black rot; 101 belonged to races 1-6, 8 and 9. Race 6 was predominant in the population of Xcc isolated in 2008 from the weedy crucifers. Evidence of new races was found in the interaction of 47 strains with the seven host differentials. Besides previously designated genes R1, R3, and R5, three new variants of R4 locus, R4A, R4B, and R4C were identified in turnip cultivars Just Right, Tokyo Cross, and Seven Top Green, respectively. Six matching pairs of avirulence genes in the different strains and resistance genes in the differential cultivars supported a gene-for-gene relationship between the pathogen and host. Evaluation of strain-specific reactions of the seven additional cabbage cultivars support the presence of five new putative avirulence genes in Xcc.

Relative susceptibility of six soybean genotypes against single and multiple viral infections in Nigeria

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Soybean genotypes, TGx1448-2E, TGx1440-1E, TGx1740-2F, TGx1485-1D, TGx1835-10E and TGx1987-10F were evaluated against *Cowpea mild mottle virus* (CpMMV), *Cucumber mosaic virus* (CMV), *Blackeye cowpea mosaic virus* (BlCMV), *Bean pod mottle virus* (BPMV), which are endemic and most frequently occurring viruses in soybean in Nigeria. Tests plants of each genotype were mechanically inoculated at seedling stage with each virus, and also in combinations of two or more viruses. Plants were monitored for symptoms and tested for viruses using ELISA. None of the accessions was found to be immune to the virus treatments, however, genotype response to virus treatments among accessions differed substantially ($P < 0.01$). High positive correlation was observed between symptom severity and incidence (0.77), plant height and seed weight (0.6), number of pods and pod weight (0.97), number of pods and number of seeds (0.93). Conversely, disease severity was negatively correlated with plant height (-0.52), number of pods (-0.63), pod weight (-0.68), number of seeds (-0.68), seed weight (-0.73). Plants infected with BPMV showed most severe symptoms; whereas CMV had significantly lower influence on performance of genotypes. In mixed infections, the CpMMV+CMV+BlCMV combination had the highest influence both on plant height and yield. Multiple virus infections of soybeans can result in complete loss of yield. These results underscore the need for the development of multiple virus resistance soybeans appropriate for Nigeria.

Laurel wilt of avocado: Relationships among disease severity, water conduction, and the spatial distribution of *Raffaelea lauricola*

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Laurel wilt of avocado, *Persea americana*, is caused by *Raffaelea lauricola*. Rapid wilting and vascular staining it causes suggest that xylem dysfunction and impaired water transport play roles in the development of this disease. Disease severity, water conduction and the spatial distribution of *R. lauricola* was examined in artificially inoculated plants of 'Simmonds' avocado. Stems were harvested 3, 7, 14, 21 and 42 da after inoculation and placed in acid fuschin to stain water-conducting tissue; the percentage of functional xylem was inversely correlated with disease severity. Water transport in these stems was also inversely correlated with disease severity. Average water flow rates were 0.6 ml min^{-1} in severely affected plants vs 200 ml min^{-1} in mock-inoculated control stems. With a fluorescein-conjugated wheat germ aggutinin stain, *R. lauricola* was observed primarily in discolored, nonconductive areas, usually in close association with tracheids and the walls of xylem vessels. Abundance of the fungus was greatest near the inoculation point and generally decreased as the distance from this point increased. The pathogen was observed in xylem vessels by 14 da. However, complete blockage of the lumen did not occur during the course of the study, nor was there evidence for extensive colonization of the xylem by the pathogen. Although blockage by the fungus may play a role in decreased water conduction in laurel wilt-affected avocado, additional factors are probably involved.

Histological and ultrastructural changes in avocado (*Persea Americana*) induced by *Raffaelea lauricola*

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Raffaelea lauricola causes laurel wilt, which affects avocado (*Persea Americana*) and other members of the Lauraceae. Symptoms include the wilting of stems and leaves and vascular staining of the wood. Host-pathogen interactions were examined with light and scanning electron microscopy. The 'Simmonds' and 'Choquette' avocado cultivars were inoculated and examined at 3, 14, 21, and 42 days after inoculation (dai). Samples were taken from 5, 10, 20 cm acropetal, and 5 and 10 cm basipetal to the inoculation site. At 3 dai, no external or internal symptoms were observed. After 14 dai, external symptoms were present and dark discoloration developed in the xylem tissue of inoculated 'Simmonds'. Pectic and phenolic compounds accumulated in tracheids and vessels of the stained areas and resulted in the complete blockage of vessels. Tylose formation in the vessels was observed by 14 days, and tylose numbers increased with increasing levels of disease. Mycelia and conidia were observed within lumina of vessels and fibers. In contrast, minor tylose formation and presence of the fungus was evident in 'Choquette' at all sample times. Mock-inoculated 'Simmonds' and 'Choquette' were asymptomatic internally and externally, and tylose free. Understanding these responses will ultimately enhance disease management and host resistance efforts on this crop.

Species limits in *Verticillium*, a group of vascular wilt-pathogens of global importance

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Verticillium is a small genus of plant pathogens closely related to *Glomerella* (anamorph *Colletotrichum*, the causal agents of anthracnose diseases). Like many strains of *Fusarium oxysporum*, *Verticillium* causes vascular wilts that result in severe crop losses. In California, *Verticillium dahliae* affects many different hosts including lettuce, tomato and strawberry. Of lesser importance are *V. albo-atrum*, *V. tricorpus*, *V. longisporum* as well as *V. nubilum* which is only infrequently isolated from diseased plant materials. *Verticillium* spp. form thick-walled, highly melanized resting structures that can survive in the soil for many years. Species identification is largely based on the kind of resting structures produced, i.e., microsclerotia in *V. dahliae*, resting mycelium in *V. albo-atrum*, chlamydospores in *V. nubilum*, and all three kinds of resting structures in *V. tricorpus*. In this study, we used multilocus phylogenetic and morphological analyses of type material and a global sample of *Verticillium* with emphasis on California, to investigate species limits in *Verticillium*. Molecular data indicate that resting structure morphology might be a poor indicator of species limits, as *V. albo-atrum*-like morphology is present in at least two different, unrelated phylogenetic groups.

Atypical 'deep' lesions on specialty potato tubers in western Washington caused by *Colletotrichum coccodes*

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Black dot occurs commonly on potatoes throughout the Pacific Northwest of the U.S. Symptoms on tubers typically are superficial brown-to-gray lesions with poorly defined margins that discolor the periderm in circular or irregular patterns. In western Washington in 2009 and 2010, thin-skinned cultivars of Ambra, Chieftain or Yukon Gold displayed deep, sunken, hollow lesions from which isolates of *C. coccodes* (Cc) were obtained. Pathogenicity of one isolate was confirmed on Chieftain and White Rose (3 reps/cv; 3 pots/rep) in a greenhouse test where 7- and 3-wk-old plants, respectively, were inoculated 2 cm below soil mix line with cellophane (~9 × 4.5 cm) that had been colonized by Cc on 1/2 PDA plates, 10 wk prior to use. In France in 2003, Cc caused deep lesions on inoculated tubers kept between 5 and 15°C. Thus, four reps of five field-grown tubers of Ambra with similar percentage of deep lesions were photographed and analyzed for disease severity using APS Assess digital imaging software after placing in paper bags and storing inside plastic boxes with lids at 4, 7, 11, 15 and 20°C. Image analyses on 1/5/10, 12/3/10, 1/711, revealed black dot severity changing from average 38.5% to between 56.7% (for 7°C) and 64.5% (for 4°C); only 20°C treatment had significantly (P =

0.05) higher disease severity (81.6%) compared to the other temperatures after 3 mo. Studies on possible interactions between Cc isolates, potato cultivars and deep lesion formation are now planned.

Control of late blight on tomato in western Washington using high tunnels

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Control of late blight (LB) using high tunnels (HT) was studied near Mount Vernon, 2008 to 2010, by comparing HT vs. open field (OF) grown tomatoes, exposed to natural inoculum of *Phytophthora infestans*. Six-wk-old seedlings of five susceptible (Big Beef, Early Girl, Northern Delight, Oregon Spring, Stupice) and one resistant (Legend) cultivar were transplanted into one or two blocks of HT and OF plots on 6/03/08 or 6/02/09 using four reps of six plants per cultivar. In 2010 four blocks with four reps each of Early Girl, Oregon Spring and Stupice seedlings were transplanted 5/27/10 or 6/3/10 into HT or OF. Plants were drip-irrigated, staked and pruned, and rated weekly for disease. Environmental data were recorded every 15 min. Low LB pressure precluded differentiation between HT and OF plots in 2009. However in 2008, the range of area under disease progress curve (AUDPC) values across cultivars was lower in the HT (0 to <1) vs. OF (71 to 246) blocks. Similarly in 2010, average AUDPC values and percent blighted fruit were significantly ($P = 0.001$) less in the HT (<1 and 1.1%) vs. OF (344 and 13.2%) blocks. LB disease severity values (dsv) calculated by WISDOM software (UW-IPM) were also lower, number of days to 18 dsv longer, and total hr of leaf wetness less for HT compared to OF all three years. In both 2008 and 2010, fruit yield was higher or significantly higher in HT than OF indicating HT as a desirable tomato cropping system for managing LB in the region.

Implication of early-season fungicide application on season long dollar spot control

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Extended control of dollar spot (DS), caused by *Sclerotinia homoeocarpa* F.T. Bennett, has been reported with fungicides applied weeks before traditional preventive applications. Field studies were conducted on *Agrostis stolonifera* L. maintained at 1.3 cm in Connecticut (CT) and Pennsylvania (PA) to assess the effect of an early season fungicide application on DS control programs during 2010. Main effects included preventive fungicide timing (mid-April or late-May application of vinclozolin), summer applied fungicides (chlorothalonil, vinclozolin, or boscalid), and application interval of summer fungicides (14-, 21-, or 28-d). Dollar spot severity in the study areas increased in CT and PA during early- and mid-July, respectively, although results varied by location. In CT, DS was less severe in chlorothalonil and boscalid treated turf receiving a preventive application in mid-April compared to late-May during July and August, and August respectively. Mid-April preventive application reduced DS severity in turf treated every 21 d compared to turf receiving a late-May application. However, no difference was observed in 14 d treated turf in CT and PA. Additional significant preventive timing effects were not observed in PA. These data suggest early season fungicide applications can improve DS control throughout the season; however this effect appears to be inconsistent among locations.

Potential role of grafting as a method to manage *Verticillium dahliae* race 2 in tomato production systems

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Fresh market tomato production is one of the largest vegetable economic commodities in North Carolina. More than 2,000 acres are harvested each year across the state. *Verticillium dahliae* race 2, that causes Verticillium wilt (VW), is the main pathogen that limits productivity in western NC. Host resistance is not available to race 2 as it is for race 1 forcing growers to depend on soil fumigants or abandon the land for tomato production. We hypothesized that preferred scions can be grafted to rootstocks (RS) that confer plant vigor and associated tolerance to VW damage. Maxifort RS grafted to Mt. Fresh scions reduced VW incidence in open field experiments from over 50% in non-treated plots to 20–30%. Incidence in fumigated controls was less than 10%. However, marketable yield of large, extra-large and jumbo fruit harvested from Maxifort grafted plants was similar to yields from plants in fumigated soils. Grafted plant spacing at 50% of standard spacing generated yields similar to non-grafted plants spaced 46 cm between plants. In a complimentary study using several genetically diverse RS, tomato root and stem tissues were collected to assay the extent of pathogen colonization in the rootstock and scion tissue to discern if the rootstock limits colonization or confers true tolerance. Integrating grafting technologies with other IPM tactics could provide a viable tool in place of, or as a complement to, fumigation for managing *Verticillium dahliae* race 2.

***Fvfsr1* in *Fusarium virguliforme* affects the development of SDS in soybean**

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Fusarium virguliforme is a soil-borne pathogen that causes Sudden Death Syndrome (SDS), an important disease of soybean resulting in significant losses in yields every year. Despite the importance of SDS, a clear understanding of fungal genetic factors that affect the development of the disease is still lacking. We have identified *Fvfsr1*, a *F. virguliforme* gene that encodes a protein similar to a family of striatin proteins previously reported to regulate cell differentiation and ascocarp development in several *Fusarium spp*. Characterization of the fsr1 in other *Fusaria* revealed its direct role in pathogenesis. Fvfsr1, the fsr1 homolog in *F. virguliforme*, was disrupted using a split marker approach. The resulting *FvΔfsr1* transformant showed a significant decrease in conidiation compared to the wild type. A greenhouse pathogenicity assay was conducted to determine the effect of the disruption of Fvfsr1 on the aggressiveness of *F. virguliforme* on soybean. The disruption of Fvfsr1 resulted in a significant decrease in SDS incidence and severity in the inoculated soybean plants.

Fusarium wilt of strawberry, caused by *Fusarium oxysporum* f. sp. *fragariae*, a new disease in California

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Fusarium wilt of strawberry, caused by *Fusarium oxysporum* f. sp. *fragariae* (Fof) was discovered in California in 2008. Dieback caused by Fof has been restricted to fields in which pre-plant flat fumigation with methyl bromide and chloropicrin has been discontinued. Thus it appears that alternative practices - such as bed fumigation with chloropicrin and 1, 3-dichloropropene - have allowed soil populations of the pathogen to increase to damaging levels. Our objectives are to determine the limits of the infestation in commercial fruit production fields, assess the diversity of the pathogen population, develop an assay for quantification of the pathogen in soil, and assess the efficacy of various pre- and post-plant treatments to minimize the impact of the disease. Although initially limited to Ventura County, infested fields have since been located also in Monterey County, which is within the largest strawberry production district in California. Thus far, the population of Fof in California appears to be comprised of two somatic compatibility groups. Quantification of inoculum in soil is accomplished using a soil dilution plate assay, in which pathogen colonies are identified by their distinctive colony morphology. Control measures being evaluated include adjustments of soil pH to 7.0 or above, the application of *Trichoderma* in an attempt to reduce the rate of infection, and screening strawberry genotypes for resistance to the disease.

Diversity and fungicide resistance of *Phytophthora capsici* on vegetable crops in Georgia

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Phytophthora blight caused by *Phytophthora capsici* has become an increasing concern in vegetable production in Georgia. It is imperative to understand the phenotypic and genotypic diversity and fungicide resistance of the pathogen for more efficient management of the disease. Morphological, physiological, and genetic characteristics of *P. capsici* isolates from different vegetable crops in Georgia were determined in this study. The results indicated that *P. capsici* populations in Georgia are diverse. Although isolates from different hosts did not differ morphologically, 12 pathotypes were identified based on their aggressiveness on pepper cultivars. The aggressiveness of the isolates appeared not to be associated with host origin of isolation. All isolates grew at temperatures from 10 to 36°C but not at 38°C, and no isolate was able to recover after 5 days of incubation at 38°C. Chlamydospores were not produced by the isolates in liquid culture. The isolates were divided into five groups based on randomly amplified polymorphic DNA analysis, and genetic variations were moderately associated with geographical location and host origin of the isolates. Isolates insensitive to mefenoxam and cyazofamid were identified, but all the isolates were sensitive to fluopicolide and mandipropamid. These studies provide

useful information for designing more efficient programs to manage Phytophthora blight on vegetables.

The host-specific virulence activity of *Ralstonia solanacearum* type three effector PopS

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The bacterial wilt pathogen *Ralstonia solanacearum* injects protein effectors into host plants via a type three (T3) secretion system. The *R. solanacearum* pangenome encodes over 100 T3 effectors whose virulence functions are largely unknown. PopS, a T3 effector of the AvrE/HopR family, is present in all eight sequenced bacteria in the *R. solanacearum* species complex, including strains with wide and narrow host ranges. These PopS orthologs are 80–100% identical in amino acid sequence and their phylogenetic grouping mirrors that of their strain, suggesting that PopS is a conserved ancestral T3 effector. A comparative transcriptome analysis revealed that two genetically and ecologically distinct *R. solanacearum* strains, GMI1000 (Asian phylotype I) and UW551 (American phylotype II), both highly upregulate *popS* expression while colonizing tomato stem at the onset of wilt disease. UW551 mutants lacking *popS* are moderately delayed in virulence on susceptible tomato (*Solanum lycopersicum* cv. Bonny Best) and are more dramatically impaired in virulence on a quantitatively wilt-resistant tomato line (Hawaii 7996). However, the *popS* mutant had full wild-type virulence on bittersweet nightshade (*S. dulcamara*), an epidemiologically relevant weed host. This suggests that the contribution of PopS to virulence is host-specific. We are characterizing the virulence activity of PopS in diverse *R. solanacearum* strains and determining the effect of PopS on plant defense responses.

Tank-mixing of dodine in early-season apple scab programs and possibilities for renewed use in the eastern U.S.

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In recent years, site-specific fungicide resistant *Venturia inaequalis* (apple scab) populations have become a growing problem in apple orchards throughout the eastern U.S. Few options for managing fungicide resistance are available to growers. The objective of this study was to evaluate the benefit of re-introducing dodine in tank-mixes with captan or mancozeb in orchards where resistance to dodine had been detected. Trials were conducted in several states in the eastern U.S. (e.g. New York, Michigan, Virginia, Pennsylvania and North Carolina) using dodine alone and in mixtures with captan or mancozeb in the early apple season, with at least two applications. It was demonstrated that the use of dodine in tank-mixes did not result in control failures due to reemergence of practical resistance, but provided scab control that was as effective or improved over standard programs of protectant and site-specific fungicides for managing apple scab. Because the majority of the orchard populations in the tests were composed of sensitive isolates as well as those with reduced sensitivity to dodine, dodine may still prove useful in many orchards in the eastern U.S. This research helps elucidate the uncertainty surrounding the reemergence of practical resistance to dodine and associated crop failure.

Results of long-term trials to control diseases in cereal crops

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The control of weeds, pests and fungal diseases was studied in a long-term trial in two crop rotations (12 years) to contribute to the determination of the necessary minimum of pesticides. Dominant diseases investigated were brown rust (*Puccinia recondita*) and *Rhynchosporium* leaf spot (*Rhynchosporium secalis*) in winter rye, net blotch (*Pyrenophora teres*) in winter barley, and Septoria leaf blotch (*Mycosphaerella graminicola*) in winter wheat. The severity of these and other diseases was mainly determined by the annual weather-dependent infection pressure and cultivar susceptibility to diseases. Fungicides were used as soon as a certain disease threshold was exceeded. Two intensity levels, 100% (situation-related), and 50% of them, achieved good to very good fungicidal effectiveness, with the one of the lower dosage level often being significantly lower when highly infested. Cultivar resistance proved to be the determining factor of the need for fungicide treatment. In winter rye and winter barley, where resistance to the dominant diseases was rather low, at least one fungicide treatment was necessary in all years studied. In the highly resistant winter wheat cultivar Pegassos, on the other hand, moderate to higher infestation occurred in only three years, and no fungicide treatment was required at all in other three years. Generally, fungicide use was economically beneficial only in years with high infestation levels and weather conditions favourable to yield formation.

Community structure of *Aspergillus flavus* and persistence of the atoxigenic strain *A. flavus* AF36 in applied fields

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Aflatoxins are toxic and carcinogenic metabolites produced by several fungi in *Aspergillus* Section *Flavi* that frequently contaminate crops. Aflatoxins impact the value of crops. The use of atoxigenic strains of *A. flavus* to displace aflatoxin producers is a proven method to reduce aflatoxin contamination. Previous work indicated applications benefit both treated and subsequent crops. The current study sought to determine factors that influence persistence of AF36 and reestablishment of the highly toxigenic S strain of *A. flavus*. Results indicate significant differences between treated areas for both persistence of AF36 and development of the S strain. The percent of the *A. flavus* community composed of AF36 two years after application was higher in Mohawk Valley (>70%) than in the Yuma Valley (50%), while S strain incidence was higher in the Yuma Valley (>40%) than in the Mohawk Valley (20%). Regression analyses indicate that the Percent AF36 significantly decreased, while the Percent S significantly increased in the Yuma Valley. There was no significant change in either the Percent AF36 or the Percent S in the Mohawk Valley. Crop rotation significantly affects the structure of *A. flavus* communities. Cotton and lettuce production resulted in higher AF36 retention and reduced incidence of the S strain. The results suggest growing season, area, and crop rotation all influence the fungal community structure and long-term influences of the atoxigenic strain treatments.

Cultivation and formulation of an endophytic *Beauveria bassiana* strain

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Endophytes like the entomopathogenic fungus *Beauveria bassiana* play an important role in protecting plants against herbivorous insects. Our aim is to develop a process for fermentation and formulation of *B. bassiana* isolate ATP-04 in order to mass-produce and formulate the fungus in such a fashion that it infects rape plants and protects them from insect pests. *B. bassiana* was raised at 25°C and 150 rpm at pH 5.5 in shake flask cultures to produce submerged conidiospores which are reported to show a higher shelf life than mycelium and blastospores. In mineral media with 5% sugar beet molasses as carbon source, *B. bassiana* produced 2.3×10^8 submerged conidiospores/mL (1.2×10^9 total spores/mL) in 170 h following inoculation. By adding of 50 g/L NaCl 96 h after inoculation the submerged conidiospores concentration was increased to 5.1×10^8/mL (8.0×10^8 total spores/mL) in the same time span. Different formulation methods, such as encapsulation, film-coating and spraying were investigated. The radial growth of mycelium out of beads consisting of 2% Ca-alginate, 2% wet biomass, 10% protein and 1% technical yeast extract or autoclaved baker's yeast was higher by 8% compared to the beads without yeast. Film-coating of commercial fungicide-treated rape seeds with 2×10^5 spores/seed showed that *B. bassiana* grew on 80% of the seeds. Further experiments will deal with production of submerged conidiospores by fermentation and efficacy tests.

Comparative genomic analysis of *Xanthomonas axonopodis* pv. *citrumelo* strain FL-1195 and closely related bacteria

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Xanthomonas axonopodis pv. *citrumelo* (XACM) (Syn. *X. alfalfae* pv. *citrumelonis*, *X. campestris* pv. *citrumelo*) is the causal agent of citrus bacterial spot. XACM was isolated from and is limited to the nurseries of Florida. In this study, the genome of XACM str. FL-1195 was sequenced using 454-pyrosequening, Illumina (Solexa) sequencing and Opgen optical mapping. The finished sequence of XACM (4,967, 469 bp) was annotated and curated. Our analysis revealed that xacm lacked plasmids, although they are commonly associated with other strains of *Xanthomonas*. Phylogenetic analysis based on housekeeping genes revealed a close relatedness of XACM to *Xanthomonas axonopodis* pv. *citri* str. 306 (XAC) causing citrus canker and *Xanthomonas campestris* pv. *vesicatoria* str. 85-10 (XCV) causing bacterial spot in tomato and pepper. Whole genome comparison revealed a gene order

similar to both XAC and XCV. Several genome rearrangements and insertion/deletion regions indicating genome plasticity were found. An all against all BLASTP of the complete proteomes revealed a total of 410 coding sequences unique to XACM. Comparative genomic analysis showed various changes in genes encoding effectors, cell wall-degrading enzymes, lipopolysaccharides, etc. Further molecular analysis of these features could account for differences in virulence and host specificity of these strains.

Identification, hosts, distribution and molecular phylogeny of desert truffles in Iran

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Desert truffles are the hypogeous ascocarps of some ascomycetous ectomycorrhiza, which can be found in semi-arid ranges of Middle East, especially Iran. The present investigation was carried out on the identification, hosts, distribution and phylogenetic relationships of these ectomycorrhizal symbionts in Iran. Among specimens collected from different climatic conditions, *Terfezia iclaveryi*, *Tirmania pinoyi*, *T. nivea* and *Picoa lefebvrei* were identified in different parts of Iran. *T. claveryi* was present in most parts of Iran. Truffles usually appear after the rainy season in the months of February to April in Iran. The results of physico-chemical analyses on soil samples from different parts of Fars province in Iran showed that the genus *Tirmania* was more prevalent in soil with high CaCO3 and silt percentage than the *T. claveryi*. The location of survey sites are recorded with GPS as a point or polygon in latitude and longitude. The Canonical Correspondence Analysis (CCA) indicated that soil structure were most important environmental parameter that influenced truffles distribution. Phylogenetic analyses indicated a close genetic relationship between *Tirmania* and *Terfezia*. The field, laboratory and anatomical studies showed that *Helianthemum ledifolium*, *H. salicifolium*, *H. lipii* and *Carex stenophylum* have ectomycorrhizal association with the four species in the studied areas.

Validation of real-time PCR assays for bioforensic detection of model plant pathogens

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The U.S. agricultural sector is vulnerable to bioterrorist and criminal threats. To attribute such crimes, law enforcement agencies need forensically valid detection assays for plant pathogens of concern. In this work, real time PCR assays were developed, optimized and validated for the detection of three plant pathogen model systems: *Pseudomonas syringe* pv. *tomato*, *Wheat streak mosaic virus*, and *Xylella fastidiosa*. Validation consisted of determination of the linearity and range, limit of detection, sensitivity, specificity, and exclusivity of each assay. Assay exclusivity was determined by testing target-specific primers against three panels of nucleic acids: a multi-species plant panel, a multi-species animal panel, and a near-neighbor microbe panel. Additionally, mutagenized positive control plasmids, distinguishable from native signature by restriction enzyme digestion, were developed and validated for use in forensic testing. Each assay displayed linear amplification of the target nucleic acid, was capable of detecting as little as 100fg of target nucleic acid, and was shown to be specific to the target pathogen. Similarly, linear amplification of mutagenized positive control plasmids was observed. Results obtained with model pathogen systems provide the framework for future development and validation of similar assays for plant pathogens of high consequence.

Selecting antagonists for control of postharvest brown rot of stone fruits originating from latent infections

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Phytopathology 101:S81

In contrast to biological control of postharvest decays (BCPD) of fruits originating from wound infections after harvest, BCPD originating from latent infections occurring in the orchard has not been developed. This is largely due to the lack of methodology to screen and evaluate microbes for biocontrol activity against this type of infection. Therefore, we have developed a novel approach where interactions between the pathogen structure involved in latent infection (appressorium) and the test microorganism can be evaluated *in vitro* and then *in situ* on fruit for biocontrol activity. Appressoria of the brown rot pathogen, *Monilinia fructicola*, were produced on wax membranes or parafilm, and were treated with the test microbes. Microorganisms colonizing appressoria and mycelium were further tested for biocontrol activity on fruit containing artificially induced latent infections under laboratory conditions. We found several effective antagonists using this approach. The next step is to select those antagonists that are best adapted to conditions occurring during storage and handling of fruit.

Requirements of different regions of the 5′ nontranslated region in replication of *Grapevine leafroll-associated virus* 3

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Phytopathology 101:S81

The genome of *Grapevine leafroll-associated virus* 3 (genus *Ampelovirus*, family Closteroviridae) is 18,498 nucleotides (nt) in length with a 5′ nontranslated region (NTR) of 737 nt. In order to elucidate the role of such an unusually long 5′NTR in virus replication, we built a minireplicon cDNA clone of GLRaV-3 containing the 5′NTR, the replicase module, green fluorescent protein (GFP) under the control of virus coat protein promoter sequence, and the approximately 1.3 kilobase 3′ terminal sequences. The minireplicon cDNA clone was moved into the T-DNA region of the binary vector pCAMBIA1380 in such a way that the CaMV 35S promoter with a double enhancer cassette is at the 5′ end and a hammerhead ribozyme sequence is at the 3′ end of the cDNA clone. Agrobacterium-mediated delivery of the GLRaV-3 minireplicon into *Nicotiana benthamiana* leaves showed expression of GFP only in leaves co-infiltrated with silencing suppressors. Detection of GLRaV-3 specific genomic and subgenomic(sg) RNAs and the GFP gene sgRNA in Northern blots further confirmed that the presence of the ectopically expressed silencing suppressors is required for replication of GLRaV-3 minireplicon. Agrobacterium-mediated delivery of the GLRaV-3 minireplicon containing different portions of the 5′NTR and monitoring GFP expression in leaves co-infiltrated with silencing suppressors demonstrated that sequences at both ends of the 5′NTR contain elements that are essential for virus replication.

Elimination of small fruit viruses by *in vitro* therapy

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Germplasm of *Rubus* and *Ribes* sp. must enter the U.S. through quarantine, and virus infections in the material delay or prevent its release to breeding/research programs. *In vitro* pathogen elimination protocols using heat treatment and meristem therapy are being developed for these genera. Axillary buds of virus-infected *Rubus* sp. were grown aseptically at 4-hr alternating periods of 29°C and 38°C (HT) with 14 hr day lengths for 5 weeks. *In vitro* explants were tested for *Black raspberry necrosis virus* (BRNV), *Blackberry yellow vein associated virus* and *Tomato ringspot virus* using RT-PCR. ELISA was used to detect *Raspberry bushy dwarf virus* and *Tobacco streak virus*. Elimination of BRNV in these tests (5 weeks HT) prompted experiments where BRNV-infected explants were heat treated from 1 – 5 weeks. After thermotherapy, all induced shoots were grown on culture media, transplanted to soil, and subsequently tested as virus free for 5 months under greenhouse conditions. Testing of additional BRNV-HT plants and other virus-infected *Rubus* will continue. *Ribes* species infected with *Gooseberry vein banding associated virus* (GVBaV) were grown at 29–34°C due to the high mortality of explants at 38°C. Preliminary data indicates GVBaV may be eradicated with or without thermotherapy using meristem tips of ≤1 mm. Additional GVBaV-infected explants will be subjected to meristem extraction/heat to validate these results. These protocols can be used to clean up virus-infected germplasm entering the U.S. through quarantine.

Impact of global climate change over the geographic distribution of *Ceratocystis fimbriata* of eucalyptus in Brazil

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Phytopathology 101:S81

In December 1997 a new disease was observed in clonal eucalyptus reforestation in Brazil, caused by *Ceratocystis fimbriata*. This disease has caused serious damage to the country, being responsible for more of 40% of the mortality of plants. In this sense, this work had as an objective evaluate the potential impact of global climate change over the *C. fimbriata* in Brazil. Were prepared maps with the favorability of the climate to the occurrence of *C. fimbriata* in the current period and future. The future scenarios used (A2 and B2) were centered for the decades of 2020, 2050 and 2080. These scenarios were obtained from six global climate models (GCM’s) provided by

the Intergovernmental Panel on Climate Change (IPCC). Considering the global warming scenarios provided by the IPCC, it will reduce the potential risk of occurrence of *C. fimbriata* climate in Brazil. Furthermore, the most favorable period of the disease occurrence also will tend to reduce in future decades. These reductions are predicted in both scenarios for the future, but it will occur more sharply assuming the A2 scenario. Additionally, changes in the geographical distribution of the disease will occur from one month to another, with unfavorable areas becoming favorable and vice-versa. However, in spite of these changes, extensive areas will still continue being favorable for the occurrence of *C. fimbriata*, especially in Brazil's major producing regions.

Potential impact of climate change over the occurrence of black spot of papaya in State of the Espírito Santo

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Phytopathology 101:S82

The State of the Espírito Santo is the major producer of papaya of the Brazil. However, this culture is affected by various diseases. Among the diseases caused by fungi, the black spot (*Asperisporium caricae*) is the most important economic. This disease is responsible for causing significant losses in environmental conditions favorable. In this sense, this work evaluated the potential impact of global climate change over the occurrence of black spot of papaya in Espírito Santo. Were prepared maps with the favorability of the climate to the occurrence of disease in the current period and future. The future scenarios used (A2 and B2) were centered for the decades of 2020, 2050 and 2080. These scenarios were obtained from six global climate models provided by the Intergovernmental Panel on Climate Change (IPCC). Assuming future scenarios outlined by the IPCC, will occur reduce in occurrence of climatic favorability of black spot in both future scenarios (A2 and B2). Furthermore, the period of greatest risk of black spot will tend to reduce in future decades (A2 and B2). However, these planned changes will be larger in the A2 scenario compared to the predicted scenario B2. Therefore, changes in the geographical distribution of the disease will occur from one month to another, with favorable areas becoming unfavorable. However, in spite of these changes, extensive areas will still continue being favorable for the occurrence of black spot of papaya in Espírito Santo.

Identify the pathogen of tomato yellow leaf curl disease of Jiangsu, China

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Phytopathology 101:S82

In 2007, a new tomato disease occurred in Jiangsu, China, which caused great losses to local tomato production. The result of field investigation and partial sequences analysis shows that the disease associated with a Begomovirus. DNA-A component of the typical isolate (j01) was sequenced and no DNA-B or other satellite component was detected. Sequence analysis showed that the virus shared high identities with tomato yellow leaf curl virus (FJ609655). To investigate the pathogenicity of DNA-A, an infectious clone of j01 was constructed and inoculated tomato. Tomato leaves showed chlorotic and upward curling of the leaflet margin after 15 days, typical symptoms of tomato yellow leaf curl disease, which conformed the new tomato disease occurred in Jiangsu is caused by tomato yellow leaf curl virus. This work was supported by Special Fund for Agro-scientific Research in the Public Interest (Grant No. 201003065) and Jiangsu Agricultural Scienctific Self-innovation Fund (Grant No. CX[10]415, CX[10]207).

Identification of pathogens responsible for root rot diseases of wheat and maize in Hebei, China

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Phytopathology 101:S82

In the North China Plain, semi-annual cropping of winter wheat followed by summer maize is the most common agricultural production system. A shift to retention of maize residues rather than burning has been accompanied by an increase in root rot diseases. In 2008–2010, root rot diseases of maize and wheat were investigated and pathogens were isolated in two successive year. 1464 and 1678 maize plants were assessed for root rot infection at seedling stage with disease incidences 83 and 90% in 2008 and 2009, respectively. *Fusarium verticillioides* and *Bipolaris sorokiniana* were the most common root rot pathogens (44 and 20% of 902 isolates, respectively). Low frequencies of *F. graminearum*, *Pythium* spp. and *Rhizoctonia* spp. (about 3% each), were also found. 2319 and 1233 wheat plants were assessed for root rot diseases at tillering stage in 2009 and 2010, respectively. The diseased root and stem incidences were 72 and 32% in year 2009 while 80 and 73% in year 2010, with 16% (2009) and 12% (2010) of plants having serious root browning, discoloration and stunting. Again, *F. graminearum* (35% of 741 isolates) and *B. sorokiniana* (30%) were most popular pathogen. Moreover, *Rhizoctonia* spp., *F. acuminatum*, *F. equiseti*, *Pythium* spp. and *F. verticillioides* representing 11, 9, 3, 2 and 1%, respectively. The established long-term, wheat-maize rotation clearly favours the build up of pathogens responsible for root diseases in both wheat and maize. New integrated methods are needed to control root rot diseases in North China Plain.

Linkage block and recombination suppression at the *Pi-ta* locus at the centromere region of rice chromosome 12

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The *Pi-ta* gene, located near the centromeric region of chromosome 12 is an effective resistance gene to *Magnaporthe oryzae* that causes rice blast disease. *Pi-ta* has been incorporated into diverse resistant rice cultivars by classical plant breeding in the southern U.S. and worldwide. Previously, large linkage blocks around *Pi-ta* ranging from 14 to 27 MB were observed in rice cultivars and in backcross progeny derived from an indica x japonica cross. In the present study, the same linkage block was further examined in 1600 random recombinant individuals possessing or lacking *Pi-ta* that were derived from indica x japonica, indica x indica crosses. Simple sequence repeat markers spanning *Pi-ta* and the centromeric region were used to detect recombination break points and to delimit the physical size of the linkage blocks. Large linkage blocks ranging from 4.1 to 10 MB on chromosome 12 were identified from recombinant individuals of indica x japonica crosses. However, significantly smaller blocks, ranging from less than 400 kb to 1 MB, were identified in indica x indica crosses regardless of the presence of *Pi-ta*. The large linkage blocks previously observed in rice cultivars and backcrossing progeny was predicted to be a result of recombination suppression and selection for blast resistance. These findings suggest that crosses of indica x japonica rice have significant recombination suppression at the centromeric region of chromosome 12.

Alteration of gene expression profile in maize infected with a double-stranded RNA fijivirus associated with symptom development

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Phytopathology 101:S82

Maize rough dwarf disease caused by rice black-streaked dwarf virus (RBSDV) is a major viral disease in China. It is suggested that viral infection of plants might cause distinct disease symptoms through inhibiting or activating host gene transcription. We scanned the gene expression profile of RBSDV-infected maize through oligomer-based microarrays to reveal possible expression changes associated with symptom development. Our results demonstrated that various resistance-related maize genes and cell wall- and development-related genes such as those for cellulose synthesis were among these genes whose expressions were dramatically altered. These results could shed lights to finding new strategies to protect cereal crops against viruses and revealing the molecular mechanisms for the development of specific symptoms in rough dwarf-related diseases.

Discovering putative *Phytophora palmivora* disease tolerance genes in papaya (*Carica papaya* L.)

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Phytopathology 101:S82

Phytophthora palmivora is a destructive pathogen of papaya (*Carica papaya* L.). Field study, cultivar SunUp is susceptible while Kamiya is tolerant to *P. palmivora*. In this work, a normal distribution of tolerance to *P. palmivora* in F1 (Kamiya crossed SunUp), indicating that the tolerance measured is a quantitative trait. With cutoff of 80% in tolerant F2 and 40% in susceptible F2 plants analyzed with AFLP (amplified fragment length polymorphism) using 130 primer sets, seven polymorphic fragments were linked with disease tolerance. By screening ±2 Mbp upstream/downstream from the fragments to locate them within the papaya genome, five out of seven polymorphic loci were found putative resistance (R) proteins around it. Beside the R proteins, MAP kinase, WRKY transcription factor, hypersensitive-induced reaction (HR) protein, pathogenesis-related (PR) protein, pathogen-inducible ethylene-responsive factor (ERF), multidrug resistance-associated protein (MRP), tobacco rattle virus-induced protein, and avirulence elicitor response (Avr)

protein, were also located near the biomarkers included that may be involved in papaya tolerance to *P. palmivora*. This study highlights specific functional R genes' and resistance related genes' segregation reflected by *P. palmivora*-resistance related AFLP markers were dominated those candidates pathogen resistance genes from tens/hundreds others members in the gene families.

Radar observations of the migration of *Nilaparvata lugens* S. (Delphacidae) in southern China

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Phytopathology 101:S83

The brown planthopper, *Nilaparvata lugens* S., is one of the most serious pests of rice in both temperate and tropical regions of east and south Asia. It cannot overwinter in China. The migration regularity and flight behavior of *N. lugens* were studied by using an 8.8 mm wavelength scanning entomological radar, a searchlight trap and a ground light-trap, field cages, systematic field survey and dissection of female ovarian in 2007and 2009. *N. lugens* took off at dusk and dawn. The dusk take-off with area density peaking 45 mins later can last 1 h, while the dawn take-off can last 30 mins. After mass take-off, *N. lugens* climbed up high altitude rapidly. In spring and autumn the flight altitude can reach 2200 and 1800 m respectively. *N. lugens* formed 3 dense layers which were at heights of 400 to 700 m, 700 to 1000 m and 1100 to 1700 m in spring and 300 to 500 m, 600 to 700 m and 900 to 1100 m in autumn. The thickness of autumn layer was thinner than spring layer. Wind shear was the main reason causing *N. lugens* forming dense layer, while heavy rainfall caused mass descent. Collective orientations of *N. lugens* with the typical "dumbbell" echo often at the height of 800 to 1200 m on the PPI were observed in the autumn migration. The orientation direction was 159.4 ± 15.14° with an acute angle 53.4 ± 13.74° to the wind direction.

Mycoviruses that infect plant pathogen *Sclerotinia sclerotiorum*

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Phytopathology 101:S83

Sclerotinia sclerotiorum is a ubiquitous inhabitant of soil in many parts of the world. The potential of hypovirus for biological control of chestnut blight (*Cryphonectria parasitica*) has attracted much interest, and led to discovery of new hypovirulent strains in other fungi. In our lab, five mycoviruses were characterized from *S. sclerotiorum*. A ssDNA virus, named as SsHADV-1, isolated from debilitated strain DT-8, is phylogenetically related to plant geminiviruses. This DNA virus could protect plants against infection of *S. sclerotiorum*. Two ss (+) RNA viruses, namely SsDRV and SsRV-L, co-infect debilitated strain Ep-1PN. SsDRV is associated with the debilitation. SsRV-L belong to Rubi-like subfamily, and relates to Hepatitis E virus phylogenetically, while SsDRV relates to plant Potexvirus. These two viruses have only one ORF, coding for RNA replicase. Two dsRNA mycoviruses, namely totivirus-like virus SsTV-L and partitivirus SsPV-S, co-infect strain Sunf-M which shows normal phenotypes. Surprisingly, SsPV-S CP has the highest similarity to ILR2 of *Arabidopsis thaliana*, thus, there was a gene horizontal transfer between SsPV-S like virus and ancestor of A. *thaliana*. Following this clue, we found that the horizontal gene transfer between dsRNA viruses and eukaryotes were common. Besides the five characterized viruses, 19 new viruses were identified from other *S. sclerotiorum* strains, suggesting that there is a rich dive rsity of mycoviruses in *S. sclerotiorum*.

Phylogenetic relationships among *Verticillium dahliae* vegetative compatibility groups based on IGS and polymorphic sequences

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Phytopathology 101:S83

Verticillium wilts caused by the soilborne fungus *Verticillium dahliae* are amongst the most challenging diseases to control. Populations of this pathogen comprise a few clonal lineages that correlate with vegetative compatibility groups (VCGs) and most genetic markers used to date have been unable to resolve diversity within clonal lineages. The objective of this study was to determine the phylogenetic relationships that might exist among VCGs and their subgroups. For this purpose, we analyzed sequences of the intergenic spacer region of the ribosomal DNA (IGS) and six anonymous polymorphic sequences in *V. dahliae* isolates representing the main VCGs and subgroups (VCGs 1A, 1B, 2A, 2B, 3, 4A, 4B, and 6) from different geographic origins and hosts. IGS alignments revealed a complex structure with numerous large indels. Phylogenetic analysis indicated that certain subgroups (e.g., VCGs 1A and 1B) are closely related and share a common ancestor; however, other subgroups (e.g., VCG 4A) are related more closely to members of a different VCG (e.g., VCG 2B) than to subgroups of the same VCG (VCG 4B). Furthermore, our analyses indicate that VCG 2B is polyphyletic with members placed in at least three distinct phylogenetic lineages. These results raise questions concerning the significance of VCG groups and subgroups, the need for reassessment of VCGs in *V. dahliae*, and the adequacy of using current VCG analysis for assessing genetic diversity in *V. dahliae* populations.

Genetic diversity and temporal dynamics of *Venturia inaequalis* populations following two Apple scab epidemics in Pennsylvania

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Phytopathology 101:S83

Apple scab, caused by *Venturia inaequalis*, is one of the most important diseases in apple production with increasing management challenges. Pathogen populations have developed resistance to nearly every fungicide used, and there is a limited availability of resistant cultivars with agronomic characteristics that are commercially accepted. In this study we analyzed how populations of *V. inaequalis* changed during Apple scab epidemics in PA in the absence of chemical control. Sampling was done from two cultivars differing in their resistance to Apple scab: 'Golden Delicious' (susceptible) and 'Rome Beauty' (highly susceptible), at the beginning (May) and near-end (September) of two epidemic years, 2008 and 2009. Eight populations of *V. inaequalis* (765 isolates) were analyzed using seven microsatellite markers. Overall, in 2008 we observed a significant reduction of genotypic diversity and a dramatic shift in genotype composition from May to September in 'Rome Beauty', whereas populations from 'Golden Delicious' maintained the same level of diversity throughout the epidemic. However, populations in both cultivars remained stable and did not change significantly in 2009. These results suggest that fitness competition between individuals is more intense on highly susceptible cultivars than on cultivars carrying some resistance genes. We also hypothesize that the pathogen population structure in a given year may be highly influenced by weather and disease pressure during the preceding year.

Dynamic monitor of physiological race variation for wheat stripe rust in Gansu province in China

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Phytopathology 101:S83

South Gansu is a variable physiological-race area of wheat stripe rust in China, from where a lot of dominant races are developed and prevail. In Gansu, the race monitoring of wheat stripe rust can provide advanced information for disease forecast and rust-resistance breeding. During the period of 2008~2010, there are 977 samples from 30 Gansu counties are monitored and in which 36 races are detected by Chinese differentials. The analysis to virulent gene indicates that the proportion rates of virulent strains to Yr-9, Yr3b+Yr4b, Yr-Su are respectively for 92.0%, 21.9% and 97.5%. CYR32 is firstly dominant race in the monitored 36 races, with the frequencies of 26.6%. While the second populated race is CYR31 with the frequencies of 16.9% And those races such as CRY21, CRY23, CYR25, CRY27, CYR28, CYR29, CYR31, are not the main races, and only with the frequency of 0.1%~1.7%. It's notable that in 2010, there are 20 pathogenic strains are monitored to variety Guinong 22, Zhong 4, Chuanmai 42, 92R137 and T. spelta album. At present, Guinong 22, 92R137 and T. spelta album are all immune to CYR31, CYR32 and CYR33, which are used as resistant resources in many breeding plans. The occurrence of these new strains might be a potential threat and should be paid more attention.

Studies on viability of sclerotia collected from Sclerotinia stem rot infected soybean plants in Iowa during 1995–2010

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Phytopathology 101:S83

Sclerotinia stem rot of soybean is caused by an ascomycetous fungus *Sclerotinia sclerotiorum*. In severe cases, this disease can cause up to 50% yield reduction in commercial fields. In developing management approaches of the disease, sclerotial viability plays an important role. Therefore, we studied viability of sclerotia collected during 1995–2010 from naturally infected soybean plants, northeast research and demonstration farm, Iowa and were stored in clear glass vials with screw caps at lab temperature. Viability of sclerotia was tested following germination test on PDA and apothecia production on sterilized vermiculite (20 g vermiculite +10 ml DSW). In

germination test, 20 surface sterilized sclerotia from each year were placed on PDA (5 sclerotia per plate), a week after incubation at 21 ± 1°C sclerotia was evaluated for mycelium production, after sub-culturing of that mycelium evaluated for sclerotia production. Sclerotia from 2008 to 2010 showed >90% germination and reproduction of sclerotia, while sclerotia collected between 1995 and 2007 showed low or 0% germination. Similarly, 20 surface sterilized sclerotia from each year were placed on vermiculite (5 sclerotia per plate). Weekly examination for five months did not show any apothecia. If the sclerotia were collected from the white mold infested soil and were stored in soil, might have different inferences. This study may aid in development of management strategies of white mold.

Insecticidal activity of cantharidin against *Plutella xylostella* and its toxicological mechanism in Lepidopteran cells

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Phytopathology 101:S84

Stomach toxicity of cantharidin to 3rd larvae of *Plutella xylostella* and its effects on activities of glutathione-S-transferase (GSTs) and acetylesterase (AChE) were tested in the laboratory. The results showed that the cantharidin had the stomach poisoning action on the *Plutella xylostella* larvae. At a concentration of 2 mg/mL, the corrected mortality of the larvae was 100% 72 h after cantharidin application. The LC_{50} against the 3rd larvae were 515.58 ug/mL for 72 hours. In addition, At the dosage of 12.5×10^{-3} mg/mL of the *Plutella xylostella* larvae were treated by cantharidin, the highest GSTs activity was 2825.17 $U \cdot mg^{-1}$ $prot \cdot min^{-1}$ at 12 h, while the highest AChE activities was 12.27 $U \cdot mg^{-1}$ $prot \cdot min^{-1}$ at 4 h. While the GSTs and AChE activities had significantly decreased at 24 h. The outcomes suggested cantharidin can influence GSTs and AChE activities of *Plutella xylostella* larvae. DNA damage level of *Spodoptera frugiperda* cells (sf-9) after treated by cantharidin was detected by comet assay. After different concentrations of cantharidin incubation, tail DNA, tail length of cultured sf-9 increased, and head DNA decreased while the concentration of cantharidin increased. There were significant differences compared with the control group. The results indicated that higher the dose of cantharidin, severer the DNA damage of Lepidopteran cell lines. DNA damage of cantharidin incubated sf-9 was of obvious dose-effect relation, which would indicate that DNA damage played a role in toxic effect mechanism.

Identifying genes differentially expressed during early interactions between the stem rot fungus (*Sclerotium rolfsii*) and peanut (*Arachis hypogaea*)

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Phytopathology 101:S84

Cultivated peanut is a major source of food and vegetable oil worldwide. *Sclerotium rolfsii* causes stem rot in peanut. The objective of my project is to identify genes in the fungal and peanut genomes that are differentially expressed during the early phase of the plant-pathogen interaction. By focusing on the early phase we expect to identify recognition events and cell signaling mechanisms. Four peanut cultivars varying from resistant to susceptible and one virulent fungal strain were selected. The fungal strain used is known to be virulent on peanut. Total RNA from infected stem and crown tissue was extracted, cDNA synthesized and 454 sequencing was performed, This generated 260390 sequences. Automated trimming and validation by Seqclean generated 225793 sequences. Comparisons of the sequences from each sample should highlight differential gene expression related to host plant resistance. Differentially expressed genes identified during bioinformatic analysis will be confirmed using qrt-PCR. Understanding the different genetic and biochemical pathways expressed early in the infection process will potentially provide new targets to disrupt the life cycle of S. rolfsii, stopping or slowing infection by this significant peanut disease-causing organism. Additionally, identification of pathogen responsive genes in peanut will add to the understanding of gene expression in peanut and could identify valuable sequences useful in potential future transgenic disease control strategies.

Comparative host response of grapefruit and alemow to narrow and broad host range strains of *Xanthomonas citri* subsp. *citri*

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Phytopathology 101:S84

Citrus canker, caused by *Xanthomonas citri* subsp. *citri* (*Xcc*), causes significant crop losses and economic impact in subtropical climates due to defoliation, premature fruit drop, and export regulations. The A strain of *Xcc* is highly virulent on a broad range of citrus species. Variant strains, A^W and A^*, have a restricted host range including Mexican lime (*Citrus aurantifolia*) and alemow (*C. macrophylla*), but do not cause canker lesions on hosts highly susceptible to A strain such as grapefruit (*C. paradisi*). Microarray and quantitative reverse-transcription PCR were used to evaluate the differential host response of alemow and grapefruit to *Xcc* A and *Xcc* A^W. 804 differentially expressed genes were identified 6 hours post inoculation. Of these genes, 47 are differentially regulated in grapefruit inoculated with *Xcc* A^W compared to all susceptible interactions and 129 genes are differentially regulated upon inoculation with *Xcc* A^W compared to *Xcc* A in either grapefruit or alemow. Genes are regulated in 29 functional groups based on MapMan including cell wall metabolism, protein degradation, hormone signaling and metabolism, transport, and biotic and abiotic stress including multiple leucine-rich repeat coding genes. The temporal expression changes in response to infection of a subset of genes across functional groups are being investigated with focus on those genes differentially expressed in the grapefruit-*Xcc* A^W interaction.

Possible interactions between Huanglongbing and nutrients in symptom development and bacterial movement

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Huanglongbing (HLB) is a devastating systemic disease of citrus caused by *Candidatus* Liberibacter asiaticus (*Las*). Florida growers report that supplemental foliar nutrient programs (NPs) maintain productivity of HLB-infected trees, implying that nutrients affect the infection process. The efficacy, mechanism, and sustainability of NPs have not been validated. The main cause of HLB symptoms, yield reduction, and tree decline is disruption of phloem, which blocks source-sink flow of photosynthate and nutrients. If nutrients affect bacterial movement or virulence mechanisms they should reduce replication and spread of *Las* or phloem plugging. Greenhouse trees sprayed with different nutritional treatments are being monitored for bacterial titer and development of disease symptoms, including phloem cell morphological changes, plugging, necrosis, and starch accumulation. *Las* distribution in roots and shoots differs from the untreated control for some nutrients, but overall, *Las* populations and incidence do not. Microscopy shows limited damage in midrib phloem of mainly root-colonized plants; however, visible leaf symptoms and unusual starch granules in phloem tissue were observed. Further characterization of the role of root colonization in disease development is underway. A complimentary field trial is evaluating bacterial titer, yield and tree health in a south Florida Hamlin orange grove with a mixture of healthy, asymptomatic (PCR+) and HLB symptomatic trees.

Effect of inoculum placement on alternative *Pythium* control methods for tobacco transplant production

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Phytopathology 101:S84

Pythium species limit tobacco transplant production in hydroponic greenhouses. Terramaster (etridiazole) provides excellent control, but is expensive, can result in plant growth regulator effects, and fungicide-treated nutrient solution must be appropriately disposed of after transplanting. Greenhouse tests were therefore conducted to evaluate alternative methods of *Pythium* control. Trials compared damping off and root rot of burley tobacco cultivar 'TN90' associated with various control treatments, which included incorporating Naiad (a commercially-available surfactant) or Terramaster (etridiazole) in the nutrient solution upon which seedlings were grown, or exposing nutrient solution to ultraviolet (UV) light. Curative application of Terramaster minimized *Pythium* incidence at all dates. In addition, 1000 ppm Naiad suppressed (k-ratio = 100) disease levels versus the untreated control and comparably to curative application of Terramaster. Although disease arising from inoculated trays tended to be higher than when inoculum was added to nutrient solutions, disease control from use of Naiad and Terramaster were consistent regardless of initial inoculum placement. A single exposure of nutrient solution to UV light did not acceptably control *Pythium* rot. Further, more detailed, investigation is needed to identify alternative methods to achieve commercially acceptable control of *Pythium* species on tobacco seedlings.

Phytophthora ramorum research at the National Ornamentals Research Site at Dominican University of California

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Phytopathology 101:S85

World-wide, *Phytophthora ramorum* has emerged as a quarantine-pathogen of significance, spurring emergency regulatory actions to address the spread of *P. ramorum* within ornamental nurseries and from infested nursery stock to native wildlands. After four years of collaborative efforts between California Department of Food and Agriculture, U.S. nursery industry, California Oak Mortality Task Force, National Plant Board and USDA, a site to perform research on *P. ramorum* under natural conditions was located in California. Funding via the U.S. Farm Bill (Section 10201) was secured and administered by the Center for Plant Health Science and Technology to develop the National Ornamentals Research Site at Dominican University of California (NORS-DUC). Situated in *P. ramorum*-quarantined Marin County, California, NORS-DUC was developed with safeguards to contain the pathogen and prevent spread to the environment. NORS-DUC has provided an unparalleled opportunity for researchers that are now conducting field studies to address the treatment of and prevention of *P. ramorum* in soil, the risk of asymptomatic infection in fungicide-treated plants, the effect of fungicides on inoculum production, biological control agents, and the affects of abiotic stress and ramorum blight in nursery ornamentals. While the current research at NORS-DUC focuses on *P. ramorum*, and will benefit the nursery and forestry industries, the data gathered will be applicable to the management of other pathogens.

Management of papaya mealybug, _Paracoccus marginatus_ through biological control

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Phytopathology 101:S85

Papaya mealybug, *Paracoccus marginatus* has got introduced into Tamil Nadu during July, 2008 and subsequently spread to southern states of India. The pest infested more than 80 hosts and the damage varied from 5–100 per cent. Integrated Pest Management practices helped to contain the pest below ETL for a fortnight and again the infestation reappeared in 10–15 days which necessitated the farmers to go for repeated application of insecticides. Since no effective native parasitoids are available, three parasitoids viz., A*cerophagus papayae*, *Pseudleptomastix mexicana and Anagyrus loeckii* were imported from Puerto Rico, U.S.A. *A. papayae* was mass multiplied in 57 research stations of Tamil Nadu Agricultural University located across Tamil Nadu state. The parasitoids were released from October, 2010 onwards @100/village in the infested crops of papaya, cassava and mulberry. So far three hundred thousand parasitoids were released across the state. Observations on pre and post release data revealed that there was 80 – 99 per cent reduction of mealybug in papaya, cassava and mulberry. Multiplication rate of parasitoid was 10–15, 5–10 and 8–14 times in papaya, cassava and mulberry respectively. Due to the adoption of classical biological control the mealybug is under control in Tamil Nadu, India. This is one of the success example of classical biological control in Tamil Nadu, India in recent years.

Relationship between stink bugs and seed decay in Mississippi soybean production

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Phytopathology 101:S85

In recent years, seed quality has become an important issue associated with Mississippi soybean production. Fungi, including *Phomopsis* spp. are major contributors to quality issues; however, other biotic and abiotic factors reduce seed quality. The stink bug is a major pest of soybean throughout the world. Stink bugs damage soybean by penetrating the pod hull with piercing-sucking mouth parts and extracting nutrients from the maturing seeds. However, depriving the seeds of vital nutrients is not the only risk to soybean. Damaged pod hulls likely result in the seeds being exposed to potentially pathogenic organisms that can further reduce yield and quality. The *Diaporthe-Phomopsis* complex is comprised of several fungi that cause yield and quality loss in soybean, with the primary pathogen being *P. longicolla*. To determine the relationship between stink bugs and seed decay in Mississippi soybean production; a survey of producer fields was conducted. Soybean seeds collected from 30 producer fields were divided into three categories including unblemished, damaged, and stink bug damaged. Seeds were surface disinfested and plated on acidified potato dextrose agar. The frequency of fungi was determined for each seed group. Significantly higher numbers of fungi were recovered from stink bug damaged seed compared to the other two groupings. The results indicate that stink bug damaged seeds contain a significantly higher percentage of fungi compared to unblemished seeds.

Limited effects of foliar insecticidal treatments on the spread of grapevine leafroll disease

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Phytopathology 101:S85

A field experiment was conducted at the experimental farm to investigate the effectiveness of foliar insecticide sprays on controlling the spread of grapevine leafroll disease (GLRD). The vineyard block has 20-year old Cabernet Sauvignon grapevines infected with GLRD. The symptomatic vines were tested positive for GLRaV-3 in one tube-single step RT-PCR using primers specific to a portion of the heat-shock protein-70 homolog (HSP70h). Within each row, all but one GLRaV-3-infected vines were removed and re-planted with certified Cabernet Franc cuttings at approximately 5 and 10 feet away from each infected vine. After planting, foliar insecticide treatments were applied in a randomized block design of six replications. The treatments were (i) a neonicotinoid treatment at delayed dormant stage, (ii) a neonicotinoid treatment at delayed dormant stage plus a pyrethroid treatment at bloom and (iii) control. Petiole samples were collected in 2009 and 2010 seasons from individual vines of Cabernet Franc and extracts from these samples were tested for GLRaV-3 as described above. The results from both the seasons indicated that spread of the virus from Cabernet Sauvignon to Cabernet Franc vines could occur as soon as a few months after planting of new vines. Insecticide application appeared to have effectively limited the movement of the vector during the first year, but mealybugs were observed even on the treated vines during the second year of the season.

Multiple copies of genes encoding endoglucanase inhibitor proteins are harbored in an 85kB region of potato genome

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Phytopathology 101:S85

The XEGIPs (xyloglucan-specific endoglucanase inhibitor protein) and their closest homologues, the EDGPs (extracellular dermal glycoproteins) have been reported in various plants, principally Solanaceous ones. One function of XEGIP is limiting pathogen attack by interfering with pathogen family 12 endoglucanases. The XEGIP gene from tomato and potato was believed to be a single copy, however a cluster of nine similar genes has been found on a single region of potato chromosome, independent of the single XEGIP previously reported. Tomato has a similar number, while pepper, eggplant, nightshade and tamarillo are lacking most of these nine genes. Each plant species was found to have a copy of the originally reported XEGIP. Expression patterns show differences in tissue type, with three of the XEGIP-like genes highly expressed in leaf tissue, and one of the EDGP-like genes highly expressed in potato cell culture. Differential expression was also seen in potato tubers, and tomato fruits. The large number of XEGIP/EDGP encoding genes in potato suggests a selection pressure for defense against the multiple copy family 12 endoglucanase-encoding genes found in the potato pathogen, *Phytophthora infestans*.

Pathogenicity of _Coconut cadang-cadang viroid_ (CCCVd) variants on oil palm (_Elaeis guineensis_ Jacq.) seedlings

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Phytopathology 101:S85

Coconut cadang-cadang viroid (CCCVd) that causes the Coconut cadang-cadang disease in coconut palms in the Philippines is associated with Orange spotting (OS) disease of oil palm. Hanold and Randles (1989) isolated CCCVd-like molecules from oil palm in the South Pacific and reported that the OS could cause a yield reduction up to 50% in single palm compared to healthy adjacent palm. Recent study by Vadamalai et al. (2006) confirmed that CCCVd variants were present in commercial oil palm plantation in Malaysia. Cloning and sequencing revealed that the CCCVd variants from oil palms has more than 90% sequence similarity with $CCCVd_{246}$ from coconut. However mechanical transmission of the CCCVd variants to oil palm has yet been conducted. In this study, nucleic acid extracted from symptomatic palms was inoculated into oil palm seedlings. Successful transmission of CCCVd

variants in oil palm seedlings were observed when OS symptoms were expressed 6 months after inoculation. This was further proven with dot blot assay when the nucleic acid extract of the inoculated seedlings hybridized with CCCVd full-length complementary probe. Cloning and sequencing revealed that all variants from inoculated seedlings were 246 nt in length and has 97% sequence similarity with coconut $CCCVd_{246}$. This study confirmed that CCCVd variants isolated from oil palm were pathogenic and replicating autonomously in its host.

Cherry leaf spot disease management in ornamental flowering cherry

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Phytopathology 101:S86

Cherry leaf spot (CLS) disease caused by a fungus *Blumeriella jaapii* is an important disease of sweet and sour cherries and other *Prunus* species; it has increasingly become a significant constraint in nursery production of flowering cherries causing great concern to growers in the Southeastern United States. While growers have attempted to control this disease with fungicides, efficacy of fungicide sprays has been poor because of poor timing of spray programs starting May. The objective of this study was to evaluate winter survival of the pathogen and assess the timing of infection establishment in mid-Tennessee to guide growers. In addition, previously infected plants were maintained in greenhouse environment protected from airborne inoculum and assessed for CLS disease development. Air-borne ascospores trapped from previously infested fields showed that infested leaf debris provided significant amounts of primary inoculum starting in early March with a peak in mid May. First CLS symptoms were observed in early April and fungicide spray program that started when petals start falling and new leaves start forming were highly effective in disease control. Development of cherry leaf spot symptoms in greenhouse plants showed that dormant buds constitute an important source of primary inoculum in Tennessee and the use of cuttings from infected trees may play a significant role in disease perpetuation.

Development of a *Tobacco streak virus* (TSV)-based gene silencing vector for soybean seed development

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Phytopathology 101:S86

Virus-based gene silencing systems are powerful tools for functional genomics that permit knockdown of expression of individual genes or closely related gene families. TSV shows recovery from initial symptoms and efficiently invades both meristems and developing embryos in soybean making it an excellent candidate for a virus-based silencing system for those tissues. TSV RNAs 1, 2, 3, and 4 were cloned into pHST40, a pUC-based plasmid vector, and pCASS-4RZ, an *Agrobacterium tumefaciens*-compatible binary vector. Both sets of clones were infectious in soybean and tobacco. A multicloning site was introduced into a truncated 2b gene of pHST40-RNA2 and the clone was stably seed transmitted in soybean. Magnesium chelatase (MgCh) gene fragments of 105 nt and 175 nt were inserted into the truncated 2b vector and were stable in systemic leaves of inoculated 'Williams82' plants, which exhibited pronounced leaf yellowing typical for silencing of MgCh mRNA. RNA 3 of the pCASS-4RZ clone was partitioned between two RNAs, one with only the movement protein (pCASS-R3Mp) and the other expressing only the coat protein (pCASS-R3Cp). Full-length green fluorescent protein (GFP) and phytoene desaturase (PDS) coding regions were inserted into pCASS-R3Mp and pCASS-R3Cp, respectively. Inoculated tobacco plants showed stable expression of GFP and photo-bleaching symptoms, which is consistent with silencing of PDS mRNA. The tissue specificity and persistence of silencing phenotypes are are being evaluated in soybean and tobacco.

Application of the 2-cyanoacetamide method for spectrophotometric assay of cellulase enzyme activity

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Phytopathology 101:S86

Cellulose is the most abundant form of carbon on the planet. Breakdown of cellulose in the plant cell wall is a means by which microbes gain ingress into their hosts. Cellulose degradation is also important for global carbon recycling and is the main substrate for the production of biofuels. The alkaline dinitrosalycylic acid (DNS) method is widely used to assay the enzymatic hydrolysis of cellulose, but is influenced by incubation conditions, and utilizes phenol. Therefore, we have developed a cellulase assay that is capable of detecting D-glucose using carboxymethylcellulose (CMC) as a model substrate. Our data show that this method is as linear and sensitive as the DNS test in detecting fungal cellulase activity. Other factors that may affect the detection of cellulase activity such as: compatibility with commonly used buffer systems, varying buffer pH, and methods to terminate the enzyme catalyzed reaction, will be presented. Data from this study can directly be used to accurately and efficiently assay cellulase activity in a wide range of buffer systems at various pH's without the use of potentially hazardous chemicals.

Nature of *Ceratocystis smalleyi – Scolytus quadrispinosus* interactions on stems of bitternut hickory with declining crowns

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Phytopathology 101:S86

Rapid crown decline and mortality of bitternut hickory in north central and northeastern U.S.A. forests recently have been attributed to damage of host stems by the canker fungus, *Ceratocystis smalleyi*, and the hickory bark beetle (Hbb), *Scolytus quadrispinosus*. However, the nature of the interactions between these organisms in causing disease is unclear. Three trees, between 23 and 27 cm dia, with crown decline ratings of 40, 55 and 80% were debarked and found to have 178, 997, and 1,488 Hbb attacks on the main stem, respectively, in two Wisconsin forest stands. Of these attacks 24, 105, and 551 were associated with inner bark necrosis and discolored sapwood typical of Ceratocystis canker. Only 15 cankers had no evidence of beetle attack. Isolations from Hbb-associated cankers routinely yielded *C. smalleyi*. The fungus also was commonly isolated or detected via PCR and cloning on exoskeletons of Hbb captured during initial attack of hickory in late summer. However, *C. smalleyi* was not isolated from 120 Hbb emerged from stem sections of four trees with >55% crown decline, although 3 of 41 adults obtained by bark excavation prior to emergence did yield the fungus. In summary, Ceratocystis cankers are very frequently associated with Hbb attacks but evidence for dispersal of the pathogen from diseased trees by Hbb is lacking. The hypothesis that Hbb is the primary vector of the pathogen needs further study.

Sclerotinia sclerotiorum utilizes oxalic acid to hijack defenses and manipulate the host redox environment

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Phytopathology 101:S86

Sclerotinia sclerotiorum is a necrotrophic ascomycete fungus with an extremely broad host range. This pathogen produces the non-specific phytotoxin and key pathogenicity factor, oxalic acid (OA). Our recent work indicated that this fungus and more specifically OA, can induce apoptotic-like programmed cell death (PCD) in plant hosts. Notably, this induction of PCD and disease requires generation of reactive oxygen species (ROS) in the host; a process triggered by the fungal secreted OA. Conversely, during the initial stage of infection, OA also dampens the plant oxidative burst, a host response generally associated with plant defense. We generated and used transgenic plants expressing a redox-regulated GFP reporter to show that this pathogen (via OA) generates a reducing environment in host cells that suppress host defense responses. In contrast, our non-pathogenic OA deficient mutant (A2) failed to alter host redox status. Importantly, this mutant produced hypersensitive response-like features following host inoculation including ROS induction, callose deposition, and restricted cell death. We used Lysotracker dyes, Monodansylcadaverine (MDC) staining, and transmission electron microscopy (TEM) to show that the restrictive cell death observed upon A2 challenge, involves an autophagic response. These results indicate active recognition of this mutant and further point to an OA-mediated suppression of defenses by the wild type fungus.

Siderophore loci in *Agrobacterium vitis* strain F25 are associated with its ability to provide biological control of grape crown gall

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Phytopathology 101:S86

Crown gall of grape, caused by *Agrobacterium vitis* is a limiting factor in grape production worldwide. A non-tumorigenic strain of *A. vitis*, F25, is able to prevent crown gall on grape when applied to wounds prior to the pathogen. F25 was sequenced and mutations were made in biological control candidate genes and mutants tested for activity on grapevines. Three siderophore loci were detected in F25, one that is unique among sequenced Agrobacterium

species. Knockouts within this cluster and in another cluster resulted in biological control-negative phenotypes. The knockouts did not affect the ability of F25 to cause necrosis of grape explants or induce a hypersensitive response on non-host plants. The effect of F25 and mutants on populations of the pathogen in wounded grape tissues was also evaluated.

Influence of fungicide timing and post application irrigation on dollar spot severity

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Phytopathology 101:S87

Dollar spot, caused by the pathogen *Sclerotinia homoeocarpa* F.T. Bennett, is a common disease of golf course turf. Our study was conducted to compare early versus traditional preventive applications of different fungicides and the influence of post application irrigation on disease suppression. A total of seven evaluations were conducted between 2008 and 2010 in Connecticut and Pennsylvania. All studies were designed as a 2 × 2 × 4 factorial and arranged as a randomized complete block with 4 replications. The main treatments included timing (mid-April or mid to late May), irrigation (none or 2.5 mm), and fungicide (none, propiconazole, boscalid or vinclozolin). All treatments decreased dollar spot when compared to the untreated control plots, but few differences were observed among the main effects. Of 50 rating dates assessed across all studies, dollar spot was reduced on only 5 and 4 dates in plots treated at early or traditional timings, respectively. Irrigation was only significant on 3 of 50 rating dates and in all cases, the application of post application irrigation resulted in an increase in dollar spot severity when compared to plots receiving no irrigation after application. Results of this study indicate that while early season fungicide applications may suppress dollar spot infection centers, they may offer little benefit over properly timed preventive fungicide applications.

Correlation of environmental and edaphic factors to the isolation frequency of *Rhizoctonia* and *Chrysorhiza* from seashore paspalum

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Phytopathology 101:S87

Hymenomycete genera include the anamorphs *Rhizoctonia* and *Chrysorhiza*. Species of fungi in these genera have similar anamorphic characteristics, and their respective teleomorphic structures rarely are observed in nature. Comparison of conserved DNA sequences such as rDNA that includes internal transcribed spacer (ITS) 1, the 5.8S ribosomal subunit, and ITS2 can help identify some of these fungi to species. 74 isolates within this class of fungi were recovered from turf tissue samples taken from eight different Florida golf courses. Soil and canopy temperatures, soil electrical conductivity (EC) as a measurement of total soluble salts and soil pH data were taken at each sample date and each location. The species, variety and/or anastomosis group (AG) of the hymenomycete isolates were identified. Soil temperature had a significant effect on isolation of *R. solani* AG 2-2LP ($P < 0.0001$) and was negatively correlated (Pearson correlation coefficient, $r = –0.61$). Soil temperature did not significantly affect isolation frequency of any *Chrysorhiza* sp. Soluble salt concentration was positively correlated ($r = 0.33$) with isolation frequency of all hymenomycetes ($P = 0.009$). *Rhizoctonia solani* AG 2-2LP recovery was more likely to occur during periods of lower soil temperatures. *Chrysorhiza* spp. were isolated over a wider range of temperatures. Increasing levels of soil salinity were observed to correlate to higher frequencies of isolation of *Rhizoctonia* and *Chrysorhiza* fungi.

Yield loss in spring wheat due to disease caused by *Xanthomonas campestris* pv. *translucens*

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Phytopathology 101:S87

Occurrence of Bacterial leaf streak and black chaff caused by *Xanthomonas campestris* pv. *translucens* has increased in the Northern Great Plains in recent years, however the potential for crop loss has not been studied. This study was conducted in Brookings, SD with an objective to estimate the losses due to the disease in spring wheat. Five spring wheat genotypes with various level of resistance were planted under inoculated and uninoculated conditions. A virulent Isolate *XctSD17* was inoculated at tillering stage to induce disease in inoculated plots. Disease development was faster and more severe in inoculated plots though some disease was also present in uninoculated plots. Grain yield was significantly different between inoculated and uninoculated plots in all the genotypes. Inoculated plots had 12 to 32% lower yields compared to uninoculated plots. Yield difference between inoculated and uninoculated plots were higher in susceptible genotypes SD3948 (32% loss) and Russ (29% loss) where as moderately resistant SD4148 had the lowest yield reduction (12%) among the tested genotypes. Test weight was poor in all genotypes however inoculated had lower test weights than uninoculated plots, with up to 7% reduction observed. These findings showed that significant yield loss due to the disease can be expected if the conditions are conducive. Additionally, this study highlighted the potential importance of the disease in a breeding program since no effective in-season control is available.

Red potato cultivar (*Solanum tuberosum* L.) susceptibility to the root-knot nematode *Meloidogyne incognita*

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Phytopathology 101:S87

Yield loss in some potato cultivars (*Solanum tuberosum* L.) by *Meloidogyne* spp. (root knot nematodes) may reach 80% in tropical and subtropical areas. Susceptibility of red potato cultivars to *M. incognita* is generally unknown and requires investigation to appropriately manage yield losses. In a greenhouse, four population densities of *M. incognita* were evaluated on eight red skinned potato cultivars. Tuber weight (TW) and nematode reproduction factor (Rf) (Rf = Pf/Pi) were used to indicate tolerance and resistance. TW of cultivars Desiree, Pink Pearl and Mountain Rose were above the overall mean by 36%, 27%, and 9%, respectively. TW was less than overall mean for Durango, Red Thumb, All Red, Colorado Rose and Rote Ersting (20%, 20%, 11%, 11%, and 9%, respectively). A ranking of Rf showed that Desiree, Pink Pearl and Red Thumb were consistently among the cultivars supporting the greatest nematode reproduction regardless of Pi (Rf ? 4). Durango, Rote Ersting and Mountain Rose were consistently among the most resistant cultivars (Rf < 1). Desiree and Pink Pearl placed in a tolerant-susceptible category, whereas; Mountain Rose was a tolerant-resistant cultivar. Red Thumb was intolerant-susceptible and all other cultivars were intolerant-resistant. This information on red skinned potato cultivars will be useful for breeders and growers in selecting cultivars tolerant and resistant to *M. incognita*, mitigating yield loss.

Viruses associated with yellow vein and vein enation disease of citrus

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Citrus yellow vein (YV) and vein enation (VE) are graft-transmissible diseases of unknown etiology. YV symptoms include yellowing of the main veins and adjacent petiole areas in almost all citrus species whereas synergism between YV and other graft-transmissible citrus diseases such as VE significantly enhance symptom expression. VE symptoms include vein enation and gall formation in susceptible hosts, such as Mexican lime (ML) and rough lemon with reports indicating that a luteovirus may be associated with VE. ML seedlings were graft inoculated with blind buds from YV and VE symptomatic plants and developed typical YV and VE symptoms eight weeks post inoculation. High molecular weight double stranded RNAs were isolated from the inoculated MLs, but not from the un-inoculated controls. After shotgun cloning and sequencing, several sequenced clones showed high degree of homology with umbraviruses and luteoviruses. The YV luteovirus species was also identified in clones obtained from the VE inoculated plants. It is proposed that the luteovirus is a major component of both YV and VE and that VE evolves to YV as a result of the synergism between the umbra- and the luteovirus.

Performance of recombinant inbred line populations segregating for *Fusarium virguliforme* resistance in soybean (*Glycine max* (L.) Merr.)

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Sudden death syndrome (SDS) caused by *Fusarium virguliforme* is a devastating disease in soybean (*Glycine max* (L.) Merr.) that causes yield losses up to 70% depending on the developmental stage that plants become infected. Characterization of resistance is greatly significant for genetics and breeding studies. Two populations were developed for this study by crossing two lines from Southern Illinois germplasm collection, LS90-1920 and LS97-1610 with 'Spencer', a line susceptible to SDS. Ninety-six $F_{5:8}$ recombinant inbred lines from each population (LS90-1920 x Spencer, and LS97-1610 x Spencer) were evaluated for two years (2009 and 2010) at three diverse locations (Carbondale, New Haven and Valmeyer IL) in Southern Illinois. Population statistics, genotype x environment interaction, and broad-sense heritability were used to reveal any major resistance genes. Genetic correlation coefficients of SDS resistance with important agronomic traits such as lodging, pubescence, growth habit, and plant height were also calculated. The information from this study will be helpful to breeders in developing mapping populations and enforcing selection practices.

Anthracnose of sweet pepper caused by *Colletotrichum simmondsii* found in Japan

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In September 2009, severe fruit rot of sweet pepper were found in Hyogo prefecture, Japan. The symptoms were circular to ellipsoid and sunken spots of 5–30 mm diameter, with concentric rings of gray-brown to black, and abundant orange masses of conidia. Stems and leaves sometimes infected of which symptom closely resembled to "Cercospora leaf spot". Two isolates obtained from the diseased sweet pepper were examined in an inoculation test by attaching mycelial disks to artificially wounded fruits. The symptoms were reproduced and the same fungus was re-isolated. The two isolates formed neither sclerotia nor seta. Conidia were subcylindrical, attenuated and blunt pointed ends without guttle. The conidia were 11.3–25.7 micrometers length and 2.7–5.2 micrometers breadth. L/B = 3.9. Appressoria were smooth, grayish brown obovoid to ellipsoid and 4-17 micrometers length and 3.2–6.8 micrometers breadth. Colonies on PDA that are gray cottony and in reverse pale gray to pale orange sometimes with dark flecking. The morphological and cultural characteristics were in accordance with *Colletotrichum simmondsii*. rDNA ITS and beta-tublin-2 sequences of the isolates were identical and had high similarity to those of *C. simmondsii*. These isolates were also virulent to tomato, string bean, and strawberry.

Genetic diversity of *Potato virus* Y^O and origin of recombinant PVY strains

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The ordinary strain of *Potato virus Y* (PVY), PVY^O, induces necrosis and severe stunting in potato cultivars carrying the *Ny* gene. A novel sub-strain of PVY^O, PVY^O-O5, has been found spreading in the U.S.A. molecular study of PVY^O and PVY^O-O5 isolates from a North American collection of PVY was conducted through whole genome sequencing and phylogenetic analysis. Forty-four PVY^O isolates were sequenced, including 31 from the PVY^O-O5 group. PVY^O-O5 isolates formed a separate, novel evolutionary lineage of PVY from potato. To shed light on the origin of the three most common PVY recombinants, a more detailed phylogenetic analysis of a sequence fragment, nt 2,406-5,821, that is present in all recombinant and non-recombinant PVY^O genomes was conducted. The analysis revealed that $PVY^{N:O}$ and $PVY^{N\text{-}Wi}$ recombinants acquired their PVY^O segments from two separate PVY^O lineages, while the PVY^{NTN} recombinant acquired its PVY^O segment from the same lineage as $PVY^{N:O}$. These data suggest that $PVY^{N:O}$ and $PVY^{N\text{-}Wi}$ recombinants originated from two separate recombination events involving two different PVY^O parental genomes, while the PVY^{NTN} recombinants likely originated from the $PVY^{N:O}$ genome via additional recombination events.

Genetic diversity of *Fusarium verticillioides* isolated from Corn in Iran

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Phytopathology 101:S88

Fusarium verticillioides (teleomorph: *Gibberella moniliformis = G. fujikuroi* mating population A) is a common fungal pathogen of Maize worldwide. Rep–PCR was used to determine genetic diversity of 41 isolates of *Fusarium verticillioides* from infected ear and stem of *Zea maize* from different corn producing areas of Iran including: Damghan, Fars, Isfahan, Tehran, Kermanshah and Khoozestan that were identified morphologically and molecularly (applying specific primers) before. Reproducible genomic fingerprint were amplified in each strain by PCRs of enterobacterial repetitive interganic consensus (ERIC) and BOX sequences. Totally all 41 isolates were evaluated and similarly comparison were shown as a dendrogram which produced by UPGMA cluster analysis based on the jaccard's similarity coefficient. Forty-one isolates divided into 30 group, 5 group with 2 individuals, 2 group with 3 individuals and 22 isolates formed single member in 65% similarity. Cluster analysis shown none agreement with the distance of sampling location. Our result shown that Rep-PCR is a convenient and rapid for genetic diversity analysis and strain differentiation in *Fusarium verticillioides*. Universal primers theoretically anneals to the intergenic target sites which are randomly dispersed in genomes and provide the amplification of different lengths fragments. Rep-PCR application are widespread in plant pathogenic bacteria and tested in different fungal strains.

Analysis of the association between *Fusarium verticillioides* isolates isolated from rice and corn in Iran

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Fusarium verticillioides (Teleomorph: *Gibberella moniliformis = G. fujikuroi* mating population A) cause considerable effect on corn and rice as pathogen. In this study 55 *F. verticillioides* isolates collected from infected rice and corn were identified morphologically as *F. verticillioides*. All of the isolates collected from infected corn were determined by specific primers while none of the isolate collected from infected rice were determined by specific primers (which were designed for corn isolates) as *F. verticillioisdes*. However, they were identified by mating type methods (Applying standard isolates). 6 out of 14 rice isolates characterized as MAT1, while others were MAT2. In present study, DNA primers (BOX and ERIC), corresponding to conserve repetitive element motifs in the genomes of divers bacterial species were used in order to comparing *F. verticillioides* isolates in rice and corn. Totally all 55 isolates were evaluated and similarly comparison were shown as a dendrogram which produced by UPGMA cluster analysis based on the jaccard's similarity coefficient. Cluster analysis showed that the isolates divided into two group in 70% similarity based on host. Results suggested that the *F. verticillioides* isolates from rice and corn are different genetically and probably belong to the two *forma speciales*.

Functional characterization of the PidS/PidR two-component regulatory system of *Burkholderia glumae*

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Phytopathology 101:S88

Burkholderia glumae is the major causative agent of bacterial panicle blight of rice, which frequently causes significant yield losses in many rice-producing countries. Environmental factors, especially high temperature and humidity, are thought to be closely related to outbreaks of this disease. Little is known, however, about the signaling mechanism that perceives and transduces environmental signals that induce expression of virulence genes in this pathogen. Recently, we found that some *B. glumae* strains produce multiple pigments in certain nutritional conditions, including CPG medium. Furthermore, the production of these pigments is abolished by mutation of the genes encoding a two-component regulatory system (TCRS) comprised of *pidS* and *pidR* that encode a sensor histidine kinase and a response regulator, respectively. Remarkably, *pidS* and *pidR* mutants failed to elicit an hypersensitive response on tobacco leaves and showed attenuated virulence in rice. In addition, these mutants produced reduced amounts of the phytotoxin, toxoflavin, a major virulence factor of *B. glumae*. This is the first report of a TCRS involved in the pathogenesis of *B. glumae*. To better understand the regulatory function of the PidS/PidR TCRS, expression patterns of virulence genes and other regulatory genes of this pathogen in *pidS* and *pidR* mutant backgrounds are currently being investigated with a *gus* reporter system and a quantitative-PCR technique.

Comparative genomics of a lucerne and non-lucerne isolate of *Verticillium albo-atrum*

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Verticillium albo-atrum is a major fungal plant pathogen that causes a wilt disease of trees, herbaceous plants, and agricultural crops. Because of its significance as a plant pathogen, both the mitochondrial (29.49 Kb) and nuclear genome (32.83 Mb) of *V. albo-atrum* isolate VaMs.102 from lucerne (alfalfa) were sequenced by Broad Institute in 2008. However, previous studies based on nuclear markers clearly revealed two homologous populations (lucerne and non-lucerne) within *V. albo-atrum*. Without

sequenced genomes for both intra-specific populations, differences in pathogenic adaptation and allelic variability in virulence-associated genes remain incomplete. Utilizing both 454 and Illumina sequencing technology, we generated a combined 14X genome assembly of *V. albo-atrum* PSU140 from *Ailanthus* for both mapping against the reference (VaMs.102) and *de novo* assembly. *V. albo-atrum* PSU140 was isolated from diseased *Ailanthus altissima* in mixed oak forests in south-central Pennsylvania and has been characterized with regard to efficacy and host specificity. Here we show the 32.4-megabase (Mb) genome assembly of PSU140 in comparison with the 32.83 Mb draft genome of VaMs.102. Pairwise comparisons of total mappable contigs between VaMs.102 and PSU140 reveals sequence similarity of 96.51%. Through these comparisons, we can begin to elucidate the molecular mechanisms that underlie pathogenicity, differentiation, and host-adapted virulence in PSU140.

Risk analysis for *Verticillium albo-atrum* isolate PSU 140, causal agent of Verticillium wilt of tree-of-heaven (*Ailanthus altissima*)
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Phytopathology 101:S89

Unprecedented wilt and mortality of the invasive tree-of-heaven (*Ailanthus altissima*) by *Verticillium albo-atrum* is currently epidemic in south-central Pennsylvania. Because *V. albo-atrum* causes wilt diseases of trees, herbaceous plants, and agricultural crops, >70 species were stem-inoculated with isolate PSU140 in the field and greenhouse. The following species exhibited wilt and vascular discoloration following inoculation: *Ailanthus*, Amur corktree, autumn olive, blackberry, black locust, corkwood, crossvine, devil's walkingstick, elderberry, honey locust, Japanese barberry, Japanese maple, catalpa, Norway maple, poison-ivy, redbud, sassafras, staghorn sumac, striped maple, and tree-of-paradise. Of these, only six species had >10% mortality following wilt: *Ailanthus*, blackberry, poison-ivy, redbud, striped maple, and sumac. Furthermore, natural spread of *V. albo-atrum* within diseased *Ailanthus* stands was observed only for *Ailanthus* (100%), devil's walkingstick (22%), and striped maple (<4%). Vascular discoloration following inoculation, but without wilt or mortality, was observed on >20 species. Although artificial inoculations provide an evaluation of potential damage to non-target hosts, the low incidence of disease and mortality of these non-target hosts among inoculated *Ailanthus* offer support that PSU140 may be host adapted. Pending the outcome of host-range and molecular studies, *V. albo-atrum* should be considered as a potential biocontrol for the invasive tree-of-heaven.

Suppression of Fusarium spp. in tissue culture (TC) banana established in field soils inoculated with commercial biological products
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Fusarium oxysporum sp. cubense threatens the survival of TC Gros mitchel banana worldwide. Control by fungicides has failed with breeding rather than control of pathogen preferred. A study funded by BMGF was conducted by CIAT-Tropical Soil Biology and fertility institute to evaluate commercial biological and chemical products for use in Africa. A complete randomized design experiment under greenhouse conditions evaluated the potential of Rhizatech (mycorrhiza) and EcoT (Trichoderma hazianum) on suppression of Fusarium spp. on vertisols, eutric nitosols and humic nitisols from banana growing regions in Kenya. Fusarium spp. were isolated using Peptone Pentachloronitrobenzene agar. Identification manual for Fusarium by Burgess using cultural and microscopic characteristics distinguished isolates as Fusarium oxysporum. The isolates were white and pink in vertisol, white in eutric nitosol and purple with white tint and white in humic nitisol. Colony forming units (CFU) were significantly ($p < 0.05$) different. The CFU before inoculation was 8.0 × 102 for eutric nitosol, and vertisol and 2.5 × 102 in humic nitisol. Rhizatech reduced CFU in eutric nitosol and humic nitisol by 87.5% and 36% respectively. EcoT reduced CFU in vertisol and humic nitisol by 12.5% and 44% respectively. Response to products depends on soil type and there is potential in use of products to suppress disease.

Controlling gummy stem blight in the greenhouse on watermelon seedlings grafted onto cucurbit rootstocks
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Phytopathology 101:S89

Interspecific hybrid squash (*Cucurbita moschata x C. maxima*) and bottle gourd (*Lagenaria siceraria*) are used as rootstocks for grafting seedless watermelon. Both rootstocks and watermelons are susceptible to gummy stem blight, caused by *Didymella bryoniae*, especially during healing, when grafted seedlings are held at high relative humidity or misted for 1 week while the vascular bundles of rootstocks and scions connect. The objective of this study was to control gummy stem blight on grafted seedlings with fungicides. To assess phytotoxicity, rootstock seedlings were sprayed twice at weekly intervals with labeled rates of fungicides. Four of 9 fungicides injured 3–35% of the surface area of cotyledons on one or both rootstock species, and tebuconazole also stunted *Cucurbita*. Five fungicides were applied to 'SS 7187HQ' seedless watermelon, 'Strong Tosa' hybrid squash, and 'Emphasis' bottle gourd. The next day scions and rootstocks treated with the same fungicide were grafted together and placed in a humidity chamber. One day later grafted seedlings were inoculated and then held for 6 more days. All fungicides reduced incidence and severity of gummy stem blight compared with the water control (94% incidence, 9.7% severity). Difenoconazole and cyprodinil (<13% incidence, ≤0.1% severity) were more effective than mancozeb or cyprodinil+fludioxonil, which were more effective than thiophanate-methyl ($P = 0.01$).

Screening for powdery mildew resistance in 'Ohelo berry germplasm in Hawaii
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Phytopathology 101:S89

'Ohelo, *Vaccinium reticulatum* (Smith), is an endemic Hawaiian shrub, less than 1 m (3.3 ft) tall, and grows between 640 and 3700 m (2,100 to 12,100 ft) elevation on disturbed volcanic sites on the islands of Maui and Hawaii. Concerns have arisen about human impacts to the environment during the wild gathering of fruits that include spreading of exotic weeds, damaging native vegetation, and reducing a food source of the endemic nene goose, *Banta sandvicensis* (Vigor). As an alternative to wild harvest, 'Ohelo cultivars for small-scale cultivation as ornamentals and for edible berries in Hawaii were identified, evaluated and selected. The main disease pressure that may limit berry production and ornamental qualities is from powdery mildew. Numerous disease resistance screens of diverse 'Ohelo berry germplasm were conducted to identify powdery mildew resistance. Controlled inoculations were made onto leaf discs, detached leaves and potted seedlings. The results were compared with natural epidemics in two locations on Hawaii Island, Mealani and Lalamilo, approximately 55 miles north of Hilo. There was a good correlation of ratings between the field and potted plant ratings. No accession rated in all four screens was immune from infection. However, one cultivar ('Kilauea') was consistently rated as tolerant across three independent studies. The results emphasize the importance of uniform testing in multiple environments using the most appropriate host material available.

Leaf blight and stem canker of Mangosteen in Hawaii
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Phytopathology 101:S89

Mangosteen (*Garcinia mangostana* Linn.) is a tropical evergreen tree that produces one of the most praised tropical fruits, commonly known as the "Queen of Fruit". Mangosteen has the potential to occupy a rapidly expanding niche market in Hawaii. The aim of this study was to identify the causative agent of the leaf spots and stem cankers in Mangosteen trees ranging in age from newly planted to 6+ years old. Symptoms were observed in a Mangosteen orchard on the Hamakua Coast of the Island of Hawaii, approximately 6 miles north of Hilo. Single spore isolations from leaf lesions and stem cankers resulted in pure cultures of the fungus. The fungus was identified as *Pestalotiopsis* sp. based on morphological characteristics and molecular analysis. Pathogenicity tests with the isolated fungus showed identical leaf symptoms on 3 year old seedlings growing in a hoophouse. *Pestalotiopsis* leaf blight has already been reported in other countries growing Mangosteen. However, this is the first report of *Pestalotiopsis* leaf blight and stem canker on Mangosteen in Hawaii.

Comparing foliar and drench application of azoxystrobin for controlling *Rhizoctonia* root rot of sugar beet
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Phytopathology 101:S89

Rhizoctonia crown and root rot caused by *Rhizoctonia solani* Kühn is the most important sugar beet disease for growers in Minnesota and North Dakota. Crown rot was common with conventional varieties since cultivation for weed control led to crown inoculation. Root rot is more common with transgenic varieties which are on 95% of the acreage. Foliar application of azoxystrobin before infection occurs will control the disease. The objective of this study was to evaluate and compare the effect of foliar and hypocotyl drench applications of azoxystrobin for controlling Rhizoctonia root rot caused by *R. solani* AG 2-2 IIIB. Treatments included a non-inoculated check; an

inoculated check; azoxystrobin at 0.672 L/ha in 121 L/ha solution applied by a spray system in an 18 cm foliar band or by a micropipette as a hypocotyl drench. Inoculations were done using two (~ 0.08 g) barley grains colonized with *R. solani* AG 2-2 IIIB. Inoculum was buried at 2.0 cm below soil surface and in close proximity with plant roots. Inoculated plants without fungicides had significantly higher root rot than inoculated plants treated with azoxystrobin. Root rot severity for foliar and hypocotyl drench of azoxystrobin were not significantly different. However, azoxystrobin applied as a hypocotyl drench resulted in the least amount of root rot since it was not different from the non-inoculated check.

Potential of *Paecilomyces lilacinus* strain 251 to control the root-knot nematode *Meloidogyne enterolobii*, a new quarantine species for the EPPO region

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Phytopathology 101:S90

Meloidogyne enterolobii (syn. *M. mayaguensis)* was recently added to the EPPO list as an A.2 quarantine organism. This species is causing severe damage on vegetables such as tomato and is particularly difficult to manage as it overcomes resistance against tropical root-knot nematodes. As organic farmers have no options to control *M. enterolobii*, *P. lilacinus* strain 251 (PL251) was tested in the water dispersible granule (Melocon WG) and a new water dispersible powder formulation (WP) in on-farm and semi-commercial yield trials, and under controlled environmental conditions. All studies demonstrated a high biocontrol efficacy of PL251 independent from the type of formulation used. Furthermore, the use of a surfactant or soy meal and sugar as additional food supplements affected the efficacy of PL251. Conversely to previous studies with *M. incognita*, stronger reduction of nematode damage by PL251 was observed at higher inoculum levels. Furthermore, high egg parasitism rates indicated an increased rhizosphere competence of PL251 which was never observed before. However, sufficient biocontrol efficacy still requires a pre-planting soil treatment to reduce initial nematode inoculum. It was demonstrated that the novel WP formulation of PL251 was equal or superior in its efficacy to control *M. enterolobii* on tomato when compared to the WG type formulation which, in combination with a 10fold higher concentration and increased shelf-life, makes it a viable option for control of *M. enterolobii*.

QBOL - Barcoding as a new tool for identification of quarantine nematodes and their close relatives

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Identification of quarantine plant pests needs to be fast and accurate to enable timely plant protection measures. False diagnostics could cause serious financial losses for trade and producers. Genetically based diagnostics is a reliable alternative to the classical identification generally based on morphological features requiring expert taxonomic skills. Genetic diagnostics through the use of DNA-barcodes, stretches of DNA that contain taxon-specific information, can be performed by any skilled lab-worker. The European Union 7th Framework project QBOL: "Development of a new diagnostic tool using DNA barcoding to identify quarantine organisms in support of plant health" aims to establish DNA-barcodes for all European quarantine organisms and their close relatives, including plant parasitic nematodes. For quarantine nematodes, several gene regions such as COI, COII, SSU, LSU and RNA polymerase subunit II are being evaluated for their barcoding potential. The results and protocols will be made available through a database, Q-bank, freely accessible to all interested users. For each group of quarantine organisms, a consortium of curators will ensure that data incorporated into Q-bank are confirmed for correctness and linked to specimen in reference collections.

Identification of the tropical root-knot nematode species *Meloidogyne incognita*, *M. arenaria* and *M. javanica* by a multiplex PCR protocol

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Phytopathology 101:S90

Meloidogyne incognita, *M. javanica* and *M. arenaria* are considered to be the economically most important root-knot nematode species due to a wide host range and high damage potential. Next to the detection of quarantine root-knot nematodes, identification of the morphologically similar tropical species is needed in routine testing. Therefore, a reliable multiplex PCR protocol was developed for rapid identification of *M. incognita*, *M. javanica* and *M. arenaria*. To guarantee a high specificity and reproducibility, primers which had been routinely used in our lab and produced reliable results in routine diagnostics were the basis for a multiplex PCR protocol. The SCAR primers Mjav/Fjav and Far/Rar produce species specific products of 720 bp and 420 bp for *M. javanica* and *M. arenaria*, respectively. A complementary primer for *M. incognita* was developed based on the 399 bp product of the SCAR primers inc-K14-F/R. Following sequencing of the amplicon, primers Mi2F4/Mi2R1 were designed to produce a product of 300 bp. This primer combination produced reliable results in multiplex PCR assays with 14 different populations from 5 countries. No cross reaction was found with *M. hapla*, *M. fallax*, *M. chitwoodi*, *M. enterolobii* and *M. ethiopica*. Furthermore, the amplified species specific products allow separation by high-resolution capillary electrophoresis and might be used in high-resolution-melting-curve analysis assays.

Severity risk spatial model for *Phytophthora* diseases in woody ornamental nurseries in southern Middle Tennessee

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Phytopathology 101:S90

Middle Tennessee has the largest concentrations of nurseries in the state. Many of the ornamentals trees and shrubs are highly susceptible to a variety of *Phytophthora* species. Disease severity risk map was calculated from a risk model based on the parameters that can be directly correlated to *Phytophthora* disease occurrence, pathogen reproduction and spread. These parameters include availability of susceptible host species, soil drainage, proximity to roads, and integrated moisture index (IMI). Technologies such as Global Positioning System (GPS), geospatial information systems (GIS), and remote sensing have been utilized to create a severity risk map for *Phytophthora*. Maps of each environmental parameter were created and overlaid to show the cumulative severity risk to *Phytophthora*. A final map was categorized into six risk levels of no risk, very low risk, low risk, moderate risk, high risk, and very high risk; nurseries were then added using GPS and remote sensing. A buffer area was applied to the GIS to surround each nursery. The percent of each severity risk level was calculated for each buffer region. Nurseries with the the highest risk categories were at the highest risk for *Phytophthora* diseases. To validate this model, a survey for *Phytophthora* occurrences was done in nine counties. The severity risk model developed in this project will allow growers to predict *Phytophthora* risks in their areas for disease management preparedness.

First report of bacterial leaf spot on milk vetch (*Astragalus sinicus*) caused by *Pseudomonas viridiflava* in Korea

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Phytopathology 101:S90

Bacterial leaf spot disease occurred on milk vetch (*Astragalus sinicus*) grown in Namwon city and Wanjugun areas, Jeollabukdo, South Korea in April 2010, Symptoms typically appeared as in the form of dark brown discoloration on the surface of leaves and water soaked brown lesions. A number of small dark brown spots were observed on the leaves and gradually enlarged larger cylindrical dark brown lesions. Disease incidence was estimated as approximately 5%. Bacterias isolated from bacterial spot lesions on milk vetch leaves were used for pathogenicity test. Bacterias were grown on TSA for 48 hr at 25°C. Pathogenicity of the bacteria was confirmed on milk vetch leaves with needle inoculation of bacterial suspension containg 10^6 CFU per ml in sterile distilled water. Sterile distilled water was used as control. Inoculated plants were placed in a humid chamber with 100% relative humidity at 20°C for 14 days. Symptoms were assessed about 14 days after inoculation. Bacterial leaf spot symptoms of milk vetch plants produced by artificial inoculation were essentially identical with those in the field. The Bacteria was reisolated from those lesions. Bacteria isolates causing leaf spot were detected and subsequently identifed as *Pseudomonas vridiflava* using the Biolog system and 16S rDNA phylogenetic analysis. This is the first report of bacterial spot of milk vetch caused by *Pseudomonas vridiflava* in Korea.

Multiple resistance phenotypes of *Botrytis cinerea* in apple orchards and effects on control of gray mold in stored apples with postharvest fungicides

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Phytopathology 101:S90

Botrytis cinerea is the cause of gray mold in stored apples. To investigate fungicide resistant phenotypes of *B. cinerea* in apple orchards and their impacts on gray mold control in stored apples, isolates of *B. cinerea* were obtained from symptomless fruit from orchards where methyl benzimidazole carbamates (MBCs); cyprodinil, an anilinopyrimidine (AP); quinone outside inhibitors (QoIs); and boscalid, a succinate dehydrogenase inhibitor (SDHI)

had been used. All isolates were tested for resistance to these fungicides. Of the 219 isolates tested, 5.5% were resistant to all 4 fungicide classes; 12.8% were resistant to MBC, QoI and SDHI; 0.5% were resistant to QoI and SDHI; 0.5% were resistant to QoI; and 80.8% were sensitive to all 4 fungicide classes. Effectiveness of postharvest fungicides thiabendazole (TBZ), pyrimethanil (PYR), and fludioxonil (FLU) for control of gray mold on apple fruit incited by different phenotypes was evaluated. TBZ and PYR failed to control gray mold incited by TBZ- and PYR-resistant isolates, respectively; while FLU effectively controlled both quadruple- and triple-resistant isolates. All three postharvest fungicides effectively controlled gray mold caused by QoI- and SDHI-resistant isolates. The results indicated that multiple resistance of *B. cinerea* has developed in the orchards and that strategies for resistance management should be implemented to avoid the failure of gray mold control in stored apples with postharvest fungicides.

Competitive interactions between the biocontrol fungus *Trichoderma harzianum* and *Fusarium solani* f. sp. *pisi* in soil

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Phytopathology 101:S91

Competitive interactions were evaluated between the potential biocontrol fungus *Trichoderma harzianum* ThzID1-M3, as well as other indigenous *Trichoderma* spp. in soil, and the plant pathogen *Fusarium solani* f. sp. *pisi* (Fsp), both in soil and in the rhizosphere. Alginate pellets containing ThzID1-M3 (a green fluorescent protein-expressing recombinant strain), along with *Fsp* (1×10^5 conidia/g soil), were added to non-sterile silt loam soil into which pea seeds were then planted. Treatments were either ThzID1-M3 or *Fsp* alone, both organisms together, or neither organism added. Using quantitative real-time PCR, *Fsp*, ThzID1-M3 and other (indigenous) *Trichoderma* spp. were quantified in soil and on pea roots over a 21-day period. Addition of ThzID1-M3 to soil significantly ($P < 0.05$) reduced *Fsp* biomass in soil, and also reduced pea root colonization by *Fsp*. Addition of *Fsp* resulted in significantly lower biomass of both ThzID1-M3 and other *Trichoderma* spp. These results suggest that competition between *Trichoderma* and *Fusarium solani* f. sp. *pisi* negatively affects the establishment of both organisms in soil.

Comparative analyses of Korean isolates of *Cucumber mosaic virus*

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Phytopathology 101:S91

Cucumber mosaic virus (CMV) occurs naturally worldwide and has the broadest host range of any known virus. We have used twelve isolates of CMV; each two isolates from tomato and *Virginia pepperweed* and each one isolate from cucumber, zucchini, red pepper, tobacco, r*udbeckia*, a*ngelica*, P*haseolus nipponensis* and a*dzuki bean* in Korea. Those isolates were investigated and classified by using the selective differential hosts from indicator host species and RNA analyses of CMV genomic RNAs 1, 2 and 3. Relationships of twelve isolates of CMV were also compared using pathogenicity on host plants, cytopathological alterations and phylogenetic analyses. To determinate the pathogenicity of virus isolates and the symptoms, all the host plants in the same families were not found to be equally susceptible to CMV isolates of the same hosts. Phylogenetic analyses of the RNAs 2 and 3 ORFs, twelve CMV isolates could be clearly divided into three clades that correspond to subgroups IA, IB and II. However, according to the pattern of the nucleotide sequences of 1a, CMV isolates of subgroup I could be divided into three clades (IA, IB and IC). For relationship of cytopathological alterations and CMV genome RNAs, tissues and cells were observed with infected leaves of twelve CMV isolates by light and electron microscopy. No specific tissue and cell was observed by light microscopy, but virus particles and inclusion bodies could be easily found in cuticle, epidermis, parenchyma, collenchyma and vacuole.

Biological and molecular characterization of *Ribgrass mosaic tobamovirus* infecting *Rehmannia glutinosa*

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Phytopathology 101:S91

Rehmannia glutinosa is a member of the S*crophulariaceae* family and is an important herbaceous medicinal plant in Korea. A virus causing symptoms of mosaic, stunt and malformation on *Rehmannia glutinosa* occurred around Hwasun area in Korea. Virus diseased plants were analyzed RT-PCR and electron microscopy. The sample was infected *Broad bean wilts virus* (BBWV2), *Tobamovirus* and other unidentified filamentous virus. This *Tobamovirus* isolates was passed through three repeated single-lesion transfers on Nicotiana tabacum cv. Xanthi-nc. This isolate caused large necrotic spots on the inoculated leaves and could not infect systemically on *N. tabacum* cv. Xanthi-nc, *N. rustica*, *N. benthamiana*, *Datura stramonium* and *Tetragonia expansa*. *Chenopodium quinoa* and *N. tabacum* cv. Samsun showed necrotic local lesions on inoculated leaves and systemic necrotic local on upper leaves. Analyses of complete nucleotide sequences of the genome were comparable with other members of the genus *Tobamoviruses*. This isolate had very higher identity to crucifer species than to other Tobamoviruses. Especially, this isolate had very high homology (90–99% identical nucleotides) to those of *Youcai mosaic virus* (YoMV) and *Ribgrass mosaic virus* (Shanghai and Impatiens isolates). The isolate is most similar to YoMV but it was seen to different biological characteristic according to host range and symptoms. These results showed that *Tobamovirus* isolate were collected from *Rehmannia glutinosa* is closely related to *Ribgrass mosaic tobamovirus*.

Fungicide resistance mechanisms of *Fusarium fujikuroi* strains against prochloraz

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Phytopathology 101:S91

Fusarium fujikuroi is a fungal plant pathogen that causes rice Bakanae disease. A number of cases on fungicide resistance of the pathogen have been reported. Understanding the resistance mechanism is of essentials for successful disease control. This study was performed to investigate resistance mechanisms of *F. fujikuroi* strains against prochloraz. Fungal growth was examined in PDB with prochloraz or ATP-Binding Cassette (ABC) transporter inhibitors and PDB with prochloraz and each inhibitor. Remaining prochloraz in PDB was determined by LC-TOF/MS. Growth inhibition of the pathogens was observed in PDB containing prochloraz and each inhibitor. The concentration of prochloraz in PDB containing prochloraz and each inhibitor remained unchanged. The ratio of saturated/unsaturated fatty acids of membrane lipids of the pathogens grown with or without prochloraz is similar, suggesting membrane lipid are not responsible for the resistant mechanism. Existence of efflux pump gene was determined by amplifying gDNA with the primer designed with the conserved sequence related to ABC transporter. Sequence analyses showed 75% similarity to *BcatrD* gene of *Botryotinia fuckeliana*, a resistant strain against conazole fungicides. These data indicate that the pathogens are capable of pumping prochloraz out of cells to decrease its toxicity. The application of prochloraz combined with ABC transporter-inhibiting chemicals would be a effective strategy to control Bakanae disease.

Genetic diversity and host range of Colletotrichum acutatum isolates obtained from several crops in South Korea

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Phytopathology 101:S91

Among 51 isolates of Colletotrichum obtained from several plants, such as pepper, Chinese matrimony vine, pear, peach, apple, avocado, and grape, 47 isolates were identified as C. acutatum by using mycological characteristics and PCR products with species-specific primers. The others were done as C. gloeosporioides. The phylogenetic relationship of all isolates of Colletotrichum spp. used in this study was investigated through the methods as AFLP, RAPD-PCR, and partial sequencing of the 5.8S-ITS regions and the β-tubulin 2 gene. Showing the result of each molecular method, all isolates obtained from pepper, peach and grape were belonged to A2 group, while the others from pear, apple and chinese matrimony vine, avocado were belonged to A3 group. In the pathogenicity test with fruits of pepper, peach, and apple in vitro, 15 isolates of Colletotrichum acutatum from pepper were able to infect pepper strongly, and 8 isolates among them could do the fruit of apple weakly. With peach, only 3 isolates among pepper isolates showed pathogenicity on peach. These results showed that there was no strong specificity of host range in C. acutatum.

Overwintering of Chrysanthemum white rust caused by *Puccinia horiana* in Pennsylvania and challenges in its management

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Phytopathology 101:S91

In 1977, Chrysanthemum white rust, caused by *Puccinia horiana*, was first detected and eradicated in PA. Until 2004, no further cases were reported. In 2004 and 2006–2010, *P. horiana* was detected at 82 sites (139 samples), including nurseries, greenhouses, retail stores, and residential areas, in 22 PA counties. At these sites, the PA Department of Agriculture and USDA attempted eradication plans involving quarantine, destruction, treatment, and

inspection, but encountered the following challenges. First, plant inspectors were unable to conduct all trace forward inspections after infected chrysanthemums had been sold at retail stores including big-box stores. In 2006 alone, 50% of infection sites were big-box stores. Second, although diagnostic PCR primers were available since 2009, their reliability for early detection of *P. horiana* has not been formally confirmed. Thirdly, *P. horiana* has been shown to survive winters. A northern York county nursery sold a portion of mature containerized mums to a southern York county nursery in fall of 2009. The mums at both nurseries over-wintered in outdoors and exhibited severe symptoms in spring of 2010. In 2004–2010, the occurrence of *P. horiana* peaked in fall with the seasonal distribution of infested sites being 4.6% in Apr-May, 18.1% in Jun-Aug, and 77.3% in Sep-Dec.

Soil suppressiveness against Fusarium crown and root rot of cucumber in organic-amended soil: Occurrence and possible mechanisms

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Phytopathology 101:S92

Soil suppressiveness (SUPP) to soilborne pathogens can evolve following the incorporation of plant residues in the soil. It is characterized by reducing disease incidence and severity, despite the presence of a potent pathogen, a susceptible host and appropriate conditions for disease development. Residues of various crucifer and herb plants effectively induced SUPP to crown and root rot in cucumber plants inoculated with Fusarium oxysporum f. sp. radicis-cucumerinum (FORC) macroconidia when seedlings were planted in the tested soils 30 months after amendment. SUPP continued to be evident after repeated inoculations and plantings in the same soil. We studied the potential mechanisms which are involved in the evolution of SUPP after incorporation of Diplotaxis tenuifolia (wild rocket). The survival of chlamydospores of FORC decreased, by 50%, in the suppressive soil after one month of incubation. Induced systemic resistance against either FORC or Botrytis cinerea, in cucumber transplants was not evident. The composition of bacterial communities, especially Streptomyces, in roots of plants which were grown in suppressive soil, was significantly different from those in nonsuppressive one as indicated by PCR-DGGE analysis. Quantitative PCR showed that colonization of the root tissue by FORC in soil treatments was similar at day 3 from inoculation but decreased by 60% in day 6 in the suppressive soil. Apparently, root colonization by specific microbial communities controls pathogen infection.

Interrelationships among SA, MeSA, lipids, and light in systemic acquired resistance (SAR)

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Phytopathology 101:S92

SAR is a state of heightened defense induced throughout a plant following local infection by a pathogen. Development of SAR involves synthesis of a mobile signal(s) at the site of infection and its translocation through the vascular system to distal tissues. Results from our group argue that methyl salicylate (MeSA) serves as this mobile SAR signal in tobacco, Arabidopsis, and potato (Park et al., Science, 2007; Vlot et al., Plant Journal, 2008; Park et al., JBC, 2009; Liu et al., MPMI, 2010; Manosalva et al., MPMI, 2010). In contrast, Zeier and coworkers presented results which suggest that MeSA is not essential for SAR in Arabidopsis (Attaran et al., Plant Cell, 2009). We have identified the difference in experimental design which accounts for these conflicting results. Under certain light conditions MeSA is required for SAR signaling while under others it is not. In addition to MeSA, one or more lipid-based mobile signals have been implicated in systemic immunity by several groups. Our analyses of mutants in the lipid-transfer protein DIR1 and of plants over expressing BA/SAMT1 suggests that SAR is activated via the interplay between at least two mobile signals, MeSA and a complex formed between DIR1 and a lipid or lipid derivative. The function of this complex is to suppress expression and/or activity of BA/SAMT1 in the distal tissue to facilitate conversion by MeSA esterases of the translocated MeSA to biologically active SA for induction and potentiation of defense responses (Liu et al., Plant Physiol. 2011).

Potential invasiveness of *Armillaria solidipes*, a tree-root-disease pathogen with a circumboreal distribution

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Phytopathology 101:S92

Armillaria solidipes (= *A. ostoyae*) is a root-disease pathogen that causes severe losses in growth and productivity of forest trees throughout the Northern Hemisphere. However, this species is genetically diverse with variable disease activities across different regions of the world. In North America, *A. solidipes* in the Colorado Plateau exists in drier habitats and causes more disease on hardwoods in comparison with *A. solidipes* in the northwestern U.S.A. In China and Japan, *A. solidipes* causes severe root disease on *Larix* spp., whereas this pathogen only rarely impacts *Larix* spp. in North America. These examples indicate that *A. solidipes* could represent an invasive species risk, especially under changing climate scenarios where Armillaria root disease is predicted to be more severe on trees that are maladapted to climate-induced stress. Furthermore, intraspecific and interspecific hybridization could create pathogens with novel ecological behavior, disease activity, and genetic adaptation throughout its circumboreal distribution. Studies are underway to assess global suitable climate space for *A. solidipes* derived from North America. Continued studies that determine the phylogeography of *A. solidipes* across the Northern Hemisphere and the suitable climate space for each phylogenetic group can help assess potential invasive risks associated with intercontinental and interregional movement of *A. solidipes*.

Evaluation of wild walnut *Juglans* spp. for resistance to crown gall disease

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Phytopathology 101:S92

Crown gall (CG) disease of walnut is caused by the ubiquitous soil-borne bacterium, <i*Agrobacterium tumefaciens*>i. The most widely used rootstock Paradox, an interspecific hybrid between <i*Juglans hindsii*>i and <i*Juglans regia*>I, is typically highly susceptible to <i*A. tumefaciens*>i. Identification of a durable source of resistance in wild <i*Juglans*>i species could be introgressed into commercially viable rootstocks, as an effective strategy for controlling crown gall in walnut. CG tolerant *Juglans* and *Pterocarya* spp. have been identified in a disease resistance screen conducted under greenhouse conditions. A wide range of variability in tumor formation was observed among different host genotypes. Even though CG resistance appeared to be rare in the germplasm accessions tested, *Juglans microcarpa* accessions were consistently the most resistant. Two *J. microcarpa* mother trees both generated open pollinated seedlings which exhibited increased tolerance to CG development. Rooted dormant cuttings from CG resistant selections were propagated, inoculated with *A. tumefaciens*, and continue to show CG resistance. These promising candidates are being further examined to confirm the stability of the observed resistance and to be used in directed crosses with commercially viable parents as a first step towards development of crown gall resistant rootstocks.

Weed control with flaming and cultivation in corn

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Phytopathology 101:S92

Propane flaming and cultivation could be combined into a single operation as an additional tool for weed control in organic maize (Zea mays L.) Field experiments were conducted at the Haskell Agricultural Laboratory of the University of Nebraska, Concord, NE in 2010, and will be repeated in 2011 to determine the level of weed control and maize response to propane flaming utilized alone, or in combination with inter-row cultivation. Total of seven treatments were applied at several growth stages of maize (V3 (3-leaf) and V6 (6-leaf) with the propane doses of 20 and 45 kg/ha for the banded and broadcast flaming, respectively. Overall, weed control and maize response varied among treatments and growth stages. Cultivation at the V3 stage only, provided the poorest weed control (20%) and the lowest yield (9.7 t/ha) due to weed competition from uncontrolled weeds. The best treatment was a combination of cultivation and banded flaming conducted twice, at the V3 and V6 stages of maize. Such treatmentprovided above 95% weed control and yielded about 27% more than cultivation alone conducted at the same time (12.6 t/ha vs. 9.9 t/ha). All other treatments provided significantly lower weed control levels, ranging from 20–80%. Based on data from just the first year of this study, it appears that the most promising season-long weed control was achieved with a combination of flaming and cultivation treatment applied twice in field maize, at V3 and V6 stages.

Plant diseases monitoring system based on Web GIS in Jeonnnam Province, Korea

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Phytopathology 101:S92

Plant diseases monitoring system, which input the scouting data, analyse the disease occurrences and then inform to farmers automatically, was constructed to respond to outbreak diseases promptly. Barley, wheat and pepper diseases were scouted and analysed the disease information in real-time based on Web GIS technology in Jeonnam Province. Scab disease was not showed in middle of May, but that of wheat, naked barley, hulled barley, and malting barley showed 4.0%, 2.2%, 2.2%, and 1.2% of diseased panicles, respectively. Phytophthra blight of pepper was appeared in early of June and the severely occurred from middle of August to early of September. Anchranose was initially occurred in middle of July but was not increase severely after that. Virus disease was first showed in early June and drastically increased from middle of July. When the disease information is shared by web GIS system, it is possible to respond effectively to outbreak diseases of plant.

Glyphosate activity on plant diseases and potential impact on plant health and yield in Roundup Ready® cropping systems

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Phytopathology 101:S93

Laboratory and field studies in the early 2000's suggested that glyphosate was active against rusts in glyphosate resistant wheat (P. triticina, P. striiformis) and Asian soybean rust, caused by Phakopsora pachyrhizi, in glyphosate-resistant soybeans. Further investigations into the disease control activity of glyphosate against a broader spectrum of plant pathogens demonstrated that that the application of glyphosate as technical material or as Roundup® formulations can suppress the incidence and/or severity of a range of plant diseases. Experiments under growth chamber and field conditions have shown that glyphosate can suppress symptoms of disease from a range of economically important pathogens, and that applications of Roundup products have the potential to reduce yield loss in the presence of significant disease pressure. The fungicidal mode of action of glyphosate is attributed to inhibition of fungal EPSPS, with disease suppression primarily provided by systemic glyphosate. Suppression of disease may be provided from both pre and post infection applications of glyphosate. Our results indicate that glyphosate has the potential to suppress diseases caused by a wide range of fungal pathogens, and could provide incremental disease control benefits in glyphosate-resistant cropping systems.

Common ragweed (*Ambrosia artemisiifolia*) – a worldwide problem

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Phytopathology 101:S93

The main cause of allergy and pollen asthma in North America and Central Europe is pollen from ragweed (*Ambrosia*) a genus in the Asteraceae. Currently short or common ragweed (*Ambrosia artemisiifolia*, L.) is rapidly spreading in Europe and has the highest weed densities in the Carpathian basin: Croatia, Hungary, and Serbia. Despite continuous efforts by the Hungarian government during the last ten years to eradicate ragweed, levels of its pollen in the air did not diminish. Ragweed infestation is heaviest in sunflower (Helianthus annuus L., the third most important crop in Hungary) fields, producing the overwhelming majority of allergenic pollen in the air (in the end of the summer pollen counts reach 1000 grains m-3) even in urban areas. In the presentation we show the current situation in Europe, focusing on Hungary and discuss the most recent measures and the strategic program based on remote sensing and precision weed management methods we developed for controlling ragweed and suppressing its pollen production.

Role of *rsmA* in virulence of phytotoxin-producing pathovars of *Pseudomonas syringae*

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Phytopathology 101:S93

The *gacS/gacA* two-component system functions mechanistically in conjunction with the global transcriptional regulator RsmA to allow pseudomonads and other bacteria to adapt to changing environmental stimuli. Analysis of this pathway in phytotoxin-producing pathovars of *Pseudomonas syringae* is incomplete, particularly with regard to *rsmA*. Our approach was to overexpress *rsmA* in *P. syringae* strains through the introduction of pSK61, a stably maintained plasmid constitutively expressing this gene. It is convenient to study RsmA regulation using overexpression approaches as opposed to the use of knock-out mutants, as some bacteria, including pseudomonads, contain two or more *rsmA* alleles with redundant functions. Disease and colonization of plant leaf tissue were consistently diminished in all *P. syringae* strains tested (pv. *phaseolicola* NPS3121, pv. *tabaci* BR2R, and pv. *syringae* B728a) when containing pSK61 relative to these isolates containing the empty expression vector pME6031. Phaseolotoxin, tabtoxin, and syringomycin were also not produced in these strains when carrying pSK61. In contrast, alginate production, biofilm formation, and the hypersensitive response were diminished in some, but not all, of these isolates under the same conditions. These results indicate that the role of *rsmA* varies with pathovar in the phytotoxin-producing strains of *P. syringae*.

Occurrence and control of Physoderma disease in China

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Phytopathology 101:S93

Physoderma disease, caused by *Physoderma maydis*, used to be a secondary disease on maize in China. In recent year, however, this disease becomes severer and severer with extension of new hybrids and stubble retention. The disease widely occurred of about 2 million ha and the disease incidence was about 30% to 50% in Henan, Hebei, Shandong and Anhui provinces in 2006, 2008 and 2010. Therefore, it is one of the major diseases on maize in these areas. The disease first occurred on leaves at the middle stage of maize and the disease peak was at 15–18 leaves stage. The yield loss was about 10% in general and the severe loss was from 30% to 40%. Resistance evaluations of 42 hybrids collected from the four provinces were conducted by artificial inoculation method. Among them, 13 high resistant hybrids were selected with the disease index lower than 11. Meanwhile, disease index of 6 susceptible hybrids were higher than 33. The result will contribute to proper usage of maize hybrids with high resistant to physoderma disease. Six kinds of fungicides including Thiophanate-methyl, Diniconazole, Triadimefon, Myclobutanil, Carbendazim and Amistar were tested in Lab condition. Results showed that all tested fungicides could restrain the germination of resting sporangia at different levels. Field trials indicated that Amistar, Diniconazole and Tebuconazole could efficient control of physoderma disease. Among them 25% Tebuconazole EC shown the best control of 90% by spraying at 8–10 leaves stage.

Survival of three quarantine pathogens in a simulated aquatic system at different levels of pH

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Phytopathology 101:S93

Phytophthora ramorum, *P. alni* and *P. kernoviae* present significant threats to plant biosecurity. As water molds, these pathogens may spread through natural waterways and irrigation systems as exemplified by *P. ramorum* detection in streams and ornamental nursery effluents. However, the knowledge of their aquatic biology is scarce. Here we investigated the survival of these three quarantine pathogens in response to different levels of pH in a simulated aquatic system. Experiments were conducted using 10% Hoagland's solution as a base medium. Treatments included a pH range from 3 to 11 and exposure times from a few seconds to 14 days. After overnight exposure, the highest recovery of *P. ramorum*, *P. alni* and *P. kernoviae* was zero, 6.9% and 12.2%, respectively. Zoospores of *P. alni* survived at low rates over 14 days across all pH levels. Similar results were obtained for *P. kernoviae* at pH 3 to 9. Although *P. ramorum* zoospores failed to survive in the system, its sporangia were tolerant to all pH levels tested, and 18–43%, except for 5.9% at pH 3, survived over 14 days. Additional experiments are underway to compare the effects of pH on plant infection by zoospores and sporangia. Implications of these data on management of these quarantine pathogens are discussed.

Characterization of small RNAs derived from *Tomato spotted wilt virus* infection by deep sequencing

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Phytopathology 101:S93

RNA silencing is a conserved eukaryotic surveillance mechanism thought to play a role in protection against invading nucleic acids such as viruses, transposons and transgenes. Virus infection leads to accumulation of viral small RNAs (vsRNAs) at high levels. The processing of vsRNAs can be from

viral dsRNA replicative intermediates, self complementary regions of the viral genome or from the action of RNA-dependent RNA polymerases on viral templates. The overall composition of the populations of vsRNAs generated by most plant viruses remains unknown. We have used deep sequencing techniques to characterize vsRNAs of *Tomato spotted wilt virus* (TSWV), a member of the genus *Tospovirus*, which causes economically important diseases in numerous crops in many parts of the world. The vsRNA profiles from TSWV-infected pepper, tomato, and tobacco plants were generated. Analysis of the vsRNAs indicate multiple hot spots for small RNA production from the TSWV genome, the location of these hot spots are predominantly conserved across infections of different host species. Details of the origin, distribution and abundance of TSWV vsRNAs in infected plant tissue were compiled.

Sources of resistance to Phytophthora fruit rot in watermelon plant introductions

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Phytopathology 101:S94

Phytophthora fruit rot caused by *P. capsici* is an emerging disease in most watermelon producing regions of Southeast U.S. Plants belonging to the core collection of U.S. watermelon plant introductions (PI) were grown in a field on raised plastic beds to evaluate for fruit rot resistance in 2009. Five fruits from each PI were harvested and inoculated with a 7-mm plug from an actively growing colony of *P. capsici* on V8 juice agar. The inoculated fruit were maintained in a room with high relative humidity (>95% RH) for four days. Data on length of disease lesion and intensity of sporulation were recorded for each fruit. Of the 205 PI evaluated, majority were highly susceptible and extensive sporulation was observed on most fruit. Overall we identified 25 PI (12%) as potential sources of resistance. Twenty two (12%) of the 159 *Citrullus lanatus* var. *lanatus* PI we evaluated from the core collection, one *C. colocynthis* (PI 388770) and two *C. lanatus* var. *citroides* PI (PI 189225) showed varying levels of resistance to fruit rot. Variability in resistance reaction to fruit rot among plants of the same PI was also observed. The most resistant PI were re-evaluated in 2010. Fruit from resistant PI had significantly lower amounts of *P. capsici* DNA/g of fruit tissue compared to susceptible cultivars Sugar Baby and Black Diamond. Selections from the most resistant PI will be further evaluated using isolates from different states to confirm the stability of resistance.

Weeds as reservoir hosts of Tomato leaf curl virus (Begomovirus) in Tamil Nadu

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Phytopathology 101:S94

Weeds have been reported as important reservoirs for emerging Geminiviruses. The white-fly transmitted Geminivirus, especially Begomovirus are reported from regions that were earlier free of these viruses. During the last two decades they have emerged as more serious problems in a variety of crops in India. In particular, Tomato leaf curl virus (ToLCV) causes severe losses and leaf curl symptoms in tomato throughout the country. Aimed at identifying the reservoir hosts of the virus in tomato field from north eastern part of Tamil Nadu, India, we surveyed tomato plants as well as weeds growing in and around the tomato fields for the ToLCV infection for a year. TOLCV infection was confirmed by PCR using ToLCV coat protein and Rep protein specific primers that amplified a ~ 800 bp DNA fragment from the samples studied. The amplicons sequenced and compared with the sequences available in NCBI confirmed their presence. Based on the above, we conclude that the weeds Euphorbia hirta, and Hibiscus cannabinus L. serve as a host for ToLCV in the tomato field both during the cropping and non-cropping seasons.

Evaluation of drip applications of Revus in fungicide programs for management of Phytophthora blight (*Phytophthora capsici*) on bell pepper and squash

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Phytopathology 101:S94

REVUS® Fungicide (a.i. mandipropamid) was introduced in the U.S. in 2008 for control of downy mildews and diseases caused by *Phytophthora* spp. For all uses, including management of *P. capsici* on cucurbits and peppers, current labeling for Revus calls for foliar application. In view of the increasing interest in delivery of products through drip irrigation, studies were initiated to evaluate Revus applied via this route. Early trials have focused on bell peppers and cucurbits. A number of drip application régimes were evaluated including Revus solo (8 floz/A), in combination with Actigard® (0.25 or 1 oz/A), and in alternation with Presidio® (Valent) (4 floz/A). All programs started with a preplant application of Ridomil Gold® SL (1 pt/A) and included alternation with foliar applications of Ridomil Gold Copper (1 lb/A). Against intense disease pressure, all drip programs with Revus slowed development of Phytophthora blight, the most effective régimes providing *ca* 50 and 75% disease control on bell pepper and squash, respectively. The drip applied programs with Revus consistently performed as well or better than a foliar-only treatment and were safe on both crops. Following up on these encouraging results, we are conducting additional studies to optimize the use of Revus via drip application in programs for *P. capsici* on cucurbits, peppers and other fruiting vegetables.

Effects of pesticide treatments on SABP2 mediated systemic acquired resistance in plants

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Phytopathology 101:S94

Plants react to pathogen infection by inducing local and systemic acquired resistance (SAR). SABP2, a 29 kDa esterase like protein has been shown to play a critical role in the activation of SAR in plants. In agriculture, pesticides are widely used to control plant pests. Some of these pesticides have been shown to inhibit acetylcholinesterase (AChE) which converts acetylcholine to choline. Both AChE and SABP2 belong to the α/β hydrolase superfamily of enzymes and exhibit esterase activities. Inhibitors of esterase activity of AChE may also inhibit enzymatic activity of SABP2. This may prevent SABP2 mediated conversion of methyl salicylate to salicylic acid (SA) resulting in plants being unable to activate SA-signaling and hence become more susceptible to pathogen infection. In vitro studies showed that enzymatic acitivity of SABP2 is inhibited by organophosphate pesticides. To test, if pesticide treatment also inhibits SAR, we used 6–8 weeks old tobacco (*Nicotiana tabacum* cv. xanthi NN) plants. Results show that pesticide treatment compromises plants ability to mount robust SAR response in tobacco plants. Transgenic plants lacking SABP2 (due to RNAi silencing) did not show any effect of pesticide treatment on SAR development. Currently we are determining molecular effects of pesticide treatments on expression of defense genes. In future, emphasis while developing/choosing pesticides should be on 1) controlling pest, and 2) their effects on the pathways mediating microbial defenses of the plant.

Effect of barley chromosome addition on the susceptibility of wheat to feeding by gall-inducing leafhopper, *Cicadulina bipunctata* (Hemiptera: Cicadellidae)

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Phytopathology 101:S94

Cicadulina bipunctata Melichar, 1904 is distributed widely in tropical and subtropical regions of the Old World and is recognized as an important pest insect of maize. This species induces galls characterized by the growth stunting of leaves and severe swelling of leaf veins on various Poaceae such as wheat, rice and maize but not on barley. In order to clarify the mechanism of growth stunting and gall induction by *C. bipunctata*, we investigate the effect of barley chromosome addition on the susceptibility of wheat to feeding by *C. bipunctata*. As a result, degrees of gall induction and stunted growth were different among six barley chromosome disomic addition lines of wheat (2H-7H). Feeding by *C. bipunctata* significantly stunted the growth in 2H, 3H, 4H and 5H, but did not in 6H and 7H. Comparing to wheat, the degree of gall induction was significantly weaker in 3H and severer in 5H, respectively. Significant correlation was not detected between the degrees of growth stunting and gall induction. These results suggest that resistant genes to growth stunting exist in barley chromosomes 6 and 7, and those to gall induction are present in chromosome 3. The results also imply that growth stunting and gall induction are two independent phenomena, even though they are both induced by the feeding by *C. bipunctata*.

Gene expression profiling in *Phytophthora phaseoli* during the infection of lima bean

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Phytopathology 101:S94

Lima bean (*Phaseolus lunatus* L.) is an important legume crop to the state of Delaware and is susceptible to the oomycete pathogen *Phytophthora phaseoli* Thaxt. which causes downy mildew. In the year 2000 alone, downy mildew caused a $3 million crop loss to the industry. In this study, we have used Illumina RNA-seq to identify genes in *P. phaseoli* orthologous to several

effector genes in *P. infestans*, a close relative of *P. phaseoli*. To study the function of these effector proteins, we selected ten candidates with similarity to RxLRs, elicitins, NPP1 and crinklers, all of which are different classes of effectors. The above effector genes were validated by performing in planta qRT-PCR. Phylogenetic analyses of candidate effector genes from other oomycete pathogens confirm a close relationship of *P. phaseoli* and *P. infestans* for all the corresponding effector genes. Selected effectors were cloned into Agrobacterium and then injected into *Nicotiana benthamiana* leaves for transient assays. Results showed three elicitins (Pp_INF1, Pp_INF4 and Pp_06908) induced a hypersensitive response and one RxLR effector (Pp_17063) showed spectrum of suppression of the hypersensitive cell death response induced by INF1. Currently, we are performing functional characterization of RxLR effectors and other effector genes, which will help us to gain a better understanding of this pathosystem and will serve as a basis for future research.

Search for *Candidatus* Liberibacter spp. in citrus and orange jasmine plants and psyllids in Texas by field surveys and multi-loci PCR assays

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Phytopathology 101:S95

Citrus huanglongbing (HLB) is associated with three species of '*Candidatus* Liberibacter', '*Ca*. L. asiaticus' (Las), '*Ca*. L. africanus' and '*Ca*. L. americanus'. In Florida HLB was detected 5 years after the vector, the Asian citrus psyllid (ACP) was found. In Mexico and other Central America and Caribbean countries, HLB was detected 6–8 years after ACP was found. ACP was first reported in Texas in 2001, which triggered HLB surveys in citrus production and residential areas in the state. Citrus species were found adjacent to jasmine trees in dooryards. Some jasmine samples produced high Ct values above 32 in real-time PCR (qPCR). Suspect samples were recollected from these plants along with ACP adults and nymphs for DNA extraction. The primers and probe based on 16S rRNA were used to identify Liberibacters by conventional (cPCR) and TaqMan qPCR. New TaqMan primer/probe sets based on other Las genes were used to confirm the 16S qPCR results. Nested cPCR was attempted to obtain bands from high qPCR Ct value samples. Of the 16 citrus, 90 jasmine and 22 ACP samples tested, 3 jasmine trees yielded high qPCR Ct values with 16S rRNA and some of the new primer/probe combinations from Las genes, but tested negative with cPCR and had no typical symptoms and will be monitored. The combination of qPCR primer sets may help resolve the high Ct value samples during subsequent testing.

First report of Sweet orange scab in U.S.A.

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Sweet orange scab caused by *Elsinoe australis* was first identified in Brazil in 1937, and has also been reported from Korea in 2001 (natsudaidai pathotype). It is reported to mainly affect the fruit of sweet oranges and mandarins, and until the development of molecular based assays, was distinguished from *Elsinoe fawcettii*, the causal fungus of common scab, by host range studies and the rarity of foliar symptoms. In 2010, a lemon fruit with scab symptoms collected in Spring TX (near Houston) gave a positive PCR result for *E. australis*. An additional positive result was obtained with a symptomatic mandarin sample from Orange TX. Samples were then collected by USDA-APHIS-PPQ and sent to the Mol. Diag. Lab in Beltsville MD which confirmed the PCR result and cultured the fungus. USDA-APHIS-PPQ then collected samples from several counties, including the three in the Lower Rio Grande Valley where most commercial production occurs. Grapefruit with what is termed 'late season wind scar' were collected from several locations and tested for *E. australis*. Several gave positive results. Cultures have since been obtained from sweet orange and confirmed by PCR and sequencing to be *E. australis* (natsudaidai pathotype), and fruit and leaf inoculation studies are in progress.

Functional analysis of NSs and NSm genes of *Impatiens necrotic spot virus* found in Salinas valley, California

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In the 2006–2007, lettuce plants in Monterey County California showed necrotic spotting and mottle/mosaic symptoms, which were subsequently shown to be caused by *Impatiens necrotic spot virus* (INSV). Sequence analysis of N and NSs genes confirmed that lettuce-infecting INSV isolates were similar to previously characterized INSV isolates, and RT-PCR was used to investigate the INSV inoculum source(s). INSV was detected in thrips (*Frankliniella occidentalis*) from infected and non-infected lettuce and some asymptomatic weeds, such as malva and shepherd's purse. This indicates that weeds are an inoculum source. To investigate properties of INSV proteins, the NSs and NSm were expressed alone or as fusions with GFP for subcellular localization and silencing suppressor experiments. Both NSs and NSm were expressed in *Nicotiana benthamiana* plants via *Agrobacterium tumefaciens*-mediated transient expression. Confocal laser scanning microscopy results showed that NSm formed punctate bodies in the cell membrane or wall, whereas NSm localized at the nuclear periphery and cytoplasm. Silencing suppressor experiments in *N. benthamiana* 16c GFP transgenic plants, and RNA binding assays with NSs and NSm are ongoing. Based on our preliminary data and previous studies on other tospoviruses, NSs may be the silencing suppressor of INSV.

The detection of *Ceratocystis fagacearum* in Texas live oak using real-time polymerase chain reaction

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Phytopathology 101:S95

The diagnosis of oak wilt depends on isolation of the pathogen, *Ceratocystis fagacearum*, from wood tissues of symptomatic trees plated onto a growth medium such as acidified potato dextrose agar. This technique is time consuming, inefficient and has many limitations, including a long incubation period which often produces false, negative results. With the objective of developing a specific molecular detection system, 16 GenBank accessions of *Ceratocystis* ITS DNA sequences were used to generate a BLAST alignment to look for regions of variation that could be used to discriminate for *C. fagacearum* in a quantitative real-time PCR assay. Two regions, including 341 to 510 bp (ITS1) and 651 to 820 bp (ITS 2) were selected and further compared with 81 data bases of fungal DNA sequences. Primers and fluorescent labeled probes specific for *C. fagacearum* were designed using Primer Express® software (Applied Biosystems). These primer/probe sets successfully detected the pathogen from cultured spore suspensions and purified, target DNA without amplifying closely related, non-target fungal species. The ITS primer/probe set CfP2 consistently detected *C. fagacearum* from sampled, symptomatic trees that were confirmed by pathogen isolation. The preliminary testing of this technique demonstrates the potential for this tool to be a significant breakthrough in the diagnosis of oak wilt and invaluable in the study of this destructive tree pathogen.

Antimicrobial lipopepetide iturin induce systemic resistance of *Arabidopsis thaliana*

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Iturin is an antimicrobial lipopeptide produced by antagonistic strains of *Bacillus* spp., and is deduced to play key roles in biological control for several kinds of plant diseases; however, the function of iturin in disease suppression is still unclear. Here, we report that a novel function of iturin as an elicitor for *Arabidopsis thaliana*. The root-treatment of purified iturin to hydroponic cultured *A. thaliana* showed significantly suppressive activity for *Colletotrichum* disease on leaves. Gene expression analyses of host plant revealed that the treatment of iturin to root induced some systemic resistant related genes.

Evaluation of *Mycosphaerella polygoni-cuspidati* for classical biological control of Japanese knotweed (*Fallopia japonica*)

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Mycosphaerella polygoni-cuspidati was selected as a potential classical biological control (CBC) agent for *Fallopia japonica* according to the results of the surveys and screening. *M. polygoni-cuspidati* was redescribed and neotypified based on not only morphology but also the internal transcribed spacer (ITS) region including the 5.8S ribosomal DNA (rDNA) sequences. Field observations revealed that this pathogen had a reduced life cycle, with only spermogonia and pseudothecia being formed. Under controlled environmental conditions, disease development of *M. polygoni-cuspidati* mycelia on *F. japonica* was assessed under several factors including leaf age, dew-period temperature, dew-period duration and post-dew temperature. *F. japonica* leaves at younger leaf stages of 7–9 days after opening were more

susceptible than the older leaves, especially those at the older than 13 days after opening. When adequate dew was provided, severe defoliation was observed over the dew-period temperature range of 15 to 20°C. With a dew period of at least 18 h, leaf defoliation was occurred, but the disease incidence was greater on plants submitted to 42–48 h dew periods. The optimal post-dew temperature for disease development was 19–21°C. Preliminary host specificity testing using UK plant species showed that *M. polygoni-cuspidati* is highly specific to *F. japonica*. These results indicate that *M. polygoni-cuspidati* has high potential as a CBC agent for an invasive weed, *F. japonica*.

Is there any other elixir of life on this planet?
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Phytopathology 101:S96

Excessive and indiscriminate use of fertilizers and pesticides had caused several ecological problems. Hence, locally available natural farm products like Annamalai mixture (cow urine, cow dung, sheep dung, poultry litter and neem cake), turmeric powder, hydrated lime were tested against various plant pathogens and vectors either alone or in combination. Spray of farm natural products particularly Annamalai mixture at biweekly intervals for 15 times have resulted in manifold increase (3–7 times) in the yield of several crop plants (cereals, pulses/legumes, oil seeds, cotton and vegetables). They also significantly reduced the major diseases of rice and pulses/legumes; bacterial blight of cotton, red rot of sugarcane, anthracnose of chillies, damping – off and killed or repelled aphids, white flies, leaf hoppers and mealy bugs completely. Livestock excreta treated rhizosphere soil exhibited maximum number of bacteria, followed by actinomycetes and fungi. For fungal and bacterial diseases livestock urine whereas for viral diseases dung extracts worked well. Among all the animal excreta tested cow, sheep and poultry were found to be the best sources. The volatile ammonia, silica in livestock excrements, curcumin (turmeric powder), calcium (hydrated lime) and azadiractin (neem cake) were responsible for the toxic effect and the enhanced yield might be due to various macro and micro elements present in them. Hence, Annamalai mixture is yet another elixir of life in addition to water on this planet, earth.

Management of leaf curl diseases by eco-friendly methods
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Phytopathology 101:S96

Tomato and chillies are the important crops grown in many countries. The leaf curl caused by leaf curl virus transmitted by whiteflies (*Bemisia tabaci*) is the most important one. Hence, locally available natural products were tested against these diseases and the insect vector. Leaf extract of *Prosopis chilensis* 75%, *Azadiracta indica* 100%, cow dung 50%, sheep dung 75% and cow urine 100% recorded total mortality of whiteflies. Cow dung, cow dung plus sheep dung combination spray as well as leaf dip method (50%, 1:1v/v) recorded total prevention of whiteflies settlement. When mixed with the viral inoculum, the combined treatment caused no disease in both the systemic as well as indicator plants. No virus was recovered on back inoculation. A similar result was obtained in pre inoculation experiment also. The seedlings root dipping and ten times biweekly spray of the combination treatment completely eradicated the viral infection even after challenged with the virus. It also recorded the maximum yield in both pot and field trial conducted. Whitefly colony was not found in any of the treatments except in control. There was an increase in total and O. D phenol and reduction in reducing, non reducing and total sugar content. Volatile ammonia and biogenic silica present in the livestock excreta may be responsible for the viricidal effect. Thus, eradication of established virus infection and total protection from whiteflies by dung combination may open a new avenue in virus disease management strategy.

PCR-based detection, by use of degenerate primers, of an *EngA* cellulase gene in *Xanthomonas sacchari* from asymptomatic sugarcane
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Phytopathology 101:S96

Subjected to PCR using primers directed to intergenic 16S-23S, DNAs from *Xanthomonas* isolates from asymptomatic sugarcane produced a 450-bp amplicon with 100% identity to the spacer region of *X. sacchari*. A lack of information on pathogenicity of these microorganisms prompted us to determine whether they possess a cellulase gene, *EngA*, an essential prerequisite for virulence in *Xanthomonas*. Two sets of degenerate primers produce overlapping, about 600-bp amplicons that, when searched by BlastX, showed about 92% amino acid similarity to the protein product predicted for the *EngA* gene of *X. albilineans*. The results can be interpreted as indicative of the potential for pathogenicity on other hosts perhaps.

***Salmonella enterica* moderates *Pectobacterium carotovorum* populations and virulence on lettuce**
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Phytopathology 101:S96

Contamination by plant or human pathogens renders fresh produce unmarketable. Infection by the phytopathogen *Pectobacterium carotovorum* causes water-soaked lesions and rapid maceration of plant tissues. *Salmonella enterica* serotype Saintpaul caused multistate salmonellosis outbreaks linked to peppers in 2008 and alfalfa in 2009. This enteric human pathogen survives in the environment between warm-blooded hosts and plants have become an important vehicle to humans. Phytopathogens improve the survival of human pathogens on plants but the converse interaction is unknown. We examined the interaction of *S. enterica* Saintpaul and *P. carotovorum* on lettuce by measuring population, pH, and virulence (lesion length). Detached lettuce leaves inoculated at the midrib with 10^3 CFU of *S. enterica* Saintpaul, *P. carotovorum* WPP359, or a *P. carotovorum* Δ*budB* mutant were sampled every 24 h, up to 96 h. Alternatively, leaves were co-inoculated with 10^3 CFU each of *S. enterica* and WPP359 or Δ*budB*. At 72 h and 96 h, *S. enterica* populations were higher in the presence of *P. carotovorum* while populations of both *P. carotovorum* strains declined in the co-inoculations compared to lone inoculations. Lesion lengths were substantially larger, and pH significantly higher, in the WPP59 treatment compared to Δ*budB*, *S. enterica* co-inoculations, and controls. These results indicate a potential role for human pathogens in plant disease development with implications for plant health and food safety.

Strategies of biological and symbiotic control of citrus variegated chlorosis by endophytic bacteria
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Phytopathology 101:S96

Citrus Variegated Chlorosis (CVC) is an economically important and destructive disease caused by *Xylella fastidiosa* (*Xf*) and transmitted by sharpshooter insects. One factor that may confer resistance to CVC is the endophytic microbial community colonizing individual *C. sinensis* plants. Our results suggest that there are interactions between *Xf* and endophytic bacteria present in the xylem of sweet orange, and that these interactions, particularly with *Methylobacterium mesophilicum* (*Ms*) and *Curtobacterium flaccumfaciens* (*Cf*), may affect disease progress. Symbiotic control is a new strategy that uses symbiotic endophytes as biological control agents to antagonize or displace pathogens. Also, candidate endophytes for use in symbiotic control of CVC must occupy the xylem of host plants and attach to the precibarium of sharpshooter insects in order to have access to the phytopathogen. We demonstrated the transmission, colonization, and genetic manipulation of *Ms* as a prerequisite to examining the potential use of symbiotic control to interrupt the transmission of *Xf*. The ability demonstrated by *Cf* to colonize plant tissues in the presence of *Xf* and the reduction of disease symptoms caused by *Xf* are prerequisites for the use of these endophytic bacteria as a biocontrol agents. We propose the endophytic bacterium *Cf* as a classical biological control agent and the endophytic bacterium *Ms* as a qualified candidate for a symbiotic control strategy.

EDDMapS: The common operating platform for aggregating and using invasive species distribution data
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Phytopathology 101:S96

The Early Detection & Distribution Mapping System ("EDDMapS"; www.eddmaps.org) is a web-based tool used to map the occurrence and range of invasive species across the United States. The system provides a common platform to aggregate data from a wide variety of sources and makes it available for viewing in online maps as well as easily downloaded for use in GIS programs. The resulting collaboration between organizations that this system has fostered provides a more complete perspective on the distribution of invasive species while also crediting the original data providers. The system has been developed to also allow citizen scientists to report new occurrences, allow experts to verify new reports, allow users create alerts to

receive notification when a taxonomic group is reported in the geographic areas they are monitoring, and allow Cooperative Invasive Species Management Areas (CISMAs) to keep records on treatments to stop the spread of invasive species. This system is part of the Center for Invasive Species and Ecosystem Health at the University of Georgia. Several new projects are now underway to expand the scope of the system, the completeness of the data, and the functionality to the end user. Any groups interested in being a part of these new efforts can go to www.EDDMapS.org to learn more.

Tomato powdery mildew may be significantly reduced by choice and management of irrigation system in the Brazilian Middle West

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Powdery mildew (PM, *Leveillula taurica*) is a limiting factor for the irrigated tomato (*Solanum lycopersicon*) winter crop in the Brazilian Middlewest. The winter season is dry with a large thermal range and frequent dew formation. A detailed study of the relation between irrigation methods and *Leveillula* mildews has not been attempted. We studied the effect of irrigation methods on the severity of tomato PM for two years (2009 and 2010), in a RCBD with 3 replicates and 100 plants per plot. The following systems were studied: Drip with one line (D1L); or two lines (D2L); Drip with one line and plastic mulch (DPM); or corn mulch (DCM); Furrow (FUR); low pressure microsprinkler with one line (MIC); and conventional overhead (COV). Irrigations were performed at each one of two soil moisture tensions: 15-30 kPa (high moisture) or 30-60 kPa (moderate moisture). FUR and DCM were irrigated only at the moderate level, totalling 12 treatment-combinations. PM was evaluated weekly and the Gompertz model was adjusted to severity data. All moderately irrigated drip, tape and furrow treatments, as well as high moisture drip treatments, had high Ymax values (>87%), disease progress rates (r between 0.052 and 0.065) and AUDPCs. Microsprinkler irrigation at high moisture caused a delay in disease progress and intermediate severity levels. Lowest Ymax (<13%), r (0.013 to 0.018) and AUDPCs were observed in both overhead irrigated treatments. PM severity varied among years, but results were consistent for both years.

Transgenic plants expressing antimicrobial lactoferrin protein are resistant to *Rhizoctonia solani*

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Lactoferrin (LF), a cationic iron-binding glycoprotein of the transferring family is present in milk, tears, saliva, and mucous secretions of most mammals. LF is known to exert a broad-spectrum primary defense activity against bacteria, fungi, protozoa and viruses. The Bovine lactoferrin (BLF) gene was introduced to tobacco (*Nicotiana tabacum* var Xanthi) and *Arabidopsis* (*Arabidopsis thaliana*) plants with *Agrobacterium tumefaciens* strain C58C1 containing a plasmid construction carrying a modified BLF cDNA. Plants expressing BLF were evaluated for resistance against an economically important fungal pathogen *Rhizoctonia solani*, the causal agent of damping off diseases. The introgression of BLF cDNA into susceptible tobacco and *Arabidopsis* lines was confirmed by Southern blot and the expression of full-length LF transcript and protein was also detected by Northern and Western blots, respectively. Transgenic lines segregating for a single locus insertion were identified and used for disease resistance assays. Detached leaves of transgenic tobacco plants exhibited high levels of *Rhizoctonia* resistance. In addition, transgenic *Arabidopsis* seedlings were resistant to the *R. solani* and prevented damping off symptoms. Use of BLF gene is a potential new approach to consider for control of diseases caused by fungal pathogens.

Epistatic involvement of plasmodesmatal localized protein and malic acid transporter in aerial pathogenesis and belowground rhizobacterial recruitment

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Beneficial soil bacteria confer immunity against a wide range of foliar diseases by activating plant defenses. Our recent work demonstrated that foliar infection by *Pseudomonas syringae* pv tomato (PstDC3000) induces recruitment of *Bacillus subtilis* (FB17), a beneficial rhizobacteria. We also show the induction of a malic acid (MA) transporter (ALMT1) post PstDC3000 infection, leading to increased MA titers in the rhizophere. Intra-plant signaling under distress conditions, especially between aboveground and belowground tissues is potentially complex due to the involvement of significant physical distances. Although, cell to cell signaling through plasmodesmata has been demonstrated in various physiological and developmental plant responses, but how plasmodesmata mediates cell to cell signaling to inflict innate immunity is not well understood. Recently, a plasmodesmata localized protein (PDLP5) is shown to be transiently expressed during a foliar PstDC3000 infection. We therefore hypothesize that post PstDC3000 aerial infection; plants may relay a shoot-to-root signal involving a member of the aluminum-activated ALMT1 to recruit FB17 in the rhizosphere. Concomitantly, the PDLP5 over-expression (*35S::PDLP5*) showed higher levels of ALMT1 compared to PDLP5 knock-out (*pdlp5*). The *35S::PDLP5* secreted higher titers of MA compared to the *atalmt1* and *pdlp5*. In addition, the mutants *atalmt1and pdlp5* exhibit reduced biofilm formation by FB17. Furthermore, both *pdlp5* and *atalmt1* were susceptible to PstDC3000.

Management of peach blossom blight canker development with biorational fungicides

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Infection of peach flowers by *Monilinia* spp. can result in formation of cankers that are important sources of inoculum for subsequent development of brown rot on fruit. Results of a preliminary study in 2008 indicated that the biorational fungicides *Bacillus subtilis* (Serenade MAX), potassium bicarbonate (Kaligreen), and neem oil extract (Trilogy) provided good to excellent control of canker formation. To further examine efficacy, low and high rates of these fungicides, along with a cyprodinil (Vangard) standard, were applied to trees in an 'Encore' orchard in 2009 and 2010 using a RCBD with four blocks. Treatment applications were made at 5–10% bloom, 75–100% bloom, and 75–100% petal fall stages. Disease was assessed by counting blossom blight cankers that formed by mid-summer on 20 flowering shoots per tree. Disease incidence was expressed as percent shoots with canker and canker density as number of cankers per shoot. Analyses of combined 2009–2010 data revealed that all treatments significantly reduced canker incidence and density. Non-treated trees had an average 16.9% incidence, while treated shoots ranged from 0 to 9.4% incidence. Furthermore, disease levels for five of the six biorational treatments, which provided 59 to 85% control, were not significantly different from the standard. These results suggest that some biorational fungicides may be directly substituted for conventional fungicides during bloom without significant loss in blossom blight control.

Characterization and epidemiological aspects of a novel badnavirus infecting fig

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Fig mosaic (FM) is the most common virus disease of fig (*Ficus carica*) with symptoms varying greatly between locations and cultivars, probably because of the number of viruses infecting the crop. A novel badnavirus was discovered in FM trees in Arkansas. The complete genome was obtained using PCR-based rolling circle amplification and phylogenetic analysis revealed its close relationship to *Citrus mosaic virus* and *Cacao swollen shoot virus*. A survey of FM material from the National Germplasm Repository in Davis, CA, and Fayetteville, AR, was conducted and the new virus was widespread in both locations with over 75% FM trees (30/40 samples) tested positive but was also found in asymptomatic material. Virus diversity was investigated using 22 isolates all of which were very similar with nucleotide identities ranging from 99–100%. Infected tissue was mechanically inoculated onto 19 indicator species and the badnavirus can infect pumpkin, soybean, English pea and several tobacco species. The new virus may be of concern to the fig growing areas due to the apparent ease of mechanical transmission, which may in turn account for its widespread presence in fig trees.

Reducing damage to root-knot nematode with fluensulfone (formerly thiazosulfene) in cucumbers and peppers

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Root-knot nematode (*Meloidogyne incognita*) is the most prevalent and damaging nematode affecting vegetable crops in the southeastern U.S. Fumigants such as methyl bromide (MB) and 1,3-dichloropropene have been used extensively to control this nematode, but they are expensive, require specialized application equipment, are hazardous to handle, and are under heavy regulation. An efficacious, non-fumigant nematicide such as fluensulfone would negate many of these problems. In the fall of 2010,

fluensulfone was compared to several fumigants and oxamyl on slicing cucumber and bell pepper. Combinations of fumigant and non-fumigant nematicides were evaluated as well. Fluensulfone and oxamyl were soil-incorporated and 1,3-dichloropropene was applied with a yetter rig prior to laying plastic. All other fumigant treatments were applied using soil-injection/plastic laying equipment and all treatments were tarped with VIF plastic mulch. Fluensulfone and oxamyl were susequently applied several weeks after planting through drip irrigation. Fluensulfone alone and in combination with oxamyl significantly reduced nematode galling compared to oxamyl alone and non-treated plots in both cucumber and pepper and was similar to fumigant-treated plots. No differences in yield were noted in the cucumber trial. Pepper yields were reduced by MB, chloropicrin and metam sodium treatments due to phytotoxicity.

Components of resistance to *Phytophthora nicotianae* in doubled-haploid lines of tobacco possessing a novel source of resistance

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Black shank of tobacco, caused by the oomycete Phytophthora nicotianae, is an important disease of tobacco primarily managed by the deployment of partial and complete resistance genes. Following the widespread occurrence of race 1, new sources of resistance are needed. Beinhart 1000 (BH 1000) is highly resistant to all races of P. nicotianae. Doubled-haploid lines from a cross of BH 1000 and the susceptible variety Hicks were evaluated for black shank resistance and a linkage map with 24 linkage groups was created. QTLs on linkage groups (LG) 4 and 8 accounted for 43% of the phenotypic variation for end percent survival; the QTL on LG 4 is a novel source of resistance. Forty three doubled-haploid lines with genomic regions from BH 1000 or Hicks on LG 4 and/or LG 8 from were selected and evaluated in greenhouse tests along with both parents for incubation period, percent root rot, and secondary inoculum production. Genotypes with LGs 4 and 8 from BH 1000 had increased incubation periods and decreased root rot compared to genotypes with LGs 4 and 8 from Hicks. The effects of the two LGs were additive and genotypes with both QTLs were significantly different from genotypes with only one QTL for all measured components of resistance. The previously unidentified QTL on LG 4 may provide growers with a new source of resistance to the black shank disease.

Implication of antibiosis in the biocontrol of *Clavibacter michiganensis* causing bacterial wilt and canker of tomato by *Pseudomonas* spp.

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2,4-diacetylphloroglucinol (DAPG), phenazine-1-carboxylic acid (PCA) and hydrogen cyanide (HCN) are antimicrobial metabolites produced by *Pseudomonas* spp. which show high biological activity against fungal plant pathogens. However, we know little about the impact of these metabolites on bacterial plant pathogens and the diseases they cause. In this study, two antimicrobial metabolite-producing *Pseudomonas* spp. strains (LBUM223 producing PCA and LBUM300 producing DAPG and HCN) as well as their respective mutants (LBUM223 PCA-, LBUM300 DAPG-, and LBUM300 HCN-) were studied for their capacity in controlling *Clavibacter michiganensis* subsp. *michiganensis* (Cmm) growth using in vitro confrontational assays and in planta experiments performed under soil conditions. Under in vitro conditions, both LBUM223 and LBUM300 significantly inhibited the growth of Cmm. Larger inhibition zones were observed with LBUM223 and LBUM300 when compared to their respective mutants, indicating that PCA, DAPG and HCN all contributed to Cmm growth inhibition. In plant assays, inoculation with LBUM300 significantly reduced disease symptoms and Cmm population in the rhizosphere, while inoculation with LBUM223 did not yield any effect. Interestingly, inoculation with LBUM300 DAPG- or HCN- also did not yield any effect, suggesting that the production of both DAPG and HCN is required under rhizosphere soil conditions for the biocontrol of Cmm.

Effect glucorafano isolated of broccoli florets on the germination of *Colletotrichum gloeosporioides* spores

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Phytopathology 101:S98

Anthracnose caused by *Colletotrichum gloeosporioides*, is the most important postharvest disease in mango producing areas worldwide, the strategy most used to control disease, is the pre-and post-harvest treatment with fungicides, but their use is increasingly restricted due to public awareness of hazardous waste in the fruits. Glucosinolates are natural products containing nitrogen and sulfur and its antimicrobial activity has been shown in other research. For this work, we collected fruits of mango, with symptoms of anthracnose: from them it was isolated and identified the fungus *Colletotrichum gloeosporioides*. The spores of the pathogen were placed on PDA with different glucoraphane concentrations (1.54, 0.92, 0.46, 0.15, 0.02 y 0 mug muL^{-1}) isolated from broccoli florets. We measured spore germination until the control treatment show the highest percentage of germination. The median lethal concentration was 0.65 mug muL^{-1} and the concentration that completely inhibited the germination of spores was 0.97 mug muL^{-1}.

Use of disease-suppressive Brassica rotation crops in potato production: Overview of 10 years of field trials

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Disease-suppressive Brassica rotation crops have shown promise for management of soilborne diseases and enhanced yield in a variety of crop production systems. Over the last 10 years, numerous field trials have focused on how to best use Brassica crops in potato rotations in the Northeast, including which crops to use, what diseases are affected, and how to implement and manage these crops (as cover, harvested, or green manure crops). A summary of over 70 individual trials indicated that, although results varied by field and year, positive effects have been observed in most trials. Yield was significantly improved in 52% of the trials, with increases up to 38%. Black scurf was significantly reduced in 70% of the trials, with reductions up to 95% and an average reduction of 30% relative to typical rotation crops. Common scab was reduced in 40% of the trials, with reductions up to 50%. Powdery scab and Verticillium wilt were also reduced in most of the trials where they occurred. Overall, mustard green manures worked best for reducing most soilborne diseases, but rapeseed green manure was best for black scurf. In general, green manures provided the best results, but crops harvested for seed also significantly reduced disease. However, due to the short growing season, Brassica crops were not effective as a fall cover crop. This research demonstrated that Brassica rotation crops can substantially reduce soilborne disease problems, but cannot completely control them.

Basis for inhibition of *Pyrenophora teres* by *Laetisaria arvalis*, a scanning and transmission electron microscopic study

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The broadly occurring foliar disease of barley, net blotch is caused by *Pyrenophora teres*, an ascomycete and could result in significant yield loss under heavy disease pressure. The basidiomycete, *Laetisaria arvalis* has been reported to have biological control activity over some plant pathogens. In a preliminary experiment, *L. arvalis* inhibited growth of *P. teres* on agar plates. The observation however, did not elucidate the mechanisms of the *P. teres* inhibition by *L. arvalis*. This research was initiated to utilize electron microscopy techniques to examine the interaction between *L. arvalis* and *P. teres* to study the basis for previously observed inhibition. Scanning and transmission electron microscopy were used to examine the interaction of the two fungi. To date, our microscopy data indicated structural changes of the hyphae in both *P. teres* and *L. arvalis* as the fungi interact. Additional examination of interacting colonies of the two fungi growing on agar shows loss of structural integrity in *P. teres* hyphae, whereas the *L. arvalis* hyphae remain intact. This includes the formation of large perforations in P. teres hyphae over time indicating possible degradation of *P. teres* hyphae resulting in its growth inhibition. This observation strongly confirms the likely inhibition of *P. teres* by *L. arvalis.* Additional experiments are in progress to better understand this interaction at the subcellular level to serve as basis for the development a biological control system of *P. teres* with *L. arvalis*.

Detection of *Pyrenophora teres* in conidia and barley seed by PCR, a technique for rapid diagnosis of infestation

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Pyrenophora teres, the causal agent of net blotch of barley survives as seedborne mycelium or pseudothesia in infested host residues. Besides the common netlike and occasional spot form symptoms on barley leaves, diffused dark or pale symptoms which are difficult to distinguish from other

fungi are produced on kernels. Examination of conidia is considered the most accurate way to diagnose net blotch of barley. We developed a PCR technique to detect *P. teres* in conidia and seeds. In this technique, conidia and barley seeds showing symptoms were first homogenized in Extract-N-Amp Plant PCR Kit (Sigma-Aldrich) extraction solution and diluted with another solution from the kit to sidestep standard DNA extraction. Freeze dried mycelial cultures from *P. teres* f. *teres* and *P. teres* f. *maculata* were treated as seeds and conidia to serve as controls. Aliquots of the homogenate were added to PCR reaction and subjected to amplification using *P. teres* actin based PTACTIN980 and ITS primers. Sizes of amplicons from infested seeds and conidia which were resolved on agarose gel correlated with amplicons from the control P. teres cultures. The amplicons, purified from gels, sequenced and compared by alignment confirmed the detection of *P. teres*. The technique will accelerate positive detection of *P. teres* in infested barley seeds from diseased kennels by positively distinguishing it from other fungi and hasten the diagnosis from conidia.

Epiphytic populations and the effect of UV light on *Cladosporium* spp. found on blueberries

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Cladosporium rot (*Cladosporium* spp.) is a common disease affecting blueberries (*Vaccinium corymbosum*) and other crops in Chile. In this study, the epiphytic fungal populations on blueberries and the effect of UV light on *Cladosporium* spp. were studied. Isolations were made from samples of flowers or immature fruits that were agitated for 5 min in 1:3 or 1:0.6 w/v 0.05% Tween, respectively. The resulting suspension (0.05 ml) was plated on PDA plus antibiotics and Igepal for 14 days at 20°C to determine the fungal colonies that were present. The effect of UV-A (λ = 361 nm) and UV-C (λ = 254 nm) on the inactivation of the conidia of *C. cladosporioides* and *C. herbarum* was investigated. Conidial suspensions (10^6 conidia/ml) were first subjected to UV-A (0.0, 0.05, 0.1 or 0.15 J/cm^2) or UV-C (0.0, 0.05, 0.1 or 0.15 J/cm^2), and then 0.1 ml of the suspension was immediately plated on PDA plus Igepal for 48 h at 20°C. The total number of colonies was counted. Species of *Cladosporium*, *Botrytis*, *Penicillium*, *Alternaria* and yeasts were isolated from the flowers and berries. *Cladosporium* spp. and yeasts were the most abundant species. The conidia of *Cladosporium* spp. exhibited a high resistance to UV-A and UV-C, which drastically differed from the high UV sensitivity of *B. cinerea* that was found in this study. The melanin pigments in the conidial cell wall can confer UV resistance and protection against solar radiation, which accounts for the high Cladosporium populations obtained from blueberry plants.

***Diaporthe/Phomopsis* complex associated with stem cankers of blueberry in Chile**

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Phytopathology 101:S99

Commercial blueberry (*Vaccinium corymbosum*) plantings extend across a range of diverse climate and soil conditions in Chile, along a north-to-south axis of over 1000 km. Stem canker is a major disease of blueberry in Chile. Symptoms include apical necrosis, reddish stem cankers, internal vascular discoloration and dieback. In this study, a total of 24 plantings were surveyed between 2005 and 2011. Isolations were performed in PDA plus antibiotics and Igepal for 14 days at 20°C. Based on colony and conidia morphology, 97 *Phomopsis* (tel. *Diaporthe*) isolates were identified. Analysis of the rDNA region ITS1-5.8S-ITS2 identified five *Diaporthe* species, including *D. australafricana*, *D. perjuncta*, *D. phaseolorum*, *D. viticola* and *Diaporthe* sp., on blueberries in Chile. These *Diaporthe* species were isolated from stem cankers; there was no evidence of the presence of these fungi on sound blueberry stems, flowers or berries. Experiments have shown that *Diaporthe* isolates are pathogenic in blueberry stems and fruit. Therefore, stem canker on the blueberry plant can be associated with a *Diaporthe/Phomopsis* complex. These species were found alone or were co-isolated with Botryosphaeriaceae spp. and *Pestalotiopsis* spp. Botryosphaeriaceae spp. were the most frequently associated fungi with stem canker of the blueberry plant in Chile. Interestingly, *D. vaccinii*, a commonly found pathogen worldwide, previously identified in Chile, was not found in this study.

Host status of soybean differential genotypes to *Rotylenchulus reniformis* and *Meloidogyne incognita* race 3

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The increased profitability of soybeans has resulted in increased acreage in the southern region of the U.S. where the reniform nematode, *Rotylenchulus reniformis* and the root knot nematode *Meloidogyne incognita* predominate. Soybean differentials having resistance to *Heterodera glycines* were used to evaluate potential resistance to *Rotylenchulus reniformis* and *Meloidogyne incognita*. Four trials were established with PIs 437654, 209332, 90763, 89772, 548316, 548658, 88788, 97100, 548402, Hutcheson and Williams 82. Soybeans were inoculated with *M. incognita* or *R. reniformis* and grown for 60 days. The *R. reniformis* population, when compared to the standard PI548658, was lower on all the differentials except for PI97100. The PI97100 genotype supported a 10% higher population of *R. reniformis* than PI548658. Five of the differentials supported a 10% greater population of *M. incognita* than PI548658. The PIs 437654, 209332, 89772, 548616, and 97100 all supported higher levels of *M. incognita* than PI 548658. The PIs 90763, 88788, and 548402 supported 85 to 50% fewer root-knot. Hutcheson, supported both *R. reniformis* and *M. incognita*, while Williams 82 increased only *R. reniformis* populations. Results indicate that the genes for resistance to *H. glycines* present in some PIs parentage may also influence susceptibility and resistance to *R. reniformis* and *M. incognita*.

Fungicide resistance in Czech cucurbit powdery mildew populations

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Phytopathology 101:S99

A total of 113 cucurbit powdery mildew (CPM) isolates (67 *Golovinomyces cichoracearum* (*Gc*), 46 *Podosphaera xanthii* (*Px*)) collected in the Czech Republic (2005–2008) were screened for fungicide resistance (fenarimol, dinocap and thiophanate-methyl; benomyl - unefficient control). Sixty CPM isolates (36 *Gc*, 24 *Px*) from 2007–2008 were also tested for sensitivity to azoxystrobin. Sensitivity was determined by a modified leaf-dics bioassay with five concentrations. Significant differencies among fungicides and years were observed. Resistant and/or tolerant isolates of both CPMs were found in different locations. Isolates collected in 2005 exhibited lower sensitivity to fenarimol (Rubigan 12 EC) and dinocap (Karathane LC) compared to previous years; however in 2006–2008 a high level of sensitivity to these fungicides was detected, all isolates of both CPMs were controlled by the recommended concentration (36 µg/ml fenarimol, 105 µg/ml dinocap). Benomyl (Fundazol 50 WP) and thiophanate-methyl (Topsin M 70 WP) would be ineffective for CPM, most isolates screened were highly resistant, with limited or profuse sporulation at the recommended as well as higher concentrations. Sensitivity to azoxystrobin (Ortiva) decreased from 2007 to 2008 when 45% of CPM isolates (*Gc* and *Px*) were tolerant or resistant to the recommended concentration (500 µg/ml), most also tolerated 1000 and 2000 µg/ml. This research was supported: QH 71229, MSM 6198959215, IGA PrF_2011.

PemK toxin encoded by the *Xylella fastidiosa* IncP-1 plasmid pXF-RIV11 is a ribonuclease

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Stable inheritance of the IncP-1 plasmid pXF-RIV11 in *Xylella fastidiosa* is conferred by the *pemI/pemK* plasmid addiction system. PemK serves as a toxin inhibiting bacterial growth; PemI is the corresponding antitoxin that blocks activity of PemK toxin by direct binding. PemK toxin and PemI antitoxin were over-expressed in *Escherichia coli* and activities of each were assessed. Purified PemK toxin specifically degraded single-stranded RNA but not double-stranded RNA, double-stranded DNA, or single-stranded DNA. Addition of PemI antitoxin blocked nuclease activity of PemK toxin. Purified complexes of PemI bound to PemK exhibited minimal nuclease activity; removal of PemI antitoxin from the complex restored nuclease activity of PemK toxin. Sequencing of 5′ RACE products of RNAs digested with PemK revealed a preference for cleavage between U and A residues of the trinucleotide UAC. Nine single amino acid substitution mutants of PemK toxin were constructed and evaluated for growth inhibition, nuclease activity, and PemI binding. Three PemK point substitution mutants (R3A, G16E, and D79V) that lacked nuclease activity did not inhibit growth. All nine PemK mutants retained the ability to bind PemI antitoxin. Collectively, the results indicate that mechanism of stable inheritance conferred by the pXF-RIV11 *pemI/pemK* plasmid addiction system is similar to that of the prototype *pemI/pemK* addiction system of *E. coli* plasmid pR100.

Fungicide screening and application for the control of walnut anthracnose caused by *Glomerella cingulata*

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Anthracnose is one of the most important disease occurred in leaves, petioles, and even in nuts of Walnut tree. Infected leaves and twigs are malformed, the leaves defoliated during growing season. Severely infected nuts became black and subsequently dropped. The disease occurrence cause the dramatic decrease in yield and quality of nut production. The disease was first reported in Korea at 1987, and the damage rate is annually increasing about 15~30% in Youngdong, Gimcheon, Buyeo, Gongju, and Eumsung area, where walnut tree is densely planted, and not managed well. In order to find and apply effective fungicides for the control of the disease, 8 fungicides including azoxystrobin (20%) were screened *in vitro* at the diluted concentration of from 500 to 4,000 times. Tebuconazole (25%) and Fluazinam (50%) showed the highest inhibition in mycelial growth of the pathogenic fungus. Application of tebuconazole (emulsifiable concentrate) at the diluted concentration of 2,000 times on the infected leaves showed high control value of 92.2%, while treatment of fluazinam (wettable powder) showed satisfactory control value of 82.5% on the infected nuts.

Identification of quantitative trait loci conferring partial resistance to *Phytophthora sojae* in soybean PI 427106

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Phytopathology 101:S100

Phytophthora root and stem rot caused by *Phytophthora sojae* is a destructive disease that limits soybean yield around the world. Fifteen resistance genes (*Rps*) to *P. sojae* have been identified, but adaptation by the pathogen has made many of these *R*-genes ineffective. In addition to *Rps*-gene resistance, partial resistance controlled by quantitative trait loci (QTL) provides effective long-term defense against many pathotypes. The objective of this study was to identify QTL conferring partial resistance against *P. sojae* from a new genetic source, PI 427106. Ninety-four recombinant inbred lines (RIL) from a $F_{6:8}$ population of OX20-8 (susceptible) by PI 427106 (with high level of partial resistance) were used in this study. The population was genotyped with approximately 200 SNPs using BeadXpress system and the genetic map was constructed. To evaluate the level of partial resistance, 7-day-old seedlings (10 plants per RIL) were inoculated on the root with *P. sojae* isolate 13.S.1.2 and lesion length was measured 7 dai. The mean lesion length of ten seedlings was statistically analyzed to obtain the best linear unbiased predictor (BLUP). Using marker genotypes and BLUP values composite interval mapping located three QTL on chromosome 13, 16, and 18, which explained 4.9 ~ 16.5% of the phenotypic variation, respectively. These results suggest that this soybean accession may be an important source of partial resistance in developing germplasm for breeding new cultivars with more durable resistance to *P. sojae*.

The occurrence and diagnosis of soybean diseases in Korea

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Phytopathology 101:S100

Soybean diseases have been reported approximately 100 species worldwide and economically damaging diseases are amount of 35 species. In Korea, 22 species of fungi, 4 species of bacteria and 5 species of virus have been reported, 10 species among these diseases caused severely damage to soybean. More precisely, fungi diseases are Phytophthora rot, Black root rot, Purple blotch, Pod and stem blight, Brown leaf spot, Frogeye leaf spot and Anthracnose. Bacterial diseases are Bacterial pustule and Wildfire. Viral disease is *Soybean Mosaic Virus*. Soybean diseases occurred complex of fungi, bacteria and virus in the field. Symptoms of some soybean diseases appear slightly different according to the soybean cultivars. Also, there are soybean diseases of the similar initial symptoms. To control these diseases, the correct diagnosis was very important. We collected and summarized many data for accurate diagnosis of theses diseases using the PCR/RT-PCR method. In case of bacterial diseases, We designed new detection PCR primers with bacteriocin gene. As results, 16 primer pairs for fungi, 4 primer pairs for bacteria and 5 primer pairs for virus were used to detect soybean diseases.

Functional analyses of two acetyl coenzyme A synthetases in the ascomycete *Gibberella zeae*

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Phytopathology 101:S100

As acetyl coenzyme A (acetyl-CoA) is a crucial metabolite for energy metabolism and biosynthetic pathways, acetyl-CoA should be concisely produced in various cellular compartments spatially and temporally. Our previous study on ATP citrate lyase (ACL) in destructive plant pathogen *Gibberella zeae* revealed that ACL-dependent acetyl-CoA generation is important for histone acetylation especially for sexual development but not for lipid synthesis. We deleted another acetyl-CoA synthetic genes, acetyl-CoA synthetases (*acs1* and *acs2*), to find alternative acetyl-CoA producer for ACL. The *acs1* deletion resulted in defect of sexual development partially because of reduced lipid production which is required for perithecia maturation. Another ACS coding gene, *acs2*, has accessorial functions for *acs1* in most of the physiological processes and has also compensational function for ACL as a nuclear acetyl-CoA producer. Because ACS is a component of pyruvate-acetaldehyde-acetate pathway, this fermentation process might have crucial roles in various physiological processes even for obligate aerobic fungi. In this study, we concluded that acetate is readily generated during the whole life cycle of *G. zeae* and has central roles for fungal metabolisms.

Rice chitinase gene contributes to rice sheath blight disease resistance

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Phytopathology 101:S100

Rice chitinases co-localize with disease resistance QTL and are implicated in multiple defense responses. Previous work demonstrated that the class IV rice chitinase Os02g39330 is linked to a disease resistance QTL on chromosome 2, and that this gene is transcriptionally active in response to fungal pathogen attack. We used an RNAi silencing approach to determine if *Os02g39330* contributes to broad-spectrum disease resistance. The effect of the silencing construct was measured on expression of *Os02g39330* and two closely related chitinases, *Os04g41680* and *Os04g41620* in five transgenic lines after inoculation with *Rhizoctonia solani* and *Magnaporthe oryzae.* Three of the five transgenic lines exhibited high levels of silencing of *Os02g39330*, and little to no silencing of *Os04g41620* and *Os04g41680.* These lines showed increased sheath blight disease, but less rice blast disease, relative to control lines with no silencing suggests that *Os2g39330* contributes to *R. solani* resistance. *Os2g39330* was not associated with *M. oryzae* resistance in this study. Enhanced expression of related chitinases *Os04g41680* and *Os04g41620* in the transgenic lines was not correlated with increased resistance to sheath blight or rice blast, suggesting that these genes do not contribute to disease resistance or susceptibility. The demonstration that *Os239330* contributes to sheath blight resistance shows that this class IV chitinase is a valuable source of basal resistance for QTL breeding programs.

Management of *Phytophthora capsici* and potential human foodborne pathogens in irrigation water

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Phytopathology 101:S100

Contamination of surface water with plant pathogens and harmful human pathogens has the potential to reduce crop yield and lower the microbial quality of produce, respectively. In cooperation with local growers, the efficacy of commercial chlorine gas (Cl_2) and chlorine dioxide (ClO_2) irrigation water injection systems in killing human pathogens and *Phytophthora capsici* was evaluated. Using predefined parameters, irrigation water treated with Cl_2 was effective at reducing populations of coliforms and generic *Escherichia coli* to below EPA standards for recreational water at all sampling points in 2009, but only at the most distal emitter in 2010. The efficacy of ClO_2 in both years was variable with coliform levels never dropping below EPA standards. However, in 2010, generic *E. coli* populations were at acceptable EPA levels at the most distal emitter. Using baiting techniques in combination with *P. capsici*-specific PCR, *P. capsici* was detected in all the local irrigation water sources sampled but not on cucumbers collected from Cl_2 or ClO_2 treated or non-treated treated water in 2009. Many environmental factors contribute to the efficacy of Cl-based chemicals, including water temperature and pH and organic matter load. Finding ways to reduce the impact of these factors on the effectiveness of these treatments will be critical if such management strategies are to be both reliable and sustainable.

Development of a multivariate matrix to trace *Clavibacter michiganensis* subsp. *michiganensis* through tomato greenhouse operations

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Phytopathology 101:S101

Clavibacter michiganensis subsp. *michiganensis* (Cmm) is a seedborne pathogen that spreads rapidly through tomato greenhouse operations, causing significant losses. The genetic diversity of Cmm has been exploited in the past to discern its origin and distribution, but no formal traceability system has been developed. Using geographical information, propagation and production flow diagrams and varietal and seed source data, an industry-specific multivariate matrix that was superimposed with repPCR fingerprints of Cmm strains was designed. The multivariate matrix allows Cmm phenotypic and genotypic information to be recorded and transmitted at any specific point in the production system and the point of origin of each strain can be quickly identified. The efficacy of this system is currently being evaluated and four new Cmm clonal groups have been identified. Producer implementation of the multivariate matrix has the potential to improve production efficiency, improve phytosanitary practices by identifying possible control points in production, decrease disease management related costs and identify new and emerging strains of Cmm. This system will also allow for further advancement of our knowledge of the diversity and distribution of this pathogen throughout North America.

Responsiveness of *Striga*-susceptible and *Striga*-resistant sorghum genotypes to soil phosphorus and arbuscular mycorrhizal fungi

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Phytopathology 101:S101

Striga, a genus of obligate parasitic weeds, has been identified as the most important biological factor limiting agricultural productivity in sub-Saharan Africa. Germination of *Striga* seeds is triggered by strigolactone root exudates from host plants. Strigolactones also induce hyphal branching in arbuscular mycorrhizal (AM) fungi, which are important for plant uptake of phosphorus in low phosphorus soils. Mechanisms of *Striga* resistance based on reduced strigolactone production may also convey resistance to AM fungi which would require higher inputs of phosphorus fertilizer to attain optimal crop growth. There is evidence for genetic differences in mycorrhizal responsiveness in other grain crops; therefore it is beneficial for breeders to be aware of these differences when developing *Striga*-resistant sorghum cultivars. This project aims to determine phosphorus and mycorrhizal responsiveness of sorghum genotypes important for or developed by breeders working on *Striga* resistance. Phosphorus response curves were determined for twelve genotypes using pasteurized low phosphorus soil amended to achieve five different phosphorus levels. Analysis of variance was performed on root and shoot dry weight. Results indicate variability in phosphorus responsiveness within *Striga* resistant and susceptible genotypes. Continuing research will analyze differences in mycorrhizal responsiveness in relation to *Striga* resistance and responsiveness to phosphorus.

Characterization of a coupled termination–reinitiation strategy for downstream ORF translation in victoriviruses (family *Totiviridae*)

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Phytopathology 101:S101

The genome-length, dicistronic mRNA of the dsRNA fungal virus Helminthosporium victoriae virus 190S (genus *Victorivirus*, family *Totiviridae*) contains two long open reading frames (ORFs) that overlap in the tetranucleotide AUGA. Translation of the downstream ORF, which encodes the RNA-dependent RNA polymerase (RdRp), has been proposed to depend on ribosomal reinitiation following termination of the upstream ORF, which encodes the capsid protein. In the current study, we examined the RNA sequence determinants for RdRp translation by this virus and demonstrated that a coupled termination–reinitiation (stop–restart) strategy is indeed used. Termination–reinitiation depends on a 32-nt stretch of RNA immediately upstream of the AUGA motif, including a predicted pseudoknot structure. The close proximity by which this predicted structure is followed by the upstream ORF's stop codon appears to be especially important for promoting translation of the downstream ORF. Normal strong preferences for AUG start codons and canonical sequence context for translation initiation of the downstream ORF appear somewhat relaxed. Similar sequence motifs and predicted RNA structures in other victoriviruses suggest that they all share a related stop–restart strategy for RdRp translation. Members of genus *Victorivirus* thus provide new and unique opportunities for exploring molecular mechanisms of translational coupling, which remain only partly understood in this and other systems.

Effect of intermittent leaf wetness on incidence and severity of gray leaf spot of perennial ryegrass turf

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Phytopathology 101:S101

Gray leaf spot, caused by *Magnaporthe oryzae*, is a devastating disease of perennial ryegrass (*Lolium perenne*) turf. Free leaf moisture is required for development of the disease. The objective of this study is to determine the effects of intermittent leaf wetness periods on incidence (% leaf blades symptomatic) and severity (Index 0-10; 0 = asymptomatic; 10 ≥ 90% leaf area necrotic) of gray leaf spot of perennial ryegrass turf. Six-weeks-old perennial ryegrass plants were inoculated with *M. oryzae* (8×10^4 conidia/ml H_2O), exposed to various treatments with wet/dry periods, and maintained at 28°C to allow the disease to develop. Disease incidence and severity were assessed seven days after inoculation. The results showed that there were significant effects of wet/dry periods on disease incidence and severity. Highest disease incidence and severity were recorded on plants exposed to the longest leaf wetness period (18 h continuous leaf wetness). Increased interrupted dry periods significantly reduced gray leaf spot incidence and severity. Additionally, there were negative correlations between interrupted dry period and disease incidence or severity, and the relationships were best described by a quadratic model for disease incidence: $Y_{Inc} = -5.04X+0.10X^2 + 91.08$ and a linear model for disease severity: $Y_{Sev} = -0.27X + 9.05$ (Inc = Incidence; Sev = Severity; X = dry period). Results of this study may be applied as a component of gray leaf spot disease management strategy in perennial ryegrass fairways in golf courses.

Effects of venom alkaloids from red imported fire ants on bacterial canker of tomato in the greenhouse

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Phytopathology 101:S101

Bacterial canker of tomato caused by *Clavibacter michiganensis* subsp. *michiganensis* (CMM) has caused major economic losses in tomato production worldwide. This study reported the use of purified venom piperidine alkaloids from the red imported fire ant, S. invicta, to control bacterial canker on tomato seedlings in the greenhouse. Sterilized Redi-Earth Plug & Seedling Mix was used as growth medium. Surface disinfected germinating seeds of Better Boy and DRK7018F1 were sowed in containers. Each container has 10 seedlings. The temperature range of the greenhouse was 18–25°C, and the RH was maintained at 60%–90%. Acetone solutions containing 0, 178.56 and 357.12 µg piperidine alkaloids/ml were sprayed twice onto the tomato seedlings at the 4–5 leaf stage with a 7 day interval. Three days after the second piperidine spray, wounds on tomato leaves and stems were created by gently applying finger pressure, and then a nutrient broth containing 6 × 106 CMM cfu/ml was sprayed onto the tomato plants. Growth containers were then kept at RH 90%-100% for 5 days. Bacterial lesions on the young stems and petioles were investigated 35 days after inoculation. The results indicated that piperidine alkaloids significantly ($P < 0.05$) reduced the lesion numbers on tomato plants of both Better Boy and DRK7018F1. There was no significant ($P > 0.05$) difference between the two piperidine alkaloid concentrations. It was observed that DRK7918F1 had higher disease resistance than Better Boy.

Identification of genes involved in biofilm formation using an EZ-Tn5 mutant library of *Xanthomonas citri* ssp. *citri* strain 306

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Phytopathology 101:S101

Xanthomonas citri ssp. *citri* (*Xcc*) causes citrus canker, one of the most destructive diseases of citrus worldwide. Increasing evidence suggests that the ability to form biofilm is important for host infection by *Xcc*. However, little is known about the mechanisms of biofilm formation in *Xcc*. To identify genes involved in biofilm formation by *Xcc*, an EZ-Tn5 transposon library containing 22,000 clones of *Xcc* strain 306 were screened in 96-well polystyrene plates, and a total of 292 mutants were selected for biofilm-defective phenotypes. The transposon insertion sites were determined by sequencing analysis combined with random amplifications of transposon ends and homology search of the whole genome sequence of *Xcc* strain 306. A total of ninety-two genes and five insertions in intergenic regions were revealed to be involved in biofilm formation. Among the 92 disrupted genes, 17 encode

proteins involved in extracellular polysaccharide and/or lipopolysaccharide biosynthesis; 19 others encode proteins involved in flagellum, type IV pili biosynthesis, bacterial chemotaxis or motility; additional 9 encode metabolic enzymes, 4 others for genetic information processing, 2 involved in signal transduction, and 4 with similarity to membrane transporter; In addition, 17 hypothetic proteins with unknown function and 16 not well characterized with putative functions were identified. The genes identified in this study should be helpful for future research into the molecular mechanisms of biofilm formation by *Xcc*.

Effect of EnvZ/OmpR and GrrS/GrrA systems on *Erwinia amylovora* virulence

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Phytopathology 101:S102

Erwinia amylovora is a necrogenic enterobacterium causing fire blight disease on rosaceous plants. Early reports have shown that two-component systems in *E. amylovora* play a major role in virulence and in regulating amylovoran production, including EnvZ/OmpR and GrrS/GrrA, two widely distributed systems in gamma-proteobacteria. While both systems negatively control amylovoran biosynthesis, deletion mutants of *envZ/ompR* and *grrA/grrS* have opposite swarming motility phenotypes. In order to determine how the two systems interact, two triple mutants (*envZ/ompR/grrA* and *envZ/ompR/grrS*) were generated. Our results showed that both triple mutants had increased virulence on apple shoots as compared to that of wild type (WT) as well as mutants deleting a single system. In an *in vitro* amylovoran assay, amylovoran production was significantly increased in the two triple mutants, indicating the two systems synergistically regulate amylovoran production. In consistency with amylovoran production, *amsG* gene expression was expressed significantly higher in the triple mutants *in vitro* than those in WT as well as mutants deleting a single system. Furthermore, the triple mutants showed reduced swarming motility on swarming plates compared to *grrA/grrS* mutants and WT strain, but moved faster than *envZ/ompR* mutants, indicating that the two systems antagonistically regulate swarming motility in *E. amylovora*. Characterization of other phenotypes for the triple mutants is now underway.

Identification of species and pathotypes of cereal cyst nematode in winter wheat on the Huang-Huai floodplain of China

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Phytopathology 101:S102

The cereal cyst nematode (CCN) of wheat has become a severe disease problem in the winter wheat on the Huang-Huai floodplain, the biggest bread basket of China. Heteroderid specimens at 21 locations in Henan and adjacent provinces were identified by morphological and molecular analysis. *H. filipjevi* was found in six locations in Henan, including two mixed with *H. avenae*; *H. avenae* type "C" being found at all other locations. Thirteen CCN populations, consist of four populations belong to *H. filipjevi* and nine belong to *H. avenae* were typed using 23 standard international differentials (13 group A for main test and 10 group B for assistant test) and a common local cultivar Wenmai 19. These populations were found to be previously undescribed pathotypes. There are 4 different pathotypes for 4 *H. filipjevi* populations from Henan province, with the different virulence to 23 differential host. In nine populations of *H. avenae*, the pathotype from Xushui and Xingyang were typed for group 3, which were virulent to *Ha1* and *Ha2* genes in barley, but avirulent to *Ha3* gene; Other 7 populations were divided into group 1 pathotype, which were avirulent to *Ha1*, *Ha2* and *Ha3* genes. The resistance reaction of 13 group A differentials hosts to 4 populations from Anyang, Qingfeng, Heze and Yingshang was same, but different from Handan, Baoding and Shangqiu populations, by being avirulent in wheat cv. Iskamish K-2-light (Handan population) or barley cv. KVL191 (Baoding and Shangqiu population).

Simultaneous detection and differentiation of four sweet potato potyviruses by one-step RT-PCR

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Phytopathology 101:S102

At least 20 viruses are reported to infect sweet potato and cause serious yield losses worldwide, and six of them are species of the genus Potyvirus in the family Potyviridae. *Sweet potato feathery mottle virus* (SPFMV) is the most common one among them, and it is closely related to Sweet potato virus C (SPVC), *Sweet potato virus* G (SPVG) and *Sweet potato virus Y* (SPVY), three newly recognized viruses. Identification and detection of these viruses is complicated by high similarity among their genomic sequences, frequent occurrence as mixed infections and low titer in many sweet potato cultivars. A one-tube quadruplex reverse transcription (RT)-PCR assay was developed for the simultaneous detection and differentiation as SPFMV, SPVC, SPVG and SPVY. Four virus-specific forward primers and one reverse primer based on the region conserved in all four viruses were selected. The assay was optimized for primer concentration, cycle number, conditions of annealing and elongation steps. The assay was first tested using a sweet potato plant naturally infected with all four viruses, and then evaluated using other single- and mix-infected plants in our collection and field samples from southwestern China. This RT-PCR is reliable and sensitive as a simple, rapid and cost-effective method to detect these pathogens in sweet potato. The assay will be useful to quarantine and certification programs as well as virus surveys when large numbers of samples need to be tested.

Phylogenetic relationships of closely related potyviruses infecting sweet potato determined by genomic characterization of *Sweet potato virus 2* and *Sweet potato virus G*

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Phytopathology 101:S102

Several closely related potyviruses including *Sweet potato virus 2* (SPV2) and *Sweet potato virus G* (SPVG) are reported to cause disease in sweet potato, and their classification is confusing. In this study, complete nucleotide sequences of a SPV2 isolate and two SPVG isolates were determined to be 10,732 and 10,800 nucleotides, respectively, excluding the 3′-poly(A) tail. Their genomic organization is typical of potyviruses, encoding a polyprotein with conserved motifs found in members of the genus *Potyvirus*. Pairwise comparisons of the genomic and deduced polyprotein and individual protein sequences of the two viruses with those of 72 other potyviruses confirm that both SPV2 and SPVG are distinct species of the genus *Potyvirus* in the family *Potyviridae*. Phylogentic trees constructed from the genomic, polyprotein sequences show that SPV2 and SPVG are most closely related to each other, and they form a distinct clade with *Sweet potato feathery mottle virus* (SPFMV) and Sweet potato virus C (SPVC). Phylogenetic analysis based on the deduced amino acid sequences of the 3′-partial genome containing a partial NIb and CP suggests that the viruses in this clade can be divided into five species, SPFMV, SPVC, SPVG, SPVY and Sweet potato virus-Zimbabwe. The analysis also reveals that Sweet potato virus G and Ipomoea vein mosaic virus are grouped with SPV2 as one species, and these two viruses should be consolidated as SPV2.

454-Pyrosequencing reveals the influence of organic and conventional farming systems on beneficial bacterial communities to enhance plant health

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Phytopathology 101:S102

Bacterial communities in soil exert versatile functions in maintaining soil and plant health. We investigated the long-term impacts of crop rotation on the beneficial bacteria to enhance plant health. Soil samples were collected from Canada's oldest organic-conventional study Glenlea, Manitoba. The main plots were two crop rotations; flax-oat-fababean-wheat (grain only) and wheat-alfalfa-alfalfa-flax (grain forage), and certificated organic and conventional methods served as subplots. A total of 123,316 sequences were generated. A total of 14 phyla were represented in the dataset, with *Proteobacteria*, *Actinobacteria*, *Acidobacteria*, *Bacteroidetes*, *Chloroflexi*, *Gemmatimonadetes*, and *Firmicutes* forming the most dominant phyla. *Proteobacteria* was significantly high under grain only organic system (44.45%), while it was only 27.25% under the forage grain conventional farming system. *Actinobacteria* were 43.12% under the forage grain conventional system, while grain only organic system had a lower percentage (32.48%). When bacteria were analyzed at the genus level, the relative abundances of different genera belonging to phyla *Actinobacteria* and *Proteobacteria* varied among the samples under different treatments. The genus belonging to *Proteobacteria*, such as *Pseudomonas*, *Stenotrophomonas*, *Brevundimonas*, were more frequently found in organic farming systems. Pyrosequencing revealed beneficial soil bacterial communities shifts resulting from different cropping systems that could have an impact on plant health.

Pathogenic *Embellisia astragali* on *Astragalus adsurgens* is very closely related to locoweed endophyte

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Phytopathology 101:S102

Embellisia astragali which resides within *Astragalus adsurgens* is a pathogenic fungal species recently identified in China. Its conidia, colony morphology and growth rate are similar to that of the locoweed endophyte, *Undifilum oxytropis*. The two fungi were compared using morphology and genetics. DNA of both fungi was assessed using a pair of *Undifilum*-specific primers, OR1 and ITS5 which amplify a portion of the ITS region. The amplicons from both fungi were tested for digestion with the restriction endonucleaseery close genetic relationship.

A new *Botrytis* sp. causing grey mold on blackberry

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Phytopathology 101:S103

Botrytis spp. cause blossom and fruit diseases on many crops, including blackberry. In a survey of 4 locations in the state of South Carolina, 86 isolates of *Botrytis* spp. were collected from blackberry fruit and single spores were characterized. Two distinct species, *B. cinerea* and another undescribed species, were identified based on examination of the non-coding ITS region and the coding G3PDH, HSP60 and RPB2 genes and morphological characters. The new species formed pale yellow to white colonies with short aerial mycelia and produced black sclerotia on PDA at 20°C. Phylogenic analysis based on combined DNA sequence data of three nuclear genes (G3PDH, HSP60 and RPB2) showed that the novel *Botrytis* sp. is most closely related to *B. fabiopsis*, the causal agent of gray mold disease of broad bean, and *B. galanthina*. Its conidia, however, are smaller than conidia of *B. fabiopsis* and *B. galanthina* and sequence analysis of genes encoding necrosis and ethylene-inducing proteins (NEPs) also indicated that the novel *Botrytis* spp. is distinct from *B. fabiopsis*. The new species is pathogenic on broad bean leaves, which distinguishes it from *B. galanthina*. Inoculation of blackberry fruit with conidia caused typical gray mold symptoms but compared to *B. cinerea* the latent period was significantly longer. In conclusion, we discovered a new species of Botrytis on blackberry which is potentially pathogenic on other crops judged by its pathogenicity on broad bean leaves.

Identification of soybean accessions with resistance to Phomopsis seed decay: Joint effort from USDA and University scientists

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Phytopathology 101:S103

Soybean Phomopsis seed decay (PSD) is primarily caused by *Phomopsis longicolla* along with other *Phomopsis* and *Diaporthe* spp. This disease causes poor seed quality and suppresses yield in most soybean-growing states in the United States. In 2009, PSD caused yield loss of over 12 million bushels in 16 southern states. To identify new sources of resistance to PSD, seed of 208 representative maturity group V soybean lines, obtained from the USDA Soybean Germplasm Collection in 2006, were plated and assayed for the percentage of Phomopsis seed infection. Based on the results of seed assays from 2006 and 2007, 14 accessions were selected for further evaluation with inoculated and non-inoculated treatments in 2008 and 2009. In addition, 135 soybean germplasm lines (maturity groups III, IV, and V) from 28 countries were field screened by natural infection in 2009 at Kibler, AR, Stoneville, MS and Portageville, MO. Based on the seed assay in 2009, 42 lines along with six resistant and susceptible checks were selected and field-tested with inoculated and non-inoculated treatments in these states in 2010. In 2009, frequent rainfall during seed maturation led to high levels of seed infection by Phomopsis (up to 80%) and other fungal pathogens for most soybean lines but several lines were identified that had low percentage of seed infection, good visual quality, and high germination rates. These resistant sources will be used to develop cultivars resistant to PSD.

Identification and virulence differentiation of *Colletotrichum gloeosporioides*, the causal agent of grapevine anthracnose in China

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Phytopathology 101:S103

Grape anthracnose become more and more serve in many of warm and moist regions in China these years. Disease samples were collected from Beijing and other 6 provinces, and 135 isolates were pured. On the characterization of colony, conidia, acervulus and rDNA-ITS sequences, the isolates were identified as *Colletotrichum gloeosporioides* (Penz.) Sacc. With the characterization of colony, sporulation, mycelium growth and pathogenicity to 14 grapevine cultivars, the isolates were classed to three distinct virulence phenotypes, Group I, Group II and Group III, the ratios are 33.75%, 13.75% and 52.% respectively. Three representative isolates belongs to different groups were used to analysis with 64 pairs of AFLP primer. There are 1059 bands in the gel and 489 are polymorphism bands, the polymorphism ratio is 32.41%. The results shown that there are some differences between the different groups in molecular level. Then, 20 random isolates were selected to identify molecular markers linked to different groups, at last, the primer E13/M14 were isolated, and the similarity is 85% between phenotypes and AFLP marker.

Use of an integrated system for disease monitoring and forecasting of wheat stripe rust in China

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Phytopathology 101:S103

Optimization of management resources at national level and maximization of beneficial returns from control measurements are top priorities in the national management program of wheat stripe rust in China. In 2010 spring, a new disease forecasting system based on mid-term weather forecasts and regional disease modeling approaches was incorporated into the current disease monitoring system. The integrated system can predict long distance spore dispersal and a weather favorability of disease development. The forecasting is driven by modeled daily weather data and field monitoring disease data updated weekly. Forecasting results are mapped to show weekly spore dispersion and monthly weather favorability to show the disease risks to guild extension specialists and farmers for disease scouting and fungicide spray. The forecast system was successful in predicting several localized occurrences in several regions so that timely control was taken to prevent disease build-up. Field surveys in the late season showed that the disease was at low level nationwide as predicted.

Volatile organic compounds produced by *Ceratocystis fimbriata* and their inhibition on plant pathogenic fungi

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Ceratocystis fimbriata is the pathogen of Pomegranate Wilt which occurred in Mengzi country of Yunnan province China recently and first reported in 2003. It has been indicated that *C. fimbriata* has the ability of generating a complex variety of aromas and distributed in the soil. Volatile organic compounds (VOCs) can be identified and quantified by headspace GC-MS. This study revealed that headspace analysis is a perfect way to detect the VOCs production from *C. fimbriata* strains isolated from infected pomegranate and sweet potato. Ten compounds, of which ethanol, aceticacidbutylester and ethyl acetate were the most abundant aroma volatiles, and carbon disulfide was first reported. The production of total volatile reached 6660.86 ng/mL. By means of dual test and vertical dual test *in vitro* without physical contact or diffusion through the culture medium, it showed that VOCs produced by *C. fimbriata* inhibited significantly the growth of experimental target fungi, including *Botrytis cinerea*, *Monilinia fructicola*, *Valsa mali*, *Fusarium verticilliodes*, *Fusarium oxysporum*, *Fusarium* sp. and *Curvularia* sp. There was an increasing inhibitory activity as the culture time lasting longer with more volatile compounds produced by *C. fimbriata*. The fruity flavour also affected pigments producing of *Fusarium*. Construction of VOCs-*C. fimbriata* bioreactor will be a potential method for controlling plant diseases.

The synergy between Bombyx mori gut bacteria and insecticidal crystal proten of Bacillus thuringiensis to its larvae

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Phytopathology 101:S103

In recent years, some researchers began to study the roles of gut bacteria on insecticidal activity of Bacillus thuringiensis (Bt), and put forward new evidences and views that gut bacteria of insect host have synergistic or antagonistic effect, it may promote pest control. This work stated the roles of gut bacteria on insecticidal activity of Bt in Bombyx mori, providing a foundation to illustrate the roles of gut bacteria and insecticidal mechanism of Bt. Presently, we will discuss the following progress. Seven dominant bacteria were identified their characteristics preliminarily. Bioassay showed Cry toxin can be lethal to sterile larvae, but its value of LC50 was higher 4 times than normal larvae approximately. This indicated that the intestinal bacteria are not necessary for Bt insecticide, but the intestinal bacteria may play a synergistic

role. The influence of intestinal flora to Cry toxin may be related to species-specific. Through comparing bioassay, we found the intestinal bacteria MB1 synergize to Cry toxin significantly. We found the MB1 played a role in accelerating the activation of protoxin. In addition, MB1 still led a higher mortality than the control. Therefore, MB1 have a complex impact on the pathogenicity of larvae.

Protein extraction methods and proteomic analysis of the locoweed filamentous fungus *Undifilum oxytropis*
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Phytopathology 101:S104

Locoism, caused by swainsonine, is a serious animal disease around the world. Swainsonine is synthesized by the fungus *Undifilum oxytropis* in locoweed plants. Consumption of locoweeds causes significant livestock poisoning and severe economic losses in the western United States and China. Details about how swainsonine is synthesized by *U. oxytropis* endophyte, and the interaction between the fungus and locoweed are poorly understood. Information the *U. oxytropis* proteome could be particularly valuable to help address the problem. Protein sample preparation is critical and challenging for two dimensional gel electrophoresis for protein analysis. Unfortunately, there is no single protein extraction method that can be universally applied to all kinds of organisms ananlysed by 2-DE. To develop an optimized protein extraction protocol for *U. oxytropis*, five protein extraction methods were evaluated. To our knowledge the present study is the first proteome analysis using 2-DE for *U. oxytropis*, and the resolution of the 2-DE reference map is a useful approach for proteomic analysis. This proteome map has aided identification of proteins from *U. oxytropis* and faciliated further studies into the swainsonime biosynthesis pathway.

Oxalate-minus mutants of *Sclerotinia sclerotiorum* via random mutagenesis retain pathogenicity
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Phytopathology 101:S104

Sclerotinia sclerotiorum is a ubiquitous necrotrophic plant pathogen capable of infecting over 400 plant species including many economically important crops. Oxalic acid production has been shown in numerous studies to be a pathogenicity factor for *Sclerotinia sclerotiorum* through several mechanisms. Random mutagenesis through Agrobacterium-mediated transformation (AMT) was used to study pathogenic mechanisms in *Sclerotinia sclerotiorum*. Screening several hundreds of AMT transformants identified three stable mutants that were unable to produce oxalic acid. The mutants did not lower pH of agar plates, and no oxalic acid was detected in liquid medium or in mycelium of the mutants using HPLC. However, the oxalate-minus mutants showed similar levels of virulence comparable to the wild type strain in colonizing pea leaves in detached leaf assays. Southern hybridization blots showed the mutation was due to a single T-DNA insertion and the T-DNA insertion site was identified to be located in the gene of oxaloacetate acetylhydrolase of *S. sclerotiorum*. The results showed that oxalic acid is not required for pathogenicity of *Sclerotinia sclerotiorum*.

Random T-DNA mutagenesis identifies a Cu-Zn-superoxide dismutase gene as a virulence factor of *Sclerotinia sclerotiorum*
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Phytopathology 101:S104

The Ascomycetous fungus *Sclerotinia sclerotiorum* is a devastating pathogen capable of infecting more than 400 plant species including many economically important crops. In order to gain a better mechanistic understanding of its non-specific host-pathogen interactions, random mutagenesis through Agrobacterium-mediated transformation (AMT) was used to identify potential virulence/pathogenicity factors in *S. sclerotiorum*. Screening several hundreds of AMT transformants identified two stable mutants that showed significantly less virulence in comparison with the wild type strain as measured by colonizing pea leaves in detached leaf assays. Southern hybridization analysis showed that the mutation was due to a single T-DNA insertion, and inverse PCR and DNA sequencing identified that the T-DNA insertion site was in the gene of the Cu-Zn-superoxide dismutase (SOD, SS1G00699) of *S. sclerotiorum*. This SOD gene consists of an open reading frame of 465 bps, and its expression levels were significantly induced under oxidative stresses or during infection of pea plants. These results suggest that this SOD gene plays critical roles in detoxification of reactive oxygen species during host-pathogen interactions and is an important virulence factor of *S. sclerotiorum* in pathogenesis.

Evidence of genetic diversity and heterothallism in Sclerotinia homoeocarpa, the causal agent of dollar spot disease on turfgrass
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Phytopathology 101:S104

Sclerotinia homoeocarpa F.T. Bennett causes dollar spot disease (DSD) on warm season and cool season turfgrasses in Florida and in northern states. The morphological and genetic relationship among DSD isolates collected from warm and cool season turfgrasses is unclear. We characterized a collection of 47 isolates of *S. homoeocarpa* from both warm and cool season turfgrass species in Florida and northern states. Screening the collection for morphological characters showed that the Florida collection mainly was represented by a novel strain of *S. homoeocarpa*. We found differences in mycelia pigmentation, stroma formation, and symptom development on St. Augustinegrass, as well as a strong vegetative incompatibility reaction between the Floridian and northern strains. We characterized and sequenced the *MAT1-2* high-mobility group and *MAT1-1* α box mating-type gene loci from the collection. Our data demonstrate an idiomorphic structure and an equal distribution of mating types in our collection characteristic of out crossing heterothallic fungi. Phylogenies established with the *MAT* locus and variable regions of the ribosomal DNA provided additional support for the observed morphological distinction between the strains. These findings provide new insight into the genetic diversity of this pathogen and its geographic distribution.

Malvaviscus yellow mosaic virus, a weed-infecting begomovirus carrying a nanovirus-like nonanucleotide and a modified stem-loop structure
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Phytopathology 101:S104

Begomoviruses (family *Geminiviridae*) have a circular, ssDNA genome encapsidated in twinned icosahedral particles. In Brazil, a number of begomoviruses infecting weeds have been described, and evidence suggests that they have given rise to the viruses currently found in crop plants. Here we describe a novel begomovirus infecting a *Malvaviscus arboreus* plant showing a bright yellow mosaic, collected at the experimental farm of the Campinas Agronomical Institute (IAC), in Campinas, Brazil, in May 2005. Total DNA was extracted and the viral genome was amplified by RCA, cloned and sequenced. Sequence analysis indicated that the virus corresponds to a novel species, for which the name *Malvaviscus yellow mosaic virus* (MalYMV) is proposed. Strikingly, MalYMV has a nanovirus- and alphasatellite-like nonanucleotide (TAGTATTAC). Moreover, a short sequence located 5′ of the nonanucleotide forms a minor hairpin structure embedded in the major hairpin. Nevertheless, the loop where the nonanucleotide is located is similar in size to the conserved begomovirus structure. The relevance of this modified structure is unknown. Although MalYMV has been collected in Brazil, it is phylogenetically closer to viruses from Central and North America. The *M. arboreus* plant has been displaying the observed yellow mosaic symptoms since at least the 1960's (as noted by the sixth author), which suggests that MalYMV may be poorly transmitted (or not transmitted at all) by local whitefly populations.

Discovery the new synthesized of PTGS-related small RNAs by an ultrasensitive silicon nanowire field-effect transister and Next-Generation Sequence
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Phytopathology 101:S104

Post-transcriptional gene silencing (PTGS) is an antiviral strategy of the plant to avoid the virus infection. However, the virus produces a viral suppressor to suppress the PTGS for preventing the silencing machinery to attack the virus. P19 of Tombusvirus (19 kDa) has been identified as a viral suppressor and specifically binds 21 nt double-stranded siRNA (ds-siRNA). In this study, we combined the nanotechnology and the RNA binding ability of p19 to develop a high sensitivity biosensor that immobilized p19 protein on silicon nanowire field-effect transistor (SiNW-FET) probe. This p19-SiNW-FET sensor has very high sensitivity to detected 21 nt ds-siRNA. Besides, the sensor has an ability to distinguish the various secondary structures of ds-siRNA, such as the size or mismatch on the ds-siRNA. The ds-siRNAs that bound on the p19-SiNW sensor also can be recovered after detection analyzed by Next-Generation Sequence (NGS). The deep sequence of ds-siRNA profile indicated that the PTGS-related ds-siRNAs has been collected and concentrated in the SiWN-FET probe. Interestingly, the most of the recovered

ds-siRNA were belong to the new-synthesized siRNA by PTGS pathway whereas some species of siRNAs were unable to detect in the input of total small RNA profile. The SiNW-FET combined with NGS technology provides a good strategy to study the protein-RNA interaction between PTGS and viral suppressor and also able to discover the new synthesized PTGS-related ds-siRNAs in the plant.

Genotypic classification of pathogenic variants of *Xanthomonas axonopodis* pv. *citri* from Taiwan by various DNA typing methods

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Phytopathology 101:S105

Molecular typing was applied for genotypic classification for three pathogenic variants of *Xanthomonas axonopodis* pv. *citri* (*Xac*) from Taiwan. These three novel variants of atypical symptom-producing *Xac* were designated as *Xac*-A^f, -A^p and -A^r. Based on the polymerase chain reaction (PCR) with primers specific to *Xac*, leucin-responsive regulatory protein (*lrp*) gene assay and DNA fingerprintings generated by repetitive-sequence PCR (rep-PCR) and amplified fragment length polymorphism (AFLP) were optimized to compare strains including the three types of atypical symptom-producing strains *Xac*-A^f, -A^p and -A^r, and additional reference strains from pathotypes *Xac*-A, -A*, -A^w, *X. axonopodis* pv. *auruantifolii* and *X. axonopodis* pv. *citrumelo*. These three types of *Xac* variants could be detected with six sets of primer specific for *Xac*. Cluster analyses by *lrp* sequence assay, AFLP and combing the band patterns of rep-PCR clearly grouped these variants in types *Xac*-A^f, -A^p and -A^r into the same cluster with typical symptom-producing strains in pathotype *Xac*-A. These three types of *Xac* variants could be excluded from strains of *Xac*-A* and -A^w in these genotypic analyses. Strains of *Xac*-A* and -A^w were closely related to *Xac*-A strains in our results. No Taiwan isolate was related to *X. axonopodis* pv. *auruantifolii* or *X. axonopodis* pv. *citrumelo*. The results further confirm the atypical symptom-producing variants of *Xac* in Taiwan belong to pathotype *Xac*-A.

One-step multiplex RT-PCR assay for simultaneous detection of two viroids and *Plum bark necrosis stem pitting-associated virus* in stone fruit trees

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Phytopathology 101:S105

Hop stunt viroid (HSVd), *Peach latent mosaic viroid* (PLMVd) and *Plum bark necrosis stem pitting-associated virus* (PBNSPaV) infect stone fruit trees. A single-step multiplex RT-PCR assay (mRT-PCR) was developed for the simultaneous detection of these pathogens. Three pairs of primers were designed to yield pathogen-specific amplicons, and amplification of a plant 18S rRNA fragment was included as a control. The expected products of 400 bp (PBNSPaV), 297 bp (HSVd), and 207 bp (PLMVd) were amplified from plants infected with each pathogen by both uniplex and mRT-PCR. The sensitivity and specificity of mRT-PCR was similar to that of the uniplex RT-PCR. The method was validated using samples from our collections that included single trees infected with one, two or three of the pathogens, as well as samples from the National Clean Plant Network. HSVd, PLMVd and PBNSPaV were detected from a co-infected peach tree, and both HSVd and PBNSPaV were detected from a co-infected myrobalan plum tree. The mRT-PCR was also evaluated by testing field samples from four different sources, and from these tests both PLMVd and PBNSPaV were detected as single infections. This assay is reliable, sensitive, and is a rapid and cost-effective method to detect these pathogens in stone fruit trees. The procedure is useful for certification, quarantine and genebank programs that test germplasm before distribution, and is especially applicable when many samples are tested for all three pathogens.

Development of a real-time RT-PCR assay to detect *Peach latent mosaic viroid* infections in stone fruit trees

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Phytopathology 101:S105

Peach latent mosaic viroid (PLMVd) infects *Prunus* and *Pyrus* species. It is distributed worldwide and can cause economic damage through reductions in fruit quality and yield. A single tube real-time TaqMan RT-PCR assay was developed for the detection of PLMVd, and compared to a conventional RT-PCR assay. Total nucleic acids were extracted from healthy and infected fruit trees using a CTAB method. The assay included a fluorogenic cytochrome oxidase gene probe (COX) as an internal control to validate the quality of the total RNA template samples. The assay was evaluated using PLMVd isolates collected from different geographic origins and field samples from commercial orchards in Colorado. The results of the TaqMan RT-PCR correlated with those from conventional RT-PCR and the sensitivity of this assay was 10^{-7}, a 10-fold increase in sensitivity over the conventional assay. This assay could be useful as a fast and sensitive option for PLMVd detection in quarantine and certification programs. It decreases the risk of contamination by performing the entire test in a single tube. It also requires less reaction time and avoids post RT-PCR electrophoresis, which reduces the labor involved when a large number of samples are tested.

The effect of *Potato virus S* infection on late blight severity in selected potato genotypes

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Phytopathology 101:S105

Late blight caused by *Phytophthora infestans* is an important disease of potato. cv. Defender is the only cultivar with foliar resistance to late blight. However, this cultivar exhibited susceptibility to infection by *Potato virus S* (PVS). PVS infection is very common in commercial potato fields in the Columbia Basin of Pacific Northwestern U.S.A. To investigate the potential interactions between these two pathogens and the resulting response of various potato genotypes, 'Defender' and Ranger Russet were inoculated with both *P. infestans* and PVS. The amount of sporulation and the extent of lesion expansion on inoculated leaves were measured to estimate the incidence of late blight. 'Defender' showed restricted spot lesions and had twenty times less amount of sporangia compared to 'Ranger Russet' when inoculated with *P. infestans* only. However, lesion expansion and sporulation increased significantly when 'Defender' was infected with PVS followed by inoculation with *P. infestans*. The increased late blight in PVS-infected 'Defender' suggests potential interaction between PVS and Defender impacting the outcome to late blight infection.

Complete genome sequence analyses and functional predictions for '*Ca.* L. solanacearum', the bacterium associated with potato zebra chip disease

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Phytopathology 101:S105

Zebra Chip (ZC) is an emerging plant disease that causes the decline of potato shoots and generally results in unusable tubers. ZC is characterized by a patterned discoloration within the tuber and fried chips from ZC-diseased tubers are commercially unacceptable. The disease has significantly impacted the U.S. potato industry and potato growing regions around the world. ZC is associated with '*Candidatus* Liberibacter solanacearum' (Lso), a fastidious alpha-proteobacterium that is transmitted by a phloem-feeding psyllid vector, *Bactericera cockerelli* Sulc. Taxonomically, Lso is related to '*Ca.* Liberibacter asiaticus' (Las), the putative causal agent of citrus huanglongbing. Research on this disease has been hampered by the fact that the bacterium is unculturable, making it impossible to obtain pure bacterial genomic DNA. In spite of these limitations, high quality genomic DNA was obtained using an immuno-capture technique. The complete 1.26 Mbp metagenome sequence of Lso was determined based on Lso DNA isolated from potato psyllids. The coding inventory of the Lso genome was analyzed and compared to other bacteria within the *Rhizobiaceae* family to identify genes and predict possible physiological functions. The analyse revealed a number of unique transporters and metabolic pathways, all potentially contributing to ZC pathogenesis. Information derived from this study will facilitate development of effective strategies for controlling ZC disease.

Proteomic analysis of grapevines in response to *Xylella fastidiosa* infection

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Phytopathology 101:S105

Xylella fastidiosa (*Xf*) is the bacterial causal agent of Pierce's disease (PD) of grapevines, as well as of other economically important diseases in a number of agronomic, horticultural and ornamental plants. The objective of this research was to tentatively identify proteins that are expressed in grapevines and involved in disease development or defense responses to *Xf* infection. A comparative analyses were carried out to identify proteins differentially expressed in *Xf*-infected grape stems from a pair of siblings of 9621-67 (highly susceptible) and 9621-94 (highly resistant) from a cross of *Vitis rupestris* × *V. arizonica*. Total proteins were isolated from the stems of uninoculated and *Xf*-inoculated plants at 1, 6, and 12 weeks after inoculation, separated by a 2D-PAGE system, and spots representing differentially expressed proteins were analyzed and identified using LC/MS/MS. Results revealed that differential expression of proteins in response to *Xf*-infection were genotype and development stage dependent. This study provides the first

proteomic analyses of the host responses to *Xf* infection in highly resistant and susceptible genotypes of grapevines. The information obtained will aid in the understanding of the mechanisms related to the host-pathogen interactions involved in PD.

Deep sequencing of small RNAs for virus and viroid identification in tomatoes

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Phytopathology 101:S106

Viroids are the smallest (246-401 nt) self-replicating plant pathogens. Recent evidence has led to the emerging view that RNA silencing has a crucial role in viroid pathogenesis and evolution, but the small RNA (sRNA) upon viroid infection on tomato plants has not been thoroughly analyzed. The objective of the present study was to conduct deep sequencing of sRNAs in tomato and to compare the sRNA profiles upon infection by *Pepino mosaic virus* (PepMV) and three pospiviroids, *Potato spindle tuber viroid* (PSTVd), *Tomato chlorotic dwarf viroid* (TCDVd), and *Mexican papita viroid* (MPVd). sRNA libraries were prepared and sequenced using Illumina technology. Libraries were generated from four samples suspected to be infected by PSTVd, TCDVd, MPVd or PepMV. Sequencing produced from 4.8 to 6.7 million reads per library. In all four libraries, sRNAs 23 nt and 24 nt in length were most abundant, followed by 21 nt and 22 nt. Nearly 90% of the sRNA reads in each sample could be aligned to the tomato genome. The reads that did not align to the tomato genome were assembled using previously characterized PepMV and viroid genomes as scaffolds. Results showed that virus or viroid-derived sRNAs from tomato were enriched and extended over respective virus or viroid genome. Greater genetic diversity of these pathogens in field samples was also unveiled. The success of the deep-sequencing of sRNA lends itself not only to virus discovery, but also to identification of unknown viroids.

Evaluation and adaptation of CANARY technology for rapid detection of plant pathogens

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Phytopathology 101:S106

CANARY is a cell-based technology which has been applied in medical diagnostic assays and monitoring for select agents. In this study, we evaluated the method for rapid detection of *Ralstonia solanacearum* and *Phytophthora* spp. using B cell lines developed by collaborators at MIT. We determined that the analytical sensitivity of *Ralstonia* CANARY is comparable to that of a typical PCR-based assay. During the study, we observed that cross-reactivity occurred between B cell lines and non-target pathogens or healthy plant extracts. We discovered that a major type of cross-reactivity resulted from an inherent antibody (Ab) anchored on the outer membrane of the cell. In the *Ralstonia* assay, the cross-reactivity was completely abolished through a chemical Ab blocking step while maintaining the target CANARY activity. The cross-reactivity between the B cell line and plants was greatly reduced or eliminated by simple sample manipulations. In the *Phytophthora* assay, chemically blocking the interfering Ab eliminated all CANARY activity, which suggested that the B cell line selected for testing contained only the inherent Ab, not the Ab targeting *Phytophthora*. We re-screened *Phytophthora* B cell clones consisting of the recombinant DNA of the desirable Ab and identified a clone generating high CANARY activity when the cross-reactivity was blocked. We will present CANARY protocols that maximize the analytical sensitivity and minimize the assay interference.

Air sampling of three powdery mildew populations using a Burkard cyclone sampler in eastern Washington

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Phytopathology 101:S106

Powdery mildews, caused by *Erysiphe necator*, *Podosphaera clandestina*, and *P. macularis*, respectively, are the most important fungal diseases of wine grapes, sweet cherries, and hops in eastern Washington. A reliable, user-friendly air-sampling device is critical for monitoring pathogen populations, which could aid in aerobiology studies and facilitate implementation of disease control measures. A Burkard multivial cyclone sampler was evaluated for studying powdery mildews on the three aforementioned crops in 2010. The multivial cyclone, Burkard volumetric, and Rototod spore traps, were installed adjacent to a vineyard, cherry orchard, and hop yard. The Burkard cyclone and volumetric samplers were programmed to collect samples daily whereas sampling medium was changed weekly for Rotorod samplers. Samples from the multivial cyclone and Rotorod traps were subjected to DNA extraction for amplifying DNA of three pathogens using their corresponding specific real-time PCR assay. Cp values of PCR amplifications were correlated to the log transformed daily spore count data of Burkard volumetric samplers. The correlation coefficients (r) were –0.86 ($P < 0.0001$), –0.88 ($P < 0.0001$), and –0.52 ($P < 0.0001$) for powdery mildews on cherry, grape, and hop, respectively. The Cp values indicated that the cyclone sampler is more efficient for trapping spores. These data indicate that the cyclone sampler shows promise as a reliable device for collecting airborne plant pathogen spores for subsequent PCR detection and quantification.

Biodegradation of cypermethrin by Rhodopseudomonas palustris GJ-22 isolated from contaminated sludge

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Phytopathology 101:S106

Abstract: GJ-22, a strain of the photosynthetic bacterium (PSB) capable of degrading cypermethrin (CPM), was isolated from the sludge from the wastewater treatment unit of an insecticide factory. The strain showed relatively high CPM degradation ability and was identified as Rhodopseudomonas palustris on the basis of its culture characteristics, colony and cell morphology, living cell absorption spectrum analysis, physiological and biochemical characteristics, type of internal photosynthetic membrane, and 16S rRNA sequence similarity analysis. Single-factor tests showed that CPM degradation by GJ-22 was good from 25°C to 35°C, but that the optimal temperature was 30°C; a pH of 7.0 was optimal for both initial growth and CPM degradation. GJ-22 completely transformed CPM at a concentration of 50 mg/L at 30°C, pH 7.0, and 7,500 lux within 7 days. Under optimal conditions, within a week, GJ-22 degraded 83.45% of CPM, 77.09% of fenpropathrin, 71.21% of bifenthrin, and 31.10% of ethofenprox at concentrations of 100 mg/L. The metabolic products were detected by performing gas chromatography/mass spectrometry (GC/MS) analysis; the analysis showed that GJ-22 oxidatively degraded CPM, yielding 5 metabolites. These results highlight the potential of this bacterium to be used in the cleanup of contaminated pesticide waste in the environment.

Antagonism between *Trichoderma harzianum* ETS 323 and *Botrytis cinerea* associated with L-phenylalanine oxidase-induced reactive oxygen species generation

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Previous proteomic studies showed that a homogenized L-phenylalanine oxidase (Th-L-AAO; molecular mass = 67 kDa) derived from extracellular proteins of *Trichoderma harzianum* ETS 323 induces apoptosis-like damage in *Botrytis cinerea*, an important global fungal pathogen; however, its mechanism in the antifungal pathway of *T. harzianum* against *B. cinerea* has not been studied. In this study, we aimed to elucidate the mechanism underlying the Th-L-AAO-induced apoptotic process in B. cinerea. Th-L-AAO was isolated from *T. harzianum* ETS 323 in the presence of deactivated *B. cinerea*. Antagonistic assay showed that treatment with purified Th-L-AAO effectively inhibited *B. cinerea* hyphal growth and caused hyphal swelling, deformed hyphae and vacuolation within hyphae, subsequently leading to hyphal lysis. Interestingly, Th-L-AAO treatment enhanced *T. harzianum* ETS 323 hyphal growth and it subsequent sporulation. We noted a substantial increase in the amount of reactive oxygen species generated in *B. cinerea* after its treatment with Th-L-AAO, suggesting apoptosis caused by mitochondria-induced endogenous reactive oxygen species generation. Further, we evidenced condensed chromatin and DNA fragmentation, increased lipid peroxidation activity, and dissipated mitochondrial membrane potential ($\Delta\Psi m$), supporting the apoptotic process in *B. cinerea*.

Protein photocleavers chrysophanol and pachybasin involved in *Trichoderma*'s biocontrol mechanism

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Anthraquinones have shown activity against phytopathogens, but the mechanism of action is not yet well understood. This study investigated the photocleavage activity on proteins of two anthraquinone derivatives, chrysophanol and pachybasin, isolated from *Trichoderma harzianum SY.*

Between the two, pachybasin showed better protein degradation with UV irradiation. The resulting binding constants of chrysophanol to lysozyme and BSA were $6.9 \pm 0.5 \times 10^4$ M^{-1} (ΔG^o = –27.62 kJ/mol at 25°C) and $6.6 \pm 0.4 \times 10^5$ M^{-1} (ΔG^o = –33.22 kJ/mol), and those of pachybasin to lysozyme and BSA were $5.0 \pm 0.2 \times 10^4$ M^{-1} (ΔG^o = –26.82 kJ/mol) and $1.1 \pm 0.1 \times 10^4$ M^{-1} (ΔG^o = –23.07 kJ/mol), respectively. Apparently, the mode of action of these compounds against proteins was oxygen-driven random cleavage. This oxygen-dependence is due to reactive oxygen species generated by the anthraquinones and may play a role in the biocontrol mechanism of *Trichoderma*.

Study on the extraction, purification and chemical structure of the activity component from *Gymnoascus reessii* za-130

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The vegetable disease caused by root knot nematode were losing seriously and difficult to control with chemical. Applying bio-materials to control this disease are more safety. The strain of *Gymnoascus reessii* za-130 has ability to kill the root knot nematode (*Meloidogyne incognita*) and has been reported the result of the nematicidal activity from its broth filtrate and physical-chemical character in 2010. This report was studied on the extraction and purification for activity component of za-130 which including pretreatment of broth filtrate, macroporous absorption and adsorption resin to remove excess impurities, and finally 100% methanol elution, collected and evaporated to dryness; With chloroform: methanol: water dissolving, the concentrated specimen were obtained by using the full with Silica gel column, and collecting to full with HPLC the mobile phase of methanol-water. Under the 45°C constant temperature to dry oven and the brown powder with the purity of 99.766% were obtained. The structure of pure product was determined by the ultra-violet spectrograph and the maximum absorption at 275nm; Mass spectrum showed the molecular weight is 326 and the molecular formula is C14H14O9. The structure of purified component is (3E,5E)-2,5-dihydroxy-2,7-dihydrooxepine-3-carboxylic anhydride promoted through above spectral analysis and their nematicide mechanism were showed a strong inhibitory effect to esterase isozymes (including acetylcholinesterase).

Isolation, identification of scutellaria extraction by 80% ethanol and its antifungal mechenism against *Monilinia fructicola*

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Brown Rot caused by *Monilinia fructicola* is a serious disease for peach trees. Recently, there are some studies showed that the traditional Chinese herbs could have very good anti-fungi effect. However, the inhibition mechanism and activity component are still not clear. Scutellaria is one of Chinese herbs which show obviously inhibition to *M. fructicola*. The active component was isolated and identified and the antifungal mechanism was studied. Crude preparation of the antifungi active ingredient of scutellaria was extraction by 80% ethanol. Inhibition test showed that scutellaria extraction could inhibit the growth and sporulation of *M. fructicola*. We also found that the cell walls of the hyphae were significantly thicker under microscope, and conidia can't germinate and membrane permselectivity changed in scutellaria extraction. To purify the antifungal substance, the scutellaria extraction by 80% ethanol was dissolved in water after decompression concentrating; then, it was extracted by ether and separated with silica gel column. The antifungal activity part of eluent was condensed and recrystallized, and identified as a mixture of baicalein and phenols by GC-MS analysis. Thanks to funding project for academic human resources development in institutions of higher learning under the jurisdiction of beijing municipality (PHR201107135).

Breeding of the high effective biocontrol strain of *Streptomyces lydicus* against plant fungal diseases by genome shuffling

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Streptomyces lydicus strains A01 and A02 were isolated from soil in suburbs of Beijing, China. Both of them presented strong inhibitive actions against many plant pathogenic fungi by producing natamycin, a polyene macrolide antibiotic with broad-spectrum antifungal activity. In this study, the 2 strains were firstly mutagenized by using complex treatment with ultraviolet ray and lithium chloride. The positive mutants E9 and E54 from A01, C16 and C23 from A02 were obtained with the natamycin productivities increased by 20%, 18%, 27% and 30% over that of their original strains respectively. Then the genome shuffling was carried out with the 4 positive mutants as original strains. After 2 times of recursive protoplast fusion, a recombined fusant G117 was screened out from the filial generations of the 4 parents. The natamycin productivity of G117 was increased by 39% over that of A01 and 56% over that of A02 respectively. Further more, the time needed for reaching the peak of natamycin yield for strain G117 was shortened from 72 hours to 48 hours in comparison with original strains A01 and A02. The inhibitory ratio of fermented broth of G117 against *Botrytis cinerea* was increased by 31%~45% over that of the original strains under the same conditions. No remarkable changes in culture characteristics, natamycin productivity and antifungal activity were found in strain G117 after 10 continuous generations of propagation.

The effects of temperature on the development of *Amblyseius barkeri* (Hughes) (Acari:Phytoseiidae)

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The development of *Amblyseius barkeri* (Hughes) at different constant temperatures was investigated. The results indicate that the developmental duration of *A. barkeri* was shortened gradually with the increasing of temperature at the temperature range of 16~28°C, and the developmental durations for egg, larva, nymph, pre-oviposition and whole generation varied from 3.34d to 1.38d, 1.65d to 0.65d, 8.50d to 3.00d, 0.50d to 1.50d and 5.53d to 14.99d, respectively. However, at 30°C and 35°C, the developmental duration was prolonged for all life stages. The developmental duration of egg and pre-ovipostion stages were linearly correlated with temperatures, while the relationship between the developmental duration of other stages and temperatures were a parabola opening upwards. According to the priority law, the developmental threshold of pre-oviposition period was 12.11°C, and all other various stages and whole generation were lower, 6.87°C (generation) and 8.03°C (larva), with an effective thermal sum of 137.58°C for generation. Based on the model of Wang Rusong, the optimum developmental temperature for the mite was 28~30°C, critical lethal high temperature was 35.31°C~38.94°C, and critical lethal low temperature was 7.23°C~8.74°C.

Probe the interaction between SCMV PIPO with maize protein

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A cDNA library of maize (Zea mays) stems and leaves was screened with PIPO (Pretty Interesting Potyviridae ORF) cistron of SCMV-BJ to identify the ineractors by yeast two hybrid-system. Among the 51 positive candidate clonies selected, two clones shared the identical sequence of 755bp containing a single open reading frame (ORF), encoding a predicted protein of 212 amino acids. A BLAST query of the deduced amino acid(aa) sequence revealed that there was a conserved domain for the protein which was identified as maize Cytochrome P450 (CYP450). Bimolecular fluorescence complementation (BiFC) assay was used to confirm the interaction between PIPO and CYP450 in plant. the reconstituted fluorescence was observed in maize protoplasts and *Nicotiana benthamiana* leaf epidermis, respectively. The results above suggested that PIPO could indeed interact with CYP450 in living plant cells.

Metabolic profiling of xylem sap from Pierce's disease resistant and susceptible grapevines

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Pierce's Disease (PD) of grapevines is caused by a gram-negative, xylem-limited bacterium *Xylella fastidiosa* (*Xf*). All *Vitis vinifera*-based cultivars are highly susceptible to *Xf* infection. However, some grape species from the southern United States such as *V. arizonica*, *V. Shuttleworthii*, and *Muscadinia rotundifolia* are resistant. Given that *Xf* is limited to xylem vessels, it has been hypothesized that chemical composition of host xylem sap could play an important role in *Xf* pathogenesis. In this comparative study, PD resistant (9621-67) and PD susceptible (9621-94) genotypes segregating from *V. arizonica* × *V. rupestri* breeding population were used. Xylem sap samples were collected from both genotypes with and without *Xf* infection using a pressure chamber. The metabolic profiles of xylem sap samples were analyzed using high performance liquid chromatography (HPLC) and gas chromatography–mass spectrometry (GC-MS). About 20 peaks were tentatively identified as phenols, sugars, fatty acids, furfurals and furanosides by mass spectrometry. Principal component analysis (PCA) was conducted to

categorize compounds that are correlated with resistance/susceptibility and infected/healthy states. Metabolic profiling coupled with PCA analysis in this study can facilitate to identify and characterize genotypic differences between PD resistant and susceptible grapevines in response to *Xf* infection.

Fumigation and fungicide effects and qualitative and quantitative analysis of Pythium, Fusarium and Rhizoctonia on strawberry roots

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Black root rot (BRR), caused by Pythium, Fusarium and Rhizoctonia, results in significant yield reductions in strawberries. Characterization and quantification of pathogens on roots is crucial for understanding pathogen ecology and management of root diseases. Fumigation trail was set up with the following treatments: Methyl bromide+chloropicrin (50:50; MC) and Pic-Clor60 (PC), and fungicides using Switch (SW+) [or Abound (AB+)] dip applications before planting plus drip applications with Ridomil Gold after 7 days and at early spring growth and Abound at full bloom. BRR severity was lower in SW+ (5.9 Horsfall-Barret scale) and AB+ (5.3) compared to MC (6.5) and PC (6.3) and all were lower than the non-treated plots (7.0). Colony numbers of Pythium spp. on roots were lowest in SW+ at each sampling. Colony numbers of Fusarium and Rhizoctonia spp. on roots were also lowest in the SW+ and AB+ treatments at each sampling. Moreover, Pythium, Fusarium and Rhizoctonia spp. composition was rich and diverse on roots within and between sampling periods. Pythium spp. were detected from roots before planting but not Fusarium and Rhizoctonia spp. based on culture dependent and DGGE techniques. Based on DGGE, MC and PC had the similar root associated fungal populations; MC and AB+ reduced Fusarium populations; AB dips before planting reduced Rhizoctonia. Selective use of fungicides that target known pathogens offers potential BRR management in combination or in place of fumigants when incorporated into an IPM program.

Biochemical and antibacterial properties of L-amino acid oxidase derived from *Trichoderma harzianum* ETS 323

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Although L-amino oxidase (LAAO) has been reported to be a potent antibacterial agent, its antibacterial mechanisms remain unclear. A novel LAAO (Th-LAAO) was isolated from extracellular proteins of *Trichoderma harzianum* ETS 323 and is thought to be antagonistic to the plant pathogen, *Rhizoctonia solani*. Here, we show that the activity and structure of this enzyme is more stable at pHs between 6 and 8 than at pH 9 or between pH 4 and 5, with the optimal pH of Th-LAAO being 7.0. Its secondary structure is estimated to comprise 35% α-helix, 17% β-sheet, 21% β-turn, and 27% random coil. Th-LAAO inhibits the growth of both Gram-negative and Gram-positive bacteria. By confocal microscopy and flow cytometry, we observed that FITC-labeled Th-LAAO interacted with bacteria and caused permeabilization of the membrane. Additionally, increased exogenous H_2O_2 production was found in Th-LAAO-treated bacteria, which appears to be mediated by accumulation of reactive oxidative species (ROS) in the bacteria. ROS accumulation triggers damage, including lipid peroxidation and DNA strand breaks, that was found in cells after treatment with Th-LAAO, possibly resulting in bacterial growth inhibition. Overall, our results show that interaction, membrane permeabilization, and H_2O_2 production are probably involved in the antibacterial action of Th-LAAO.

Evaluation and characterization of antifungal compounds from the fermented products of Trichoderma harzianum SL-BNR1-6

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Metabolites of Trichoderma include molecules that confer resistance to host plants (elicitors) and molecules with antimicrobial activity. Six Trichoderma strains - T. harzianum ET S323, T. harzianum ET S428, T. harzianum SLBN R16, T. virens LNPA R42, T. harzianum T22, T. virens YAM were comparatively evaluated based on the dry weight of secondary metabolites they produce by fermenting sugarcane bagasse. Trichoderma harzianum SLBN R16 was found to produce higher amount of secondary metabolites on the fourteenth day of fermentation. Further, an assay guided fractionation and purification of metabolites was performed to characterize antifungal compounds of T. harzianum SL-BNR1-6. Three anthraquinones - ω-hydroxypachybasin, Phomarin and emodin; and a phenylpropanoid derivative - Methyl ρ-coumarate were identified as major antifungal compounds in the fermented products of T. harzianum SL-BNR1-6. Methyl ρ-coumarate and ω-hydroxypachybasin showed higher antifungal activity against Rhizactonia solani a well known plant pathogen. The structures were determined from respective 1H and 13C NMR spectrum.

Suppression of plant cell death and immunity by a family of *Magnaporthe oryzae* zinc-finger effectors

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Magnaporthe oryzae is one of the most important pathogens that devastate many cereal crops such as rice, wheat and barley. Elucidation of its virulence mechanisms at the molecular level is a prerequisite for developing novel disease control strategies through biotechnology. Bioinformatic and experimental analyses of *M. oryzae* genome suggest that many of the fungal proteins (so-called effectors) may enter rice cells and exploit host molecular or cellular events for the pathogen's benefits. In this study, we have identified and characterized a 7-member family of putative effectors with a typical tripartite structure: signal peptide, host-targeting site and monodactyl C2H2 zinc-finger motif. Molecular and biochemical experiments indicate that this family of the fungal effectors may enter host cells in a pathogen-free manner. In addition, transient expression of these effectors in *Nicotiana* plants was shown to inhibit the BAX-induced programmed cell death, suggesting their potential role in suppressing plant defense response. To further investigate their role in suppressing immunity and promoting disease in rice plants, we have generated stable transgenic rice lines expressing individual effectors and are currently characterizing these plants for compromised host resistance to pathogen infection. Ultimately, these efforts should lead us to a better understanding of the pathogen virulence and host immunity and facilitate the development of novel approaches for plant disease control.

Rice OsERF9 is involved in responses to biotic and abiotic stresses

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AP2/EREBP transcription factors play important roles in plant development and in the responses of plants to biotic and abiotic stresses. There are 122 members in Arabidopsis and 139 in rice. Rice *OsERF9* is induced by abscisic acid and salt treatments as well as both virulent and avirulent pathogen *Magnaporthe oryzae*, causal agent of rice blast disease. OsERF9 is localized to the nucleus, binds specifically to the GCC box sequence *in vitro* and acts as a transcriptional activator in plant and yeast cells. Knockdown *OsERF9* by means of RNAi enhanced resistance against *M. oryzae*. The elevated disease resistance of RNAi plants was associated with increased expression of *PR*, *PAL*, and phytoalexin biosynthesis related genes. On the other hand, the *OsERF9* overexpression progenies became more sensitive to salt stress in rice and tobacco plants. Interestingly, overexpression of *OsERF9* in tobacco increased disease resistance to *Pseudomonas syringae* pv *tabaci*. These results suggest that OsERF9 is integrated into the cross-talk between biotic and abiotic stress signaling networks.

Endophytic bacteria in potato tubers affected by zebra chip disease

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Potato zebra chip disease (ZCD) could drastically reduce quality and value of all market classes of potato, costing growers and processors millions of dollars in losses in North America. Endophytic bacteria colonize the internal tissue and could have both positive and negative effect on their host plants. In potato, there has been research on endophytic bacteria but few were carried out in the context of ZCD. In this study, endophytic bacteria in ZCD affected potato tubers (Cultivar Atlantic), defined by the symptom of phloem necrosis and PCR detection of "Candidatus Liberibacter solanacearum", were analyzed. A total of 85 bacterial strains belong to 17 bacterial genera were isolated by in vitro cultivation. ZCD tubers had significantly more culturable endophytic bacterial species than non-ZCD tubers. Paenibacillus sp., Microbacterium sp., Brachybacterium sp., Staphylococcus sp., Stenotrophomonas sp. and Klebsiella sp. were only isolated from ZCD potato tubes, while Bosea sp., Sphingopyxis sp., Sphingomonas sp. and Nocardia sp. were only isolated from non-ZCD potato tubers. PCR experiments using specific primers further confirmed the presence of Microbacterium spp. to ZCD tubers and Bosea sp. to non-ZCD tubers. Scanning electron microscopy revealed bacteria with morphology of bacilli, coci, and pleomorphic in the phleom tissue of potato tubers.

Cymene inhibition of *Beauveria bassiana* spore germination

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Losses due to seedling damping-off caused by *Rhizoctonia* and *Pythium* can be reduced both by seed treatment with *Beauveria bassiana* isolate TN11-98 (Bb) and by use of bioactive monarda herbages. Impact of essential oils found in monarda herbage on *Bb* spore germination is unknown. The purposes of this study were: to determine if cymene, an essential oil found in all monarda herbages, inhibits germination of *Bb*; and to develop and validate mathematical models for the impact of cymene on *Bb*. Six concentrations of cymene; ranging from 0.0005 to 500 µM, and a no cymene control were tested. Spore suspensions of *Bb* were placed on microscope slides coated with water agar, in the presence or absence of cymene-treated filters. Spore germination was observed for 24 h. After 12 h when detectable germination typically began, microscope views were photographed every 4 h; and germ tube lengths (including the diameter of the spore) were measured. Germ tube length was equal to control at concentrations at or below 0.05 µM; but germination was stimulated and germ tube length increased at 0.5 µM for all observation times and at 5 µM for most times. Spores exposed to 50 or 500 µM cymene rarely germinated. Models of spore germination tube growth rate as a function of cymene concentration were developed and model results fitted well to experimental data. Predictions of models were tested and validated by separate experiments.

Evaluation of ten leguminous cover crops as cryptic hosts for *Verticillium dahliae*

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Verticillium wilt is a vascular disease caused by *Verticillium dahliae*, challenging agronomic production of over 300 crops worldwide. The fungus produces durable microsclerotia in infected plant tissue, which can survive in soil for many years. In the absence of fumigation, suppression of soil populations of *V. dahliae* relies on natural attrition during periods of nonhost cultivation. However, rotation crops that appear to be nonhosts because they show no symptoms of disease may nevertheless support development of the pathogen and thus negate the benefit of crop rotation. This study was undertaken to evaluate the extent to which common legume cover crops are colonized by *V. dahliae* and support formation of microsclerotia. Fava bean, bell bean, sunn hemp, sesbania, black-eyed peas, field pea and four vetch species were evaluated under both greenhouse and field conditions. No visual symptoms were observed and stem height of inoculated and control plants were not significantly different. However, *V. dahliae* was recovered from the stems of all ten crops. When infected stem segments were buried in potting mix, microsclerotia formed on decomposing tissue of eight of the ten crops. The results of this study should help to guide growers in selection of cover crops that will not aggravate problems caused by *V. dahliae*.

Screening strains of *Trichoderma* spp. for decomposition of agriculture wastes

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Trichoderma spp. are one of the important groups of fungi used commercially as biocontrol agents for the management of plant diseases. Polysaccharide hydrolases released from *Trichoderma* spp. are also important source of enzymes for decomposition of agro-wastes. This study was conducted to develop a quickly technique for screening strains of *Trichoderma* spp. with strong capability of hydrolyzing cellulose through the activity of cellulase. Results of the experiments in solid/ liquid culture media using ammonium chloride as nitrogen source showed a high degree of correlation between the index of cellulase activity/mycelia growth and the activity of CMCase for the tested strains of *Trichoderma* spp. When the agricultural wastes, rice straw, rice bran, peanut shell, sugar bagasse or sawdust, were used as growth substrates, the enzyme activities of *Trichoderma* spp. varied with strains and growth substrates. On rice straw, the strain PT- MusaS24-1 released the highest amount of CMCase (119.5U), whereas the strain PTNC-WAS50-3 released the highest amount of Avicelase (29.32U) and FPA (32.05U). On peanut shell, the strain NT-TaS17-1 released the highest amount of β-glucosidase (9.53U) and on rice bran, strain PTNC-WAS50-3 released the highest amount of xylanase (176.65U). This study concludes that rice straw and rice bran are suitable substrates for production of cellulase by selected strains of *Trichoderma* spp., whereas saw dust is the most unsuitable substrates among the agricultural wastes tested.

New records of *Tospoviruses* and *Geminiviruses* in Mauritius

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Onion and tomato are crops of economic importance in Mauritius with annual production of 8500 and 18000 tonnes, respectively. Iris yellow spot virus (IYSV [family *Bunyaviridae*, genus *Tospovirus*]), was confirmed using ELISA and RT PCR followed by cloning and sequence analysis as the causal agent of a serious outbreak of a viral disease of onion in several production areas of Mauritius. An incidence of over 80% infection was noted in onion fields. In October 2009, tomato plants with reduced leaf size, leaf curling, and yellow margins associated with plant dwarfism were observed in open fields in the southern part of the island with disease incidence ranging from 5% to 50%. Tomato yellow leaf curl virus (TYLCV) (Family *Geminiviridae*, genus *Begomovirus* was confirmed by PCR and sequencing of the amplicons. TYLCV was prevalent in open-field tomato varieties 'Swaraksha' and 'Epoch'. IYSV and TYLCV are new records for Mauritius.

Exploring the Brazilian diversity of *Trichoderma* spp. with focus on biological control of white mold on common beans in the field

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The microbial culture collection kept by Embrapa Rice and Beans gathers over than 300 isolates of *Trichoderma* spp, which have being identified in the species level by means of optical microscopy and DNA barcode methods. These isolates were collected in several Brazilian regions, in order to select the best biological control agents for white mold (*Sclerotinia sclerotiorum*), a major disease of common beans (*Phaseolus vulgaris*) in the country. After dual culture, hyperparasitism and enzyme production tests carried out in partnership with the Federal University of Goias, the eight best isolates were tested in field trials in Goianira, Goias State, in 2009 and 2010. In each year, the experimental field was artificially infested with an average of 145 viable sclerotia m^{-2} and cropped to the indeterminate bush 'Perola' cultivar, under no-tillage and sprinkling irrigation. A suspension of 2×10^9 viable spore mL^{-1} was obtained after isolate growth at 25°C on autoclaved rice, and sprayed in the field plots during the crop V4 stage. The *S. sclerotiorum* inoculum density (number of apothecia m^{-2}) and disease severity were assessed, respectively, at the R5 and R7 stages. The ANOVA showed consistent results between the trials, and that the three best isolates effectively reduced the *S. sclerotiorum* apothecia up to 70%, with a correspondent decrease of disease severity. As a result of lower white mold severity, yields were higher in comparison to control plots and ineffective isolates.

The use of social media sites at the Plant Disease and Insect Clinic at North Carolina State University

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Phytopathology 101:S109

The Plant Disease and Insect Clinic (PDIC) at NC State University provides plant disease diagnostic and insect identification services to county agents, master gardeners, producers, homeowners, and the general public. Part of effective disease diagnosis is communication and outreach to clientele. At the PDIC, we felt that we were significantly lacking in this dimension of disease diagnosis. Formerly, our website functioned primarily as a reservoir of information on sample submission instructions, fees, and links to other sites. It was not a destination page that our clientele would visit and spend time exploring. In early 2011, we developed social media pages to communicate more effectively with the public and revamped our website to be more informative, user friendly, and attractive. We began using social media outlets, Facebook, Twitter, and Blogger, to broadcast current information on plant diseases and insect pests, to publicize webinars, and to alert the public to potential threats to plant life in NC. During the first two months after implementation of our social media sites, we had 963 page views on our blog, 44 followers on Twitter, and 112 monthly active users on Facebook. These sites have provided our clients with easy access to the resources and expertise of NC State University and the PDIC. Using social media sites has allowed us to reach people across the state and network with specialists, diagnosticians, and county agents from our neighboring states.

Characterization and mefenoxam sensitivity of *Pythium* species in North Carolina greenhouses

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Phytopathology 101:S110

Herbaceous ornamentals exhibiting symptoms of Pythium root rot were collected from greenhouses throughout North Carolina. Greenhouses were selected based on diagnostic records of Pythium-positive plants submitted through the Plant Disease and Insect Clinic (PDIC). Roots were assayed for *Pythium* by isolation on selective media and individual isolates were identified by morphological characterization and sequencing of the ITS rDNA region. Isolates of the predominant *Pythium* species were screened for mefenoxam sensitivity on 5% clarified V8 agar amended with 100 ppm mefenoxam in 48-well micro-titer plates. Isolates were evaluated using a rating scale of 0 (no growth) to 5 (mycelium completely colonizing entire well). Mean sensitivity scores 4 were considered insensitive to mefenoxam. The information obtained in this study will allow the PDIC to provide growers with control recommendations customized to their specific situation.

Seasonal fluctuation of '*Candidatus* Liberibacter asiaticus' titers in citrus trees

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Phytopathology 101:S110

Huanglongbing (HLB) associated with 'Ca. L. asiaticus' (Las) is the most destructive disease of citrus. First reported in 2004, HLB has already been detected in over 300 municipalities of the states of São Paulo (SPS), Paraná, and Minas Gerais. From the Araraquara region, the initial focus of the disease, HLB moved longer distances to the south than to the north/northwest SPS, despite the widespread presence of the psyllid vector and the many citrus farms. Summer air temperatures, significantly higher in the north/northwest, may be affecting bacterium multiplication and the pattern of disease spread. In fact, in growth chambers, Las titers were 100 to ten thousand fold lower in citrus kept at daily regimen of 24–38°C than at 24–32°C (Plant Dis. 93:257-262). In this work, 36 Las-inoculated potted sweet orange plants were maintained under screen for 32 months in Botucatu and Colina, south and north SPS. Symptom progress and bacterium titers were assessed every 2 to 3 months. Significant seasonal variation in average cycle threshold (Cts) was observed in both locations, with the highest values assessed during the summer (24.75 in Jan 2009 in Colina and 23.15 in Nov 2009 in Botucatu) and the lowest during the spring and fall (16.80 in Sept 2008 in Colina and 15.64 in Apr 2010 in Botucatu). Multiple regression analysis showed significant association between Cts and temperature. The higher the Ct the higher the number of hours above 32, 35 or 38°C registered just before sampling date.

Distribution and abundance of nematodes in corn fields in Illinois

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Phytopathology 101:S110

As a first step toward assessment of the effects of plant parasitic nematodes on corn production in Illinois, a survey was conducted to determine the distribution and population densities of plant-parasitic nematodes in corn fields in 2009 and 2010. A total of 587 soil samples were collected from 95 (of 102) counties. The survey revealed the presence of at least eleven genera, including *Helicotylenchus*, *Heterodera*, *Hoplolaimus*, *Longidorus*, *Meloidogyne*, *Mesocriconema*, *Paratylenchus*, *Pratylenchus*, *Trichodorus*, *Tylenchorhynchus*, *Xiphinema*, and a number of others designated "tylenchids" which were counted as a group and not yet identified to genus. The most frequently observed genera were those in the "tylenchids," along with *Helicotylenchus* and *Pratylenchus*, which occurred in 98.8%, 98.5%, and 84.2% of the samples, respectively. The next most frequently observed genera were *Heterodera*, *Tylenchorhynchus*, *Paratylenchus*, and *Hoplolaimus*, found in 57.1%, 36.8%, 23.7%, and 22.0% of the samples, respectively. Population densities of each genus often exceeded estimated damage thresholds. Prominence values (PV) (PV = population density × (√frequency of occurrence)/10) were calculated for each genus. *Helicotylenchus* had the highest PV (1634.5), followed by the tylenchids (1612.12), and *Pratylenchus* (286.7). The survey indicated that plant-parasitic nematodes are widespread in corn fields in Illinois. The economic importance of this information is yet to be determined.

Identifying *Phytophthora* species isolated from nursery irrigation water throughout North Carolina

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Phytopathology 101:S110

Species of *Phytophthora* are well adapted to aquatic environments, and have been commonly detected in rivers, canals, runoff water, ponds, reservoirs, streams, and hydroponic systems. The presence of pathogenic organisms in irrigation water sources can be a serious threat to plant health, especially if a grower uses reclaimed surface water for irrigation. During the summer and fall of 2010, irrigation water was collected from numerous ornamental plant nurseries in 13 counties of North Carolina. Water samples were assayed for *Phytophthora* by filtration then cultured on selective media so individual isolates could be identified by sequencing of the ITS rDNA region. Our collection of over 62 isolates included representatives of the described species *P. cactorum*, *P. citricola*, *P. citrophthora*, *P. cryptogea*, *P. hydropathica*, *P. nicotianae*, *P. palmivora* and *P. tropicalis*, as well as isolates in five clades of previously undescribed species. Future studies will include intensive monitoring at two nursery locations and sequencing of additional rDNA regions including the cytochrome oxidase II gene. The pathogenicity of undescribed species will be determined by inoculation of select ornamental hosts.

Comparative study of *Pythium* species causing carrot cavity spot in California and Michigan

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Phytopathology 101:S110

California (CA) and Michigan (MI) combined account for about two thirds of the carrot production in the U.S. One of the limiting factors of production is cavity spot, caused by *Pythium* spp. To characterize the pathogen by regions, *Pythium* spp. were isolated from carrot roots with cavity spot lesions from both CA and MI. The internal transcription spacer (ITS) of the ribosomal DNA region was sequenced for species identification. *Pythium violae* was predominant and isolated from more than 90% of all 122 CA samples; a few isolates of *P. sulcatum*, *P. irregulare* and *P. ultimum* were also identified in CA. In MI, four species were isolated, including *Pythium sulcatum* (38%), *P. sylvaticum* (32%), *P. intermedium* (22%) and *P. irregulare* (8%). In some cases, more than one *Pythium* species were isolated from one lesion. In virulence tests on carrot roots, *P. sulcatum* and *P. violae* were more aggressive than the other three species. Mycelia of *P. violae* and *P. sulcatum* grew slower than the other four species on V8 agar medium at room temperature. Mycelial growth inhibition was tested with three fungicides; all five species were sensitive to mefenoxam and zoxamide, but only *P. violae* was sensitive to fluopicolide. These results suggested that predominant pathogen populations were different between CA and MI, and that zoxamide might be effective in both CA and MI, but fluopicolide is not recommended for the MI carrot industry.

Risk assessment of *Phytophthora capsici* resistant to fluopicolide

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Phytopathology 101:S110

Fluopicolide is a systemic fungicide affecting oomycetes including *Phytophthora capsici*. A laboratory study was conducted to assess the risk of fluopicolide resistance in *P. capsici*. Baseline sensitivity to fluopicolide was determined using 126 *P. capsici* isolates from Michigan, U.S.A. All isolates were sensitive to fluopicolide and values of effective concentrations for 50% inhibition of mycelial growth (EC50) ranged from 0.0847 to 0.2380 µg/ml, with a mean of 0.1572 µg/ml in unimodal distribution. Resistant mutants were obtained at a mutation frequency above 1.0×10^{-7} from five *P. capsici* isolates by screening zoospores on fluopicolide-amended (5 µg/ml) agar plates. The mutants showed either intermediate (resistance factors between 3.53 to 92.63) or high resistance (resistance factors between 2245.09 to 7034.79) to fluopicolide. The fluopicolide resistance of the mutants was stable through 10 mycelial transfers on fungicide-free medium. All resistant isolates exhibited an equal overall level of fitness (zoospore production, cyst germination, and virulence on zucchini fruit or pepper seedlings) compared with their sensitive parental isolates, with few exceptions. No cross-resistance was detected between fluopicolide and five other fungicides, including cyazofamid, mandipropamid, mefenoxam, zoxamide and azoxystrobin. Based on these results, the potential for *P. capsici* populations to develop resistance to fluopicolide in the field and the resistance risk may be moderately high.

Molecular mechanism of QoI resistance in *Fusicladosporium carpophilum* – causal pathogen of almond scab in California

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Phytopathology 101:S111

Almond leaf and fruit scab caused by *Fusicladosporium carpophilum* (*Venturia carpophila*) is a common and widespread disease in California. QoI-resistance in populations of this pathogen has been reported previously in different regions of the state. The partial cytochrome b gene was sequenced and a mutation at codon 143 (G143A) was found in all isolates highly resistant (EC_{50} > 30 µg/ml) to azoxystrobin. No mutations at codon 143 or 129 were found in isolates moderately resistant (EC_{50} 1 to 8 µg/ml) to azoxystrobin. A primer pair was designed and confirmed to target the G143A mutation. Another primer pair was designed to distinguish *F. carpophilum* from five common species in the genera *Monilinia*, *Alternaria*, *Botrytis*, *Botryosphaeria*, and *Aspergillus* that occur on almond. Using these primer pairs, a real-time PCR assay was established to quantify the frequency of the G143A allele in *F. carpophilum* populations. In laboratory studies, there was a significant correlation ($r^2 = 0.97$, $P < 0.001$) between the proportion of spores from azoxystrobin-resistant to -sensitive isolates and the frequency of the G143A allele that was quantified by real-time PCR. This study demonstrated the potential of using real-time PCR to efficiently quantify QoI-resistance in *F. carpophilum* populations.

***Botryosphaeria* species complex associated with coast live oak (*Quercus agrifolia*) mortality in Southern California**

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Phytopathology 101:S111

A 2009–10 survey of oak stands in San Diego and Riverside Counties was conducted to identify and characterize pathogenicity of fungi involved in oak mortality in southern California. Coast live oak (*Quercus agrifolia*; CLO) mortality caused by the goldspotted oak borer (GSOB, *Agrilus auroguttatus*) has occurred in San Diego County, California, since 2002. *Diplodia corticola*, *Botryosphaeria sarmentorum*, *B. iberica*, and *B. stevensii* were consistently recovered from bleeding CLO trunk cankers in GSOB-infested and -uninfested sites with tree mortality. Species were confirmed by ITS4/5, β-tubulin, and EF factor rDNA sequencing and by morphology of ten isolates of each species on PDA-tet and on pine needle agar under UV light at 25°C, for characterizing mycelial and pycnidia characteristics, respectively. For each species, five one-year-old CLO seedlings were wound-inoculated with two isolates, using plugs from one-week old cultures, or sterile agar for controls. Every species was recovered from necrotic tissue. At three months, lesions were significantly longer than controls; however, within four weeks, *D. corticola* lesions extended throughout inoculated seedlings, including roots, and caused seedlings to die. Conidia occurred throughout the plant tissue, and seedlings exhibited bleeding and epicormic sprouting. Results suggest that the *Botryosphaeria* species complex is important in the decline of CLO at GSOB-infested and -uninfested sites throughout southern California.

Molecular screening of walnut backcross populations for a DNA marker linked to cherry leafroll virus resistance

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Phytopathology 101:S111

Blackline disease, a graft union disorder caused by infection of English walnut (*Juglans regia*) trees by *Cherry leafroll virus* (CLRV) is a major problem for walnut production in Northern California where scions are grafted onto virus resistant black walnut (*J. hindsii*) or 'Paradox' (*J. hindsii* × *J. regia*) rootstocks. A breeding program is currently developing CLRV-resistant English walnut cultivars by recurrent backcrossing of 'Paradox' hybrid with English walnut cultivars. We have developed primers to detect a DNA marker specific to *J. hindsii* parent linked to hypersensitivity to CLRV for marker assisted selection. In PCR assays, these primers amplified a 535-bp DNA fragment from *J. hindsii* and 'paradox' rootstocks. Analysis of nucleic acid extracts from trees of a third generation backcross population by PCR indicated association of the marker with hypersensitivity to CLRV as determined by bark patch grafting inoculations. We then screened 1,174 fourth generation backcross seedlings for the presence of the DNA marker and found that 48% (563/1174) of these seedlings were positive for the marker. The molecular screening method used in this study was able to reduce time and resources that were otherwise required for screening by patch graft testing.

Evaluation of ningnanmycin for management of dollar spot and anthracnose in turfgrasses

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Phytopathology 101:S111

Ningnanmycin, a low risk bio-pesticide produced from fermentation byproducts of the soil actinomycete *Streptomyces noursei* var. *xichangensis* n. var, was evaluated for its *in vitro* activity against *Sclerotinia homoeocarpa* (the causal agent of dollar spot) and *Colletotrichum cereale* (the causal agent of anthracnose) using a three-fold dilution from 0.81 to 0.01 µg/ml. Two isolates of *C. cereale* and six isolates of *S. homoeocarpa*, including three isolates sensitive to demethylation inhibitor (DMI) fungicide and three isolates resistant to DMI, were selected for the assay. Triticonazole was included as a standard for comparison. The mean EC_{50} values of *C. cereale*, *S. homoeocarpa* DMI-sensitive isolates, and *S. homoeocarpa* DMI-resistant isolates were 0.30, 0.01 and 0.17 µg/ml for ningnanmycin compared to 0.98, 0.05, and 0.49 µg/ml for triticonazole, respectively. A positive correlation was detected between EC_{50} values of ningnanmycin and triticonazole for isolates of *S. homoeocarpa*. In the field efficacy experiments, Ningnanmycin 10% WP at 0.29 g/m^2 applied on a 7-day interval provided the highest control followed by Ningnanmycin 10% WP at 0.15 g/m^2 (7-day interval) and Trinity 1.69S at 0.32 ml/m^2 (14-day interval) for both dollar spot and anthracnose. The results of *in vitro* sensitivity assays and field efficacy experiments demonstrate that ningnanmycin has activities against two major turfgrass pathogens and can provide similar levels of control compared to traditional fungicides.

Screening and application of bacterial isolates as biocontrol agent against powdery mildew on cucumber

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Phytopathology 101:S111

Powdery mildew is an important disease on cucumber and causes a great damage in China. The control of this disease primarily depends on the application of chemical pesticides. However, it becomes a popular and important problem that many chemicals show the potential toxic effect on foods, pathogen resistance and environment. So, it is a hot research point nowadays to find and use natural product to control this disease. Microbial fungicides show such a more potential control efficacy on plant diseases in greenhouse that the studies thereof become more popular in China. In this study, 125 bacterial isolates were tested for their ability to control this disease by pot experiment and the results showed that 12 bacterial isolates significantly decreased this disease. In field plot experiment, 10 bacterium isolates of them showed a significant controlefficacy. Among them, the bacterial isolate CAB-1, NZT-14-84 and BDT-25 expressed better control efficacy with 79.0%, 67.5% and 57.4% respectively. The isolate CAB-1 was identified as *Bacillus subtilis*. A preparation, 5×10^8 cfu/ml spores AS, was made with spores of *B. subtilis* CAB-1 and applied in greenhouse. Naturally infested, the cucumber plants were sprayed after transplanting every 7-day interval with 50-fold diluted solution of this preparation. Results showed that the treatment could significantly reduce the diseases with a control efficacy 70.5%. This study will provide a new and environment-friendly fungicide to control this disease in China.

Effects of cultural practices, *Meloidogyne incognita*, and *Thielaviopsis basicola* on cotton root morphology in the field

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Phytopathology 101:S111

A potential production constraint in many Arkansas cotton fields is a plow or hardpan resulting from one or more of several factors may exist. Water infiltration and aeration may be restricted and soil penetration resistance increased impeding root penetration and exploration, and lead to suppressed plant growth and productivity. Two soilborne pathogens *Meloidogyne incognita*) and *Thielaviopsis basicola* are also common in cotton production fields in the state. Damage to roots resulting from these pathogens may be amplified where a hardpan is also present. The objective of this study was to evaluate the topological changes in root systems resulting from these two pathogens in the presence of a compaction layer. Field studies were conducted in a cotton production field in northeastern Arkansas in 2009 and 2010. Field-length strips were subsoiled to a depth of 12–15 inches before planting, and strips of equal size adjacent to each subsoiled strip was reserved. Telone II was applied to half of each strip prior to planting each year. Excavated root systems were analyzed from each sub-plot in June and October using WinRhizo software. Nematicide application reduced galling by *M. incognita* but increased root magnitude, altitude, exterior pathlength and total surface area, root volume and length. Subsoiling generally increased the same

parameters numerically with less effects. Effects of both subsoiling and nematicide application were more evident early in the growing season.

Evaluation of organic sulfide fumigants for suppression of vegetable soilborne replanting diseases in greenhouse

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Phytopathology 101:S112

Soilborne replanting diseases are seriously limiting greenhouse vegetable production in China. Authors intended to use organic sulfide fumigants to manage the diseases. Monitor on microbial populations in field soil fumigated for 6 days showed that soil fungal density was greatly reduced by dazomet(DZM), methylene-(bis)thiocyanate(MBTC), diallyldisulfide(DADS), allylisothiocyanate(AITC), sodium tetrathiocyanate(STTC) and dimethyldisulfide(DMDS) at 30 g, 60 ml, 35 ml, 35 ml, 60 ml and 35 ml/m^2, respectively, as determined by PDA soil dilution plate method. Sequence of toxicity of the tested fumigants to soil bacteria was AITC ≥ MBTC = DZM = DADS ≥ DMDS = STTC as by NA soil dilution plate. All the tested fumigants were highly suppressive to nematodes. Among them AITC was the most suppressive one with LC_{90}<0.5 µg/ml to J_2 of *Meloidogyne incognita*. AITC and DZM also greatly reduced weed density by 73.6–94.7% in field at 35 ml and 30 g/m^2, respectively. Trials demonstrated that fumigation before seedling transplantation significantly decreased kidney bean *Fusarium* root rot and root knot diseases with control efficacy sequence AITC = MBTC = DZM ≥ DADS = DMDS ≥ STTC. Safety tests showed no injury occurred on cucumber, tomato, mask melon and cowpea when STTC, MBTC, DADS, TITC and K-Vapam were applied immediately before transplanting at less than 120, 60, 15, 15 and 15 ml/transplanting hole, respectively. These data are instructive in formulating operation procedures of the fumigants to be used in vegetable soil treatment.

Powdery mildew biological control agents exhibit endophytic characteristics

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Phytopathology 101:S112

Two bacteria (B17A and B17B) and two fungi (F13 and F16), have been observed to exhibit endophytic characteristics by invading plant tissues. Fungal hyphae of F13 invaded the intercellular spaces of parenchyma cells, with some forming vesicular and arbuscular growths in plant cells. Cells of B17B were observed under a compound microscope in the parenchyma cells, and occasionally in the vascular vessels of stained roots of young dogwood seedlings, grown in the shade house or greenhouse environments. Both B17A and F16 invaded plant cells as well, but not as frequently as either B17B or F13. These microorganisms were previously isolated from dogwoods growing in forests where no fungicides have been used. They have also shown great potential as biological control (biocontrol) agents (BCAs) in the control of powdery mildew of flowering dogwood (*Cornus florida*), in greenhouse and shade house environments. Application of these BCAs individually by foliar spraying, root inoculations and root drenching have proven effective in the control of powdery mildew of dogwood, during greenhouse and shadehouse experiments. In most trials, BCAs were not significantly different from a conventional fungicide thiophenate methyl (Cleary's 3336®), commonly used to control the disease in dogwood.

A network of field trials to test the susceptibility of rice mega-varieties to sheath blight

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Phytopathology 101:S112

Sheath blight (ShB), caused by *Rhizoctonia solani*, is a major rice yield-reducer especially in highly intensive production systems. To date, no source of complete resistance has been identified, and no rice varieties resistant to the disease have been deployed in Asia. Mega-varieties (MVs), i.e. rice varieties grown over large acreages were tested to their susceptibility to ShB. A network of field experiments was established at 5 sites for 2 successive rainy seasons from 2009 to 2010 using a common framework (inoculated line sources; split-plot design with 3 replications; disease measurements at 5 distances from the sources; four common MVs at all sites as controls; 3 observations at 10, 25, and 40 days after inoculation). Significant MV effects were consistently found, indicating that MVs differ in their susceptibility to ShB. Aside from the very strong, significant distance-to-source (D) effect, a significantly D x MV was found, indicating that disease gradients, i.e., the ability of the disease to spread, differ among MVs.

Evaluation of differentiation between *Magnaporthe grisea* and *M. oryzae* by using of specific primers

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Phytopathology 101:S112

Magnaporthe grisea (Hebert) Barr is the causal agent of rice blast and gray leaf spot of grasses. Rice blast causes economically significant crop losses annually. It is now known that *M. grisea* consists of a cryptic species complex containing at least two biological species that have clear genetic differences. Complex members isolated from the grass genus *Digitaria nomenclaturally* tied to *M. grisea*. The remaining members of the complex isolated from *Oryzae sativa* and other cultivated grasses have been renamed *M. oryzae*. Two *forma speciales* names have been applied to the anamorph of *M. grisea*. *Pyricularia grisea* was described from *Digitaria sanguinalis*, and *P. oryzae* Cavara was described from *O. sativa*. *P. oryzae* was distinguished from *P. grisea* based on its sparse, usually nonseptate hyphae and larger, biseptate conidia. The usage of the names *P. grisea* and *P. oryzae* has generally reflected the host from which the fungus was isolated rather than any morphological differences. In this study in order to evaluation of the differentiation between these species, 26 isolates of M. oryzae and 14 isolates of M. grisea collected from north rice fields of Iran. DNA were extracted and reproduced by using mif23 specific primers. PCR primers were derived from the sequence of the mif23 infection–specific gene. All isolates made a 390 bp band. So, with these specific primers no genetic difference was observed between these isolates.

Grower implementation of LAMP PCR to initiate grape powdery mildew fungicide program based on inoculum detection

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Phytopathology 101:S112

Inoculum detection for timing fungicide applications against grape powdery mildew is effective using qualitative and quantitative PCR (qPCR) approaches. However, implementation by viticulturist was impeded by the cost equipment and specialized skill required. Loop mediated isothermic PCR (LAMP) is a robust method for the detection of DNA that can be performed with minimal equipment and skill. LAMP primers were designed against the ITS2 ribosomal DNA region of *Erysiphe necator* that are specific and can detect less than one spore or less than 5 copies of target DNA in a purified plasmid. Continuously running an impaction spore traps where sampled ever 3 or 4 days. DNA extraction was accomplished by placing rods in 100 µl of TE buffer, centrifuging, boiling, vortexing and then placing 5 µl DNA extract in PCR tube with mastermix. The PCR tube was then placed at 65°C for 45 min followed by 80°C for 5 min. Positive detection was determined by the formation of white precipitate. Grower implementation was tested by placing 3 traps at each vineyard with one processed by the grower using LAMP and the others processed in the lab for LAMP and qPCR. Participating growers were more than 55% accurate in detecting 1 or 10 spores and 100% accurate in detecting 100 spores in spiked samples. They had 74% agreement in detecting *E. necator* compared to our LAMP-PCR results, and our LAMP-PCR results were 96% in agreement with our qPCR results.

Pathogen Transport and Response-tool for Agricultural Canopies (P-TRAC) - A modeling system to guide disease management decisions in perennial canopies

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Phytopathology 101:S112

We are building a system to predict the spread of a pathogen from internal and external inoculum sources at region to sub-block scales. The system will aid in targeting disease monitoring and mitigation efforts to areas within vineyards that have a high probability for deposition and disease development.

Two years of particle dispersion data indicate that traditional dispersion modeling approaches do not adequately describe dispersion in vineyards. The Large-Eddy Simulation technique was used to model the airflow and spore dispersion and deposition in vineyards. The simulations provided high-resolution time resolved 3D distributions of momentum, heat, moisture, and spore concentration that will increase our understanding of the relationship between canopy architecture, weather and disease development. The models indicated that as spores move from one row to the next, most are deflected vertically with relatively fewer deposited in or passing through the canopy. They also imply that as canopy density decreases (i.e. lower leaf area per vine and/or increased row spacing) the distance that spores travel increases but fewer spores escape the canopy. This leads to a decreased likelihood of long distance dispersion. Thus, a denser canopy would increase the focal nature of epidemics within a field but increase the potential to infect adjacent fields. In contrast, a sparser canopy will lead to an increased spread within a field but a decreased likelihood of transmission to adjacent fields.

Innate response in tissue cultured *Anthurium andraeanum* against *Radopholus similis*

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Phytopathology 101:S113

Radopholus similis causes Anthurium decline, a loss of vigor with smaller and fewer flowers. A disease complex occurs in Anthuriums where the plants are infected with *R. similis* and *Xanthomonas axonopodis* pv. *dieffenbachiae (Xad)* the causal agent of bacterial blight. Tissue cultured Anthuriums inoculated with nematodes did not experience significant weight loss. In a factorial design experiment, anthurium Marian Seefurth with an average weight of 0.72 g was inoculated at the roots with 10 nematodes and 1 ml *Xad* at 10^7 CFU/ml. Plants were weighed at 1, 3, 7, 10 days and weekly for 3 weeks and monthly for 4 month. After 120 days post inoculation, anthurium roots were cut into 1 cm pieces and *R. similis* were extracted in water. Roots inoculated with *Xad* were macerated and subjected to a dilution series. Plant weight with nematode inoculation peaked at 7 days and weighed 0.6 g at 120 pdi whereas plant weight for *Xad* inoculation peaked at 3 days with a final weight of 0.52 g. These peaks in weight could be associated with PAMPs. In the nematode only treatment, *pf/pi* for *R. similis* was 0.42 compared to nematode and *Xad* co-inoculation which was 9.2. The CFU in plant roots inoculated with *Xad* ranged from 1.0×10^6 to 3.6×10^7. Lipopolysaccharide (LPS) in *Xad* and glycosyl hydrolase from the nematode could have acted as PAMPs and triggered a response in plants to cause a peak of rapid weight gain. Further testing is needed to confirm Pattern Recognition Receptors (PRRs) in Anthuriums.

A tomato model system to study Citrus huanglongbing

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Phytopathology 101:S113

Citrus greening disease (huanglongbing, HLB) has caused severe damage to citrus industries world-wide. Low titer of the HLB associated bacteria *Candidatus* Liberibacter asiaticus (LAS) in plants, prolonged latency, slow disease progression and seasonality of infection in citrus as well as lower incidence of Las in vectors makes the citrus/HLB a challenging system to conduct research and to develop better disease management strategies. A closely related bacterium, *Candidatus* Liberibacter psyllaurous (LPS), causes "psyllid yellows" symptoms in tomatoes and several other annual crops. The disease was graft and insect transmitted to healthy tomatoes easily with symptoms showing within 2–3 weeks. Symptom expression in tomatoes was shown to be temperature dependent. Symptoms expressed well at about 25°C, and the bacterial titer decreased transiently when the plants were maintained at 36°/30°C (16h/8h) for a week. Host range studies using several members of Solanaceae with both graft and insect transmission of LPS revealed both resistant and susceptible plants. The suitability of the tomato/psyllid yellows model system to screen a large number of chemicals, antibiotics and transgenes for developing strategies for potential control of citrus HLB will be discussed. A BAC library constructed from infected tomato psyllids was used to generate genomic sequence information on LPS using metagenomic approach.

The CRT1 family participates in four distinct layers of immunity against a wide range of pathogens in Arabidopsis

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In Arabidopsis, the R protein HRT of the Di-17 ecotype indirectly recognizes the coat protein of Turnip Crinkle Virus (TCV) and triggers *R* gene-mediated resistance. CRT1, an endosomal-localized ATPase, was identified in a genetic screen of mutants Compromised for Recognition of Turnip Crinkle Virus. The *CRT1* gene family was shown to be required for *R* gene-mediated resistance against bacteria and oomycete pathogens as well as for resistance-associated cell death (Kang et al., 2008 and 2010). In addition to its involvement during several *R* gene-mediated interactions, we have assessed the role(s) of CRT1 family during other plant immune responses using an Arabidopsis double knock out (dKO) mutant *crt1-2 crh1-1*, which lacks CRT1 and its closest homolog. Basal resistance against virulent *Pseudomonas syringae* and TCV was reduced in this dKO. Callose deposition after inoculation with *P. syringae hrcC* mutant strain was also compromised. Moreover, CRT1 was found to interact with the PAMP recognition receptors FLS2 and EFR, as well as with their associated kinases BAK1 and BIK1. Interestingly, the dKO mutant was also defective in development of systemic acquired resistance. Furthermore, our results indicate a role for CRT1 in a set of pre-invasion defenses activated during the non-host interaction between Arabidopsis and *Phytophthora infestans*. Together, these findings argue that CRT1 is a critical component of four distinct layers of defenses and participates in defenses against a broad spectrum of pathogens.

***Fusarium virguliforme* genes and pathways involved in the development of sudden death syndrome in soybean**

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Phytopathology 101:S113

Fusarium virguliforme is a soil-borne pathogen that causes sudden death syndrome (SDS) in soybean. Despite the importance of the disease, little is known about the fungal genes involved in the infection process and their expression profiles in response to plant defense mechanisms. Greenhouse assays were conducted to identify *F. virguliforme* genes expressed in planta under conditions conducive to the development of SDS. Total RNA was extracted from soybean roots challenged with *F. virguliforme* 15 days after planting. Sequencing-based transcript analysis using Illumina technology was used to identify and characterize fungal transcripts expressed in the infected soybean roots. The acquired sequences cover 20% of the publicly available genomic sequence of *F. virguliforme*. Data analysis and annotation of RNA species was performed. Subsequently, annotated fungal genes were classified into different groups based on their molecular function. The expression patterns of a subset of the identified genes were confirmed by RT-PCR. This is the first report of using next=generation sequencing to identify and characterize *F. virguliforme* genes and pathways involved in the development of SDS in soybean. These results will be used to identify new target genes to disrupt in *F. virguliforme* and study their role in SDS development.

Integration of sunn hemp cover cropping and soil solarization for reniform nematode, *Rotylenchulus reniformis*, management

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Phytopathology 101:S113

Sunn hemp (SH), *Crotalaria juncea*, has been known to suppress reniform nematode, *Rotylenchulus reniformis*, while enhancing free-living nematodes involved in soil nutrient cycling. Two field trials were conducted in winter 2009 (Trial I) and summer 2010 (Trial II) in Hawaii to examine if SH cover cropping could suppress reniform nematode more efficiently if integrated with solarization. Cover cropping of SH, soil solarization (SOL), SH followed by SOL (SHSOL) were compared to weedy fallow (F) for 3 months prior to planting a reniform nematode susceptible host, *Vigna unguiculata*. Although SH and SOL consistently showing a trend of reducing abundances of reniform nematode, both treatments did not suppress the nematode significantly. Reniform nematodes were suppressed ($P < 0.05$) by SHSOL in Trial I, but not in Trial II. Thus, SOL only occasionally improves reniform nematodes suppression by SH. Differences in SH biomass among treatments and trials might have caused this difference. SH biomass was higher in SHSOL than that of SH in Trial I, but a reverse was true for Trial II. Another possibility of different performance of SHSOL was that summer SOL in Trial II generated much higher heat than that of Trial I and might have reduced the allelopathic effect of SH. Thus, integration of SH and SOL does not always suppress reniform nematodes better than each treatment alone, but could suppress the nematode better when the SH biomass is high and when SOL is not too hot.

Managing pest risk of plants for planting in international trade: U.S. import regulations at a crossroad

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The Animal and Plant Health Inspection Service (APHIS) has proposed a comprehensive revision of U.S. import regulations for plants for planting, contained in the Code of Federal Regulations, Title 7, Part 319, Subpart 37 (7 CFR 319.37). Implementation of the regulatory revisions has been initiated with three major rules, which are in different stages of development. First, a new category of regulated plants will be established, whose importation is not authorized pending pest risk analysis (NAPPRA). Pest plants and plant hosts of quarantine pests meeting criteria established by APHIS will be included in the NAPPRA category. The second rule will establish a system of controlled import permits, which will revise the Departmental Permit system to reflect current practices and appropriate pest risk management. The third rule will establish a framework for the structural reorganization and consolidation of regulations affecting imported plants for planting, and integrated measures approaches for pest risk management. These measures are being developed to reduce pest risk while minimizing adverse economic impacts on international trade in plants for planting. The U.S. is a net importer of live plants, with increasing trends in trade from all world regions.

The potency of fungal antagonists to combat root rot in industrial *Acacia mangium* plantation

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Phytopathology 101:S114

Root rot disease had caused plant death in *Acacia mangium* with increasing number of dead plants over time and rotation. The pathogen remains in the field within the infected plant roots and stumps, as well as rotting logs of the infected tumbled trees. Three types of different root rot pathogens had been identified at some root rot spots in South Sumatera Indonesia, and the antagonistic agents isolated from the area were tested separately against the three root rot pathogens using double culture method. From the 45 samples collected from the root rot damaged area, mostly in form of the pathogen fruit bodies, with few wood-skin and root cuts, 18 fungal isolates were gained, and 14 of them had antagonistic potency of 90–100% at day three towards the three most commonly found root rot pathogens, which were identified as: *Ganoderma lucidum*, *Ganoderma australe*, and *Rigidoporus microporus*. The antagonistic fungi were mainly identified as *Trichoderma* spp. and *Gliocladium* spp. Further tests showed that representatives of the *Trichoderma* spp. and *Gliocladium* spp. isolates were able to grow and sporulate on the *Acacia mangium* leaf litters to yield spores with similarly high antagonism potency to the different root rot pathogen types tested, whether applied singly or as a mixture of the two types. Therefore there is good possibility for the antagonistic fungi to be able to combat root rot in large industrial plantation such as in *Acacia mangium*.

Seasonal distribution of SI fungicide resistance in apple scab populations in Virginia

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Phytopathology 101:S114

Apple scab, caused by *Venturia inaequalis*, is an economically devastating disease that occurs wherever apples are grown. Sterol inhibitor (SI) fungicides have been dominant systemic fungicides used to manage scab. Unfortunately, *V. inaequalis* is developing resistance to the SIs. We evaluated fungicide resistance in 266 single-spored *V. inaequalis* isolates collected in Winchester, VA from 2006 to 2010. Within a given season, the mean colony growth was significantly different ($P < 0.001$) among assay treatments (0, 0.1, 0.5 or 1.0 ppm myclobutanil) and assay times (7, 14, 21 or 28 days). Sampling interval was significant ($P < 0.001$) in 2007 and 2008, and pairwise comparisons suggested variations between early and late season. When analyzed concurrently, all factors were significant ($P < 0.001$) including collection year. Percent growth suppression (PGS) – the difference in colony growth on 0 and 1 ppm myclobutanil at 28 days – was used to assess fungicide resistance. Generally, a range of resistance was seen at each sampling interval, and the average PGS was similar for treated and non-treated trees of the same cultivar. The average PGS in the July (30%) sampling interval was lower (i.e. more resistant) than in the May (60%), June (60%) or August (50%) sampling intervals. Of the total 266 isolates evaluated, approximately 8% were classified as resistant, 29% as moderately resistant and 63% as sensitive. This 5-year study provides useful information about the seasonal distribution of SI fungicide resistance in VA's apple scab population.

The USDA-APHIS quarantine programs for sugarcane, grasses, rice & bamboo

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Phytopathology 101:S114

Since its transfer to Plant Protection and Quarantine (PPQ) of the USDA-APHIS in October, 2005, the Plant Germplasm Quarantine Program (PGQP) has received an increasing number of introductions and species belonging to the Poaceae family, which are imported primarily as vegetative propagative materials and occasionally as seed. These importations include sugarcane, turf and forage grasses, *Miscanthus*, bamboo and rice. Since October 2005, PGQP has entered more than 325 sugarcane clones into quarantine, 51 of which were clones that re-entered the program after coming out of our virus elimination program. In addition, PGQP has established approximately 57 bamboo accessions, 304 *Miscanthus* and 24 *Arundo donax* introductions for biofuel evaluations, 153 turf and forage grasses, and 726 viable rice accessions. Each clonal introduction is subjected to an array of tests which include: bioassays; leaf dip assay via electron microscopy; isolation and culture; and serological and PCR-based tests to detect a specific pathogen or the pathogen group that includes grass pathogens. Germplasm that consistently tests negative for pathogens of quarantine interest and appears healthy during growth is released to the original requestor. Infected germplasm may be subjected to virus elimination via apical meristem culture. Pathogens detected from imported Poaceae germplasm will be presented.

A functional 3-hydroxy-2-butanone pathway is required for virulence in *Pectobacterium carotovorum* subsp. *carotovorum*

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Phytopathology 101:S114

The *Pectobacterium* are ubiquitous soft rot pathogens (SRP) responsible for wilting, necrosis and massive maceration symptoms in potato, vegetables and ornamental plants. Like other Enterobacteriaceae plant pathogens, such as *Dickeya*, *Enterobacter* and *Erwinia*, *Pectobacterium* species encode genes involved in the production of acetoin (3-hydroxy-2-butanone, 2H3B). 2H3B is a volatile metabolite produced via the Embden-Meyerhof pathway. Previously we found that an operon encoding 2H3B pathway enzymes, *budAB*, was highly expressed during *P. carotovorum* subsp. *carotovorum* (Pcc)-potato stem and tuber interactions. We found that a Pcc mutant with a deletion of *budB*, which encodes alpha-acetolactate synthase, is significantly reduced in its ability to macerate potato tubers compared to the wild-type strain. The mutant was not impaired in bacterial growth in tuber tissue during infection, which suggests that this pathway is not required for nutrient acquisition. Additionally, this mutant also inhibits alkalinization of growth medium and tuber tissue under anaerobic conditions. An increase of pH in the plant apoplast favors the activity of bacterial pectate lyases, which are involved in plant cell wall degradation. Thus, this data suggests that the acetoin pathway in SRP may contribute to pathogenesis through pH modulation. Finally, although 2H3B has been reported to promote plant growth, we were unable to demonstrate any effect of these compounds on potato plantlets grown in tissue culture.

Assembly of the draft genome of *Xanthomonas axonopodis* pv. *dieffenbachiae* strain V108LRUH1, a bioluminescent strain highly virulent on anthurium

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A serious limitation to the large scale production of anthurium is bacterial blight caused by *Xanthomonas axonopodis* pv. *dieffenbachiae* (*Xad*). Although some cultivars of anthurium have exhibited tolerance to the disease, high levels of resistance or immunity are not commonly observed in *Anthurium andreanum* cultivars. To gain insight into the underlying plant-microbe interaction, we have used Roche 454 sequencing technology to sequence the genome of *Xad* strain V108LRUH1, a well-characterized bioluminescent (Lux^+) strain. This sequencing process has generated 582,110 reads. Assembly of the *de novo* sequencing reads has yielded 215 contigs with intervening gaps being closed by Sanger sequencing. Preliminary analysis indicates that the genome of *Xad* is greater than 5Mbp in size with a GC content of approximately 65%, similar to other members of the *Xanthomonas* genus. Genes associated with virulence (i.e. effectors, secretion system, etc.) will be compared to those of other *Xanthomonas* strains. This is the first *X. axonopodis* pv. *dieffenbachiae* strain to be sequenced and will represent the first sequenced genetically modified xanthomonad. The genomic content of *Xad* and further phylogenomic comparisons of this strain to other

Xanthomonas genomes will provide a better understanding of bacterial evolution and plant-microbe interactions.

A new broad-spectrum fungicide for use on lentil, field pea, and chickpea crops

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Phytopathology 101:S115

BAS 703 01 F is a new broad-spectrum fungicide under development in Canada by BASF Canada for broad-spectrum control of key fungal diseases of lentils, field peas, and chickpeas. BAS 703 01 F is a premix fungicide containing two active ingredients, fluxapyroxad and pyraclostrobin in a 1:1 ratio. Fluxapyroxad is a new fungicide developed by BASF which inhibits respiration of fungi by blocking production of succinate dehydrogenase and will be classified in FRAC group 7. Field research trials have indicated BAS 703 01 F is highly effective at controlling diseases such as Ascochyta blight (*Ascochyta lentis*) and anthracnose (*Colletotrichum truncatum*) of lentil; Mycosphaerella blight (*Mycosphaerella pinodes*) of field pea; and Ascochyta blight (*Ascochyta rabiei*) of chickpea in the rate range of 150 – 200 g ai/ha. Field trial results from 2009 and 2010 and proposed directions for use will be presented. BAS 703 01 F has been submitted to the PMRA for registration.

Blueberry necrotic ring blotch, a new blueberry disease caused by a virus

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Phytopathology 101:S115

Novel symptoms have been observed on southern highbush blueberries (*Vaccinium corymbosum* interspecific hybrids) in several southeastern states. Affected plants show irregularly shaped circular spots or blotches with green centers on the top and bottoms of leaves. Diagnostic tests failed to isolate any fungal or bacterial pathogens typically associated with such symptoms. Double-stranded RNA (dsRNA) was extracted from symptomatic leaves suggesting the presence of virus(es) possibly involved in the disease. Three of five dsRNA segments observed on gels have been sequenced and used to develop diagnostic primers for detection by RT-PCR. More than 50 individual plants that exhibited necrotic ring blotch symptoms in North Carolina, Georgia and Florida were positive in the RT-PCR assay that was developed. The perfect correlation between the virus and symptoms in plants from across several states suggests that the virus this, for which we propose the name *Blueberry necrotic ring blotch virus* (BNRBV), is indeed the causal agent of the disease. Sequence analysis showed that BNRBV has conserved replicase and movement domains characteristic of other ssRNA viruses. Because no coat protein conserved domains have been identified, high throughput sequencing is being used on dsRNA preparations to determine if one of the other two dsRNA bands may code for a coat protein, or if additional virus(es) may be involved in the disease. BNRBV is related most closely to *Citrus leprosis virus*.

Identification of an emergent bacterial blight of garlic in Brazil

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Phytopathology 101:S115

Outbreaks of a bacterial blight disease occurred on garlic (*Allium sativum*) cultivars Roxo Caxiense, Quiteria and Cacador in Southern Brazil, and threatened the main production regions of Rio Grande do Sul State. Symptoms were characterized by watersoaked reddish streaks along the leaf midrib, followed by yellowing of leaves, a rot of bulbs and plant death. The disease can negatively impact seed production in infested fields. Epidemics occurred mainly during bulb formation and the preharvest period. Bacteria, fluorescent on King's B medium, were isolated from leaf tissue. Physiological tests indicated that the bacteria were *Pseudomonas marginalis*. Pathogenicity tests were performed on plantlets and detached cloves. Symptoms similar to those in the field were reproduced on the leaves. Additionally the bacteria incited a rot of cloves. Reisolates had physiological properties identical to the inoculated strains, demonstrating Koch's postulates. DNA fragment pattern analysis using DNA amplified with the BOXA1R primer, demonstrated that two different pathogens were responsible for the disease. The DNA fragment patterns were different than the type strain of *P. marginalis* and the pathotype strains *P. marginalis* pv. *pastinaceae*, *P. marginalis* pv. *alfafae*. Although the strains appear to be similar to *P. marginalis* according to physiological tests, further research is needed to determine if the bacteria represent a novel pathovar or species.

Colonization of spinach (Spinacia oleracea L.) by GFP-tagged Verticillium dahliae

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Phytopathology 101:S115

The soilborne fungus, Verticillium dahliae, causes wilt in a wide range of hosts, including spinach (Spinacia oleracea L.). The interaction between a green fluorescent protein (GFP)-tagged V. dahliae strain and spinach was studied by confocal laser scanning microscopy. The roots of spinach seedlings were inoculated with a conidial suspension of a GFP-tagged strain and pathogen colonization events were followed through seed production. At 24 to 96 hours post-inoculation (PI), conidia germinated and formed hyphal colonies on root tips and in the root elongation zones. Two weeks PI, hyphae of V. dahliae grew both intracellularly and intercellularly in the cortical tissues and penetrated into the xylem. At six to eight weeks PI, the fungus colonized the entire taproot xylem with abundant mycelia and conidia. Further colonization of the taproot and crown of inoculated plants led to vascular discoloration when foliar symptoms became apparent. At 10 weeks PI, xylem tissues of the upper stem were colonized that also extended to the inflorescence and the various spinach seed parts, including fruit wall, epicotyl meristem and integument. However, the fungus did not colonize the perisperm (the diploid maternal tissue) in the seed. This information is useful in administering effective seed treatments without compromising seed viability and ultimately the introduction of this very destructive pathogen via seed.

Egg parasitoids of *Chrysocoris javanus* Westw. (Hemiptera: Scutelleridae) on *Jatropha curcas* L. in Bogor, West, Java, Indonesia

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Phytopathology 101:S115

Physic nut (*Jatropha curcas*) is one of biofuel plants which is planned to be cultifated on large scale areas in Indonesia. *Chrysocoris javanus* is an important sucking pest attacking physic nut. To control this pest, various pest control measures have to be studies and implemented. The objectives of this research were to find out egg parasitoids which are potential as biological control agents and their parasitization level at three physic nut plantations in Bogor, West Java, Indonesia. Egg parasitoids found during the study were *Anastatus* sp. (Eupelmidae), Pteromalidae, and Scelionidae (Hymenoptera). Parasitized eggs of *C. javanus* were black in color, whereas the unparasitized eggs were orange. The pteromalid wasp was the dominant parasitoid found in two plantation. Parasitization level of three parasitoids ranged from 60.1% to 97.0%. Almost all of *C. javanus* egg clusters were parasitized (88.7% - 100%).

Relative efficacy of chemical management tools on Phytophthora crown and root rot of pepper plants

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Phytopathology 101:S115

The oomycete pathogen *Phytophthora capsici* can cause extensive losses in pepper plantings. Fungicides are an important component of this Phytophthora disease management system. Several products have recently been registered and some additional new chemistries are being developed with activity against oomycete pathogens. Two greenhouse trials were conducted in 2010 to evaluate these individual products for their ability to suppress development of crown and root rot on pepper plants in the presence of *P. capsici*. Within a series of 500 ml capacity plastic pots, a 2-month-old chile pepper transplant was placed into either a peat-based potting mix (first trial) or a silty clay loam field soil (second trial), both infested with vermiculite containing *P. capsici*. The potting mix or soil in each of the 10 replicate pots per treatment was drenched with one of the test products at the initiation of each experiment and again 14, 28 and 42 days later. Plants were watered daily for the 2-month duration of each trial. No untreated plants were alive at the conclusion of the trials. In contrast, the mean survival rate for treated chile pepper plants in both experiments was 85% for Ridomil Gold (mefenoxam), 80% for Revus (mandipropamid), 65% for Forum (dimethomorph), 60% for Omega (fluazinam) or V-10208, 55% for Presidio (fluopicolide), 45% for Zampro (ametoctradin + dimethomorph), and 25% for Ranman (cyazofamid).

Reevaluation of *Phomopsis* species affecting sunflowers in the United States

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Phytopathology 101:S115

Phomopsis Stem Canker (PSC) is a serious sunflower disease in Europe, but has remained at a low incidence in the United States until recently. In 2010, PSC affected 8.7% of the U.S. crop, and yield reductions occurred in isolated fields. *Phomopsis helianthi* was thought to be the sole incitant of PSC in Europe, but some researchers hypothesized more species were involved. PSC infected sunflower stalks were collected from infected fields in Minnesota (MN), North Dakota (ND) and South Dakota (SD) in 2010. Identification of Phomopsis isolates using morphology and molecular analysis of the internal transcribed spacer region (ITS 1, 5.8S ribosomal RNA gene, and ITS 2) revealed four *Phomopsis/Diaporthe* species – namely *Diaporthe helianthi*, *Diaporthe stewartii*, *Phomopsis longicolla* and an unknown *Diaporthe* sp. Our research indicates that multiple species are associated with PSC of sunflowers in United States.

Screening of the World *Phytophthora* Collection for viruses

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Phytopathology 101:S116

The presence of viruses in fungi and oomycetes, including a few *Phytophthora* spp. is well established. These viruses can affect host phenotypes including pathogenicity, which has led to their use as biocontrol agents. The World *Phytophthora* Collection (WPC) is maintained on the UC Riverside campus in the Department of Plant Pathology and Microbiology and includes over 10,000 accessions from multiple species and geographic locations. In order to assess the incidence and type of mycoviruses present in this collection, 200 accessions were selected for screening by double-stranded (ds) RNA analysis which can detect the presence of both double-stranded and single-stranded RNA viruses. DsRNAs have been detected in over a dozen isolates with several unique patterns, some indicating the possible presence of mixed viral infections. A large dsRNA segment (approx. 13KB) was found in five isolates and is believed to be an endornavirus that has been previously associated with *Phytophthora* spp. RT-PCR of the dsRNAs is being conducted to confirm this association. Several of the dsRNA positive cultures grow very slowly and the relationship of this phenotype to the presence of a mycovirus is being investigated. Virus particle isolations are underway as is continued screening of the WPC for dsRNAs. This wide ranging survey of *Phytophthora* isolates could result in the eventual development of a viral biocontrol agent for important plant diseases.

Understanding cellular and molecular interactions between the rice blast fungus and a putative biocontrol bacterium

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Lysobacter enzymogenes is a gram negative, soil-dwelling bacterium that interacts with many microbes and lower eukaryotes. This bacterium also produces lytic enzymes and antibiotics, and by virtue of its antagonism, has great potential as a biological control agent. The details of *L. enzymogenes*' interactions with other organisms are not well-understood. In order to better understand them, we used a model plant pathogenic fungus, *Magnaporthe oryzae*. Our goal was to investigate this interaction using molecular and cellular tools. We generated a fluorescent dsRed-C3 (wildtype strain) of *L. enzymogenes* and a Zs-green strain of *M. oryzae*. Live cell imaging using confocal microscopy was used to examine the fungal-bacterial interactions. *M. oryzae* strain 70-15 was grown on oatmeal medium for 10 days. Six ml of L. enzymogenes (1 × 106 cfu/ml) in phosphate-buffer saline solution was added to the plate. The bacterial-fungal interaction was imaged for 24 hours. Our findings reveal that the wild type bacterium readily and quickly attaches to fungal hyphae and spores, and between 3–4 hours, many more attachments are seen. At approximately 12 hours, bacteria cover 70–80% of the fungal material. No internalization was observed of bacteria into live fungal cells. mRNA-seq of fungal gene expression during early and late stages of the interaction is currently underway and will be discussed.

Detection and population dynamic analysis of biological control agent *Pseudomonas fluorescens* LRB3W1 in tomato plants from the 'Live coating seed'

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The 'Live coating seed' is the newly developed pelletized seeds, in which useful microorganisms are coated alive using a combination of decompression and dehumidification technology. This technique improves the efficacy of biological control agents (BCAs) for plant diseases. In the present study, detection and periodical movement of a BCA, *Pseudomonas fluorescens* LRB3W1 (3W1), in the tomato plants from the live coating seed, were investigated using serological and molecular biological methods. The antiserum against 3W1 used in this study was raised in a rabbit by immunization with heat treated antigen. The characteristic of the serum was tested by indirect ELISA. As a result, the titer value and specificity of anti-3W1 serum were suitable for bacterial detection. Furthermore, the immunofluorescence method using FITC conjugate was also admitted to be usefully applicable, in particular for qualitative movement analysis on specific sites of the plant. The specific PCR primer targeted 3W1's *gyrB* gene sequence was designed and tested for the specificity and detection limit. The bacterial populations in plant parts were examined by these methods. The results obtained from different methods were strongly correlated, suggesting the applicability of these methods for population dynamic analysis of BCA on plant from 'Live coating seed'.

Cultural control of maize wallaby-ear symptom: Damage avoidance by earlier planting of forage maize

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Recent global warming has brought many pests and diseases from tropical to temperate region. Maize wallaby ear symptom (MWES), which had occurred only in tropical areas, newly occurred in temperate Asian countries such as China and Japan after 1980s. In Southern part of Japan, this symptom now becomes one of the most serious problems in forage maize production. Here, we report a cultural control method to MWES in forage maize. This symptom is caused by feeding of the maize orange leafhopper (*Cicadulina bipunctata*), and degrees of MWES occurrence is depending on density of the leafhopper and plant growth stage (leaf stage): MWES occurs seriously on younger maize (less than 5 leaf stage) attacked by more *C. bipunctata*. These facts suggest that planting of forage maize in earlier season before *C. bipunctata* density increases in field is effective to avoid MWES occurrence. We verified this hypothesis by field experiments. In our census field, density of *C. bipunctata* remains low from spring to early July, then, it rapidly increases in late July and reaches maximum in September or October. No or less MWES was observed when forage maize was planted before late July, however, MWES occurred on maize planted after early August, and the degrees of MWES became serious depending on delay of planting date. These results indicated that earlier planting of forage maize can avoid MWES occurrence in Southern part of Japan.

A new disease of parsley (*Petroselinum crispum*) in California caused by a fluorescent pseudomonad related to *Pseudomonas viridiflava*

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In 2008 fluorescent bacteria were isolated from bacterial leaf spot symptoms on Italian parsley (*Petroselinum crispum*) in Ceres, California. These isolates were different from the known bacterial pathogens of parsley in California. To determine the etiology of this disease, pathogenicity was evaluated on parsley, celery (*Apium graveolens*), and coriander (*Coriandrum sativum*). Buffer (0.01 M phosphate, pH 7.0) or suspensions of bacteria in buffer (at approximately 10^8 CFU/ml) were inoculated by spraying until runoff. All plants inoculated with bacteria developed leaf spots. DNA fragment banding patterns of the original eight isolates and the fluorescent reisolates from symptomatic tissue from pathogenicity tests were identical to each other by BOX-PCR and differed from *Pseudomonas syringae* pv. *apii*, *P. syringae* pv. *coriandricola* and *P. viridiflava*. BLAST was used to compare the 16S rDNA gene sequences from the parsley isolates to those in public databases. The16S rDNA sequences from the parsley isolates were identical to the 16S rDNA sequence of the type strain of *Pseudomonas viridiflava*. Although *rpo*D and *gyr*B sequences of the parsley isolates were most similar to those of *P. viridiflava*, they were not identical. These results indicated that the unknown pathogen isolated from parsley was related to but not identical to *P. viridiflava*. Further taxonomic work is needed to determine if these isolates are variants of *P. viridiflava* or represent a new pathovar or species.

Biological control properties of Pseudomonas isolates

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Rhizoctonia solani AG-8 and *Pythium ultimum* are important soil-borne fungal pathogens of wheat that cause annual yield reductions of 5–30% in Washington State and billions of dollars in losses worldwide. Commercial wheat varieties lack resistance to these pathogens and seed treatments are generally ineffective. Common management strategies include cultural practices such as tillage, crop rotation, elimination of weeds and volunteers between plantings. Biological control is an unexplored alternative. The aim of this study was to evaluate 26 strains of *Pseudomonas* spp. for their ability to colonize the wheat rhizosphere in raw soil and to test a subset of strains for control of *R. solani* and *P. ultimum.* The strains differed in motility and production of extracellular polysaccharides, exoprotease, and cyclic lipopeptides. PCR-based screening indicated that several strains carried genes for the production of the antifungal metabolites 2,4-DAPG, PCA, pyrrolnitrin and pyoluteorin. Colonization assays revealed that nine strains were capable of maintaining populations of > log 5 CFU g^{-1} root throughout four cycles of wheat plantings. Most strains were capable of controlling *R. solan*i AG-8, but *P. ultimum* was inhibited by only three strains. The most promising strain, 15D11, was a persistent colonizer capable of controlling both pathogens *in planta*. This strain has potential for development of a consistently-performing biopesticide for control of root diseases of wheat.

Complete genomes of plant growth-promoting rhizobacteria *Pseudomonas fluorescens* strains Q8r1-96 and Q2-87

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We report here complete sequences of genomes of *P. fluorescens* strains Q8r1-96 and Q2-87, two plant growth-promoting rhizobacteria that originate from a take-all decline soil in Washington state, U.S.A. The genome sequences were obtained using a combination of Sanger and 454 pyro-sequencing technologies. The genome of Q8r1-96 is comprised of a 61%-GC circular chromosome of 6.6 Mbp and encodes 5,783 proteins, 61 tRNAs, and 9 rRNA operons. The 6.4-Mbp genome of Q2-87 has GC content of 60.7% and encodes 5,689 proteins, 62 tRNAs, and 10 rRNA operons. Both strains are plasmid-free but carry a number of mobile genetic elements. Despite the fact that Q8r1-96 and Q2-87 are very closely related, their genomes share only 82% of predicted genes. Both strains carry a number of environmental fitness and biocontrol determinants including type III and type VI protein secretion systems and genes for production of 2,4-diacetylphloroglucinol, hydrogen cyanide, CLPs, antimicrobials, and putative entomotoxins. Q8r1-96 and Q2-87 also carry putative rhizosphere colonization determinants and traits that favor plant growth. Among these are the ability to produce gluconic acid and solubilize inorganic phosphate, and to utilize plant-derived phenolics, gamma-aminobutyric acid, and volatiles acetoin and 2,3-butanediol. Q8r1-96 also carries a gene for 1-aminocyclopropane-1-carboxylic acid deaminase and can stimulate root growth by lowering plant ethylene levels.

Characterization of a rare Plum pox virus W isolate found in germplasm illegally carried to the U.S.

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Plum pox virus (PPV) was identified in GF-305 seedlings bud-grafted with three Prunus accessions from Ukraine that were illegally hand-carried to the U.S. without official permit. One of the accessions was typed as PPV D and the other two as PPV W, a rare strain described on a single plum tree in Ontario, Canada in 2003. Serological characterization using sub-group specific antibodies showed that the 44189 isolate does not belong to PPV D, M, ElAmar or Cherry subgroup. Several monoclonal antibodies (MAb), specific to PPV W isolate 3174 from Canada, were tested. PPV isolate 44189 from Ukraine tested negative with PPV W MAbs 10G7 and 2C3 and positive with 4C11 and 6B6 MAbs in triple antibody ELISA and/or Western Blot. Coat protein sequence comparison of W3174 and W44189 isolates revealed amino acid deletions and substitutions in MAbs 10G7 and 2C3 epitope sites. Full-length sequencing of the isolate 44189 genome is 85% complete. RT-PCR forward primers were designed and combined with previously described reverse primer (James and Varga, 2004, Acta Hort. 657:177-182) for identification of PPV W. Our experience with characterization of the 44189 isolate at the amino acid and nucleic acid level highlights the need for constant improvement and re-validation of existing molecular and serological tools to achieve accurate detection and identification of new and unusual PPV isolates, especially from new geographical areas.

Active manipulation of resident biology to suppress *Macrophomina phaseolina* in strawberry

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M. phaseolina is a pathogen of emerging importance in strawberry production systems. Brassicaceae seed meal amendments suppressed proliferation of *M. phaseolina* through soil systems, but optimal seed meal-induced pathogen suppression required a functional soil biology. Suppression of *M. phaseolina* was obtained with seed meal sourced from various brassicaceae species and was not associated with production of a biologically active chemistry (e.g. allyl isothiocyanate by *Brassica juncea*). Seed meal-induced disease control was temperature sensitive and suppression of *M. phaseolina* root infection attained at 28°C was abolished when assay temperature was elevated to 32°C. Wheat cultivation alone or in conjunction with *B. juncea* seed meal application was highly effective in suppressing *M. phaseolina* root infection when strawberry was planted into a naturally infested field soil. Interestingly, treatments that suppressed or abolished strawberry root infection by *M. phaseolina* did not consistently suppress quantity of the pathogen detected in bulk soil. Disease control was associated with an overall increase in density of fungi recovered from rhizosphere soil.

Advances in Brassicaceae seed meal formulation and application for replant disease control in organic apple orchards

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Brassicaceae seed meals when used independently do not provide uniform and sufficient control of the pathogen complex that incites apple replant disease. Trials were established at multiple sites to evaluate the efficacy of seed meal formulations for control of this disease in organic production systems. When amendments were applied approximately one month prior to planting and tarped with a virtually impermeable film, a *Brassica juncea/Sinapis alba* seed meal formulation significantly improved apple tree growth and suppressed the target pathogen complex at two (STM and Tukey) of the three orchard sites. At the third site (SR), seed meal amendment resulted in significant phytotoxicity and approximately 40% tree death. Application of the seed meal formulation in the autumn prior to planting at SR orchard resulted in tree growth that was equivalent to that attained in response to pre-plant soil fumigation. The seed meal formulation reduced in-row weed coverage by approximately 85% at the STM orchard and weed suppression was evident at the end of the growing season. These preliminary data indicate that the seed meal formulation may be as or more effective than Telone-C17 fumigation for control of replant disease, but that plant back periods and seasonal application requirements will vary with soil type.

Woody host plant problems in Maryland diagnostic clinics from 2008–2010

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The University of Maryland Plant Diagnostic Laboratory processes ca. 800 samples per year. Diagnoses are determined by microscopy, isolation, ELISA, Biolog, and Agdia virus testing. Woody hosts made up 37% of samples submitted to the diagnostic lab from 2008–2010. Diagnoses from this period were 29% fungal and oomycete, 25% abiotic, 11% insect-related, 5% bacterial, 2% unknown/insufficient sample, and 0.4% nematode/virus. Additionally, 27% of diagnoses were "no pathogen found," indicating that no specific abiotic factor or infectious disease agent could be identified. Common diagnoses included environmental stress, *Phytophthora* root/crown rot, *Botryosphaeria* canker, Swiss needlecast (*Phaeocryptopus gaeumannii*), scales/borers, and bacterial leaf scorch. Homeowner inquiries are received via email at a separate facility, the Home and Garden Information Center (HGIC). Because no physical samples are submitted, conclusive diagnoses are not possible at this location. From 2008–2010, 2,767 woody host questions were submitted to the HGIC. Of those, 67% dealt with plant problem issues (18.5% insect, 14.3% disease, 34.2% abiotic); the remaining 33% were on weed control and plant selection/culture. The lower amount of disease reported by the HGIC is likely due to homeowner inexperience. Though the UMD

Plant Diagnostic Lab and the HGIC address diagnostics with different methods, they work jointly to answer plant problem questions for all Maryland citizens.

Using metconazole as a seed treatment to protect sugarbeets from early season Rhizoctonia Crown and Root Rot
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Rhizoctonia solani Kuhn is found in the soil of all sugarbeet growing regions. It is the casual pathogen of Rhizoctonia Crown and Root Rot (RRCR), an economically important disease of sugarbeets. Recent reports have noted the increase in the distribution and severity of RRCR. In extreme situations, RRCR can destroy up to half of the crop. Currently there are no registered seed treatments that offer protection from *R. solani* for sugarbeets. Metconazole is a systemic, triazole fungicide that has shown activity against *R. solani* in other crops. It was applied at a rate of 0.2 grams of active ingredient per 100,000 seeds, on pelleted blank seed or incorporated into the pelleting. Metconazole has been shown to provide a reduction in infection severity of RRCR and early season survivability in the greenhouse. In the field, metconazole has increased stands and reduced infection percentage from RRCR. Metconazole, applied as a seed treatment, has been shown to provide protection from early season RRCR.

***Erwinia amylovora* CRISPR arrays provide an effective tool for evaluating species diversity and microbial source tracking**
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Clustered regularly interspaced short palindromic repeats (CRISPRS) comprise a family of short DNA repeat sequences found in approximately half of all bacterial and archeal genomes. These repeats are separated by non-repetitive spacer sequences that, in combination with a suite of Cas (CRISPR-associated) proteins, are thought to function as an adaptive immune system against invading foreign DNA. The content of each CRISPR array can differ in both repeat number and in the presence or absence of specific spacers. Seventy-two strains of *Erwinia amylovora* (Ea) varying in geographic isolation, host range, plasmid content, and streptomycin sensitivity were evaluated for CRISPR array number and spacer variability. The CRISPR repeat sequence among Ea strains consists of 29 bp and is universal despite host range or other variables. A total of 536 unique spacers were identified in the 3 CRISPR arrays present in Ea. CRISPR arrays 1, 2, and 3 could be subcategorized into 20, 16, and 2 pattern types, respectively. Spacer patterns from Michigan strains were mainly distinct from strains isolated in the western U.S. although strains from Europe and the Middle East shared the same patterns as some strains from Michigan. Host of isolation was also a factor in spacer diversity, with *Rubus* and Indian Hawthorn Ea isolate spacers distinct from those Ea isolated from pome fruit. Spacer homology was predominantly to chromosomal DNA (55%), followed by plasmids (19%), and phage (7%).

Fungicide sensitivity of *Podosphaera xanthii* and efficacy of fungicides with resistance risk for cucurbit powdery mildew in New York in 2010
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A seedling bioassay was used to determine sensitivity of the cucurbit powdery mildew (PM) fungus to currently registered fungicides in research and commercial plantings. After the last fungicide application in the research plantings, the highest frequency of strains resistant to boscalid (tolerant of 500 ppm) was in plots where Pristine (boscalid and pyraclostrobin; FRAC Code 7 and 11) was applied alone every week (70% where Pristine was applied at lowest label rate and 29% where applied at highest rate, compared to 10% where an integrated program was used) and the highest frequency of strains tolerating 10 ppm quinoxyfen (20%) was in Quintec (Code 13) plots (compared to 0% where an integrated program was used). Quintec was very effective while efficacy of Pristine and Procure (triflumizole; Code 3) declined during the season resulting in ineffective control for the entire season based on AUDPC for severity on lower leaf surfaces. The bioassay conducted in pumpkin crops mid-season on 31 Aug revealed that 0–24% (12% avg) and 40–100% (61% avg) of the pathogen populations were resistant to boscalid and code 11 fungicides, respectively, while 0–1% and 0–7% tolerated 10 ppm quinoxyfen and 120 ppm myclobutanil. The bioassay on 21 Sep at the end of the PM management period revealed that 11–70% (40% avg) of the pathogen population was boscalid-resistant and 0–2% tolerated 10 ppm quinoxyfen. Degree of control in these fields reflected frequency of boscalid resistance.

Molecular identification of *Galactomyces* species and population structure of the two postharvest sour rot pathogens of fruit crops in California
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Sour rot caused by *Galactomyces citri-aurantii* (*Gca*) and *G. geotrichum* (*Gg*) is a major postharvest decay of fruit crops in California (CA). Species-specific primers from endo-polygalacturonase and beta-tubulin genes differentiated isolates of the two morphologically similar species. Isolates were collected from agricultural soils and decaying fruit from CA and worldwide locations, and were used in population genetic studies based on AFLP markers. Four geographical sub-populations (3 CA counties and locations outside CA) among 97 isolates of *Gca* and two sub-populations (within or outside CA) among 35 isolates of *Gg* were defined. For both species, the proportion of polymorphic loci and haplotypic diversity were high. Indices of genetic differences (FST) among sub-populations within each species were all low (0.0384 to 0.2263) indicating a low level of genetic differentiation. The effective migration rate N_m was calculated as 1.709 to 2.862 for *Gca* and 12.53 for *Gg* migrations per generation. Following clone correction, mating type segregation ratios for Gca did not significantly ($P > 0.1$) deviate from a 1:1 ratio for all four sub-populations, indicating a random mating structure. Tests of the index of association IA and parsimony tree-length permutation tests also supported a random mating structure for both species. A mixed reproduction system with an out-crossing sexual mating system and a prolific asexual phase is proposed for both species.

Reducing take-all inoculum build-up during a first wheat crop
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Globally, take-all (*Gaeumannomyces graminis* var. *tritici*) is regarded as the most important root disease of wheat and when severe can be devastating to wheat productivity. The occurrence of severe take-all depends on the amount of inoculum surviving in the soil when a susceptible wheat crop is sown. The effect of wheat cultivar on take-all inoculum build-up (TAB) in the soil during a first wheat crop has been investigated in field experiments over multiple years. After harvest a soil core bioassay method is used to gauge the amount of take-all inoculum left in the soil. A cross-season REML variance analysis revealed that there are consistent differences between wheat cultivars in their ability to build-up take-all inoculum in the soil during a first wheat crop (9 cultivars tested over four growing seasons, $P < 0.01$). This trait has also been identified in a wider range of current commercial UK wheat cultivars in a 2009 field experiment (45 cultivars, $P < 0.001$). The genetic basis of the TAB trait is being explored in an Avalon (A) x Cadenza (C) double haploid (DH) mapping population. The A x C DH mapping population was shown to segregate for the trait and two putative major quantitative trait loci (QTLs) have been identified. These findings potentially provide the first genetic solution to the control of take-all. The identification of wheat cultivars that reduce take-all inoculum build-up could help to significantly reduce yield losses in consecutive wheat crops.

Characterization of novel genes involved in *Erwinia amylovora* pathogenesis
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The enteric pathogen *Erwinia amylovora* is the causative agent of fire blight. Mutational analyses of the *E. amylovora* genome have yielded important insights into the genetic determinants required for full virulence by *E. amylovora*. To date key pathogenicity factors include the exopolysaccharide amylovoran and the hypersensitive response and pathogenicity (hrp) type III secretion system (T3SS). Here we report the characterization of three novel genes involved in *E. amylovora* pathogenesis. Microarray analyses identified three novel genes (*nlpI*, *ydcN* and EAM_2938) that are regulated by HrpL, a master regulator of T3SS. YdcN is a predicted XRE transcriptional regulator that may regulate genes important in *E. amylovora* pathogenesis. NlpI is a lipoprotein implicated in the secretion of extracellular DNA (eDNA), suggesting a novel role for eDNA in plant-microbe interactions. EAM_2938 encodes a putative membrane protein of unknown function. Chromosomal deletions in *nlpI*, *ydcN* and EAM_2938 genes were generated and also subjected to phenotypic analyses. Ea1189Δ*nlpI*, Ea1189Δ*ydcN* and Ea1189ΔEAM_2938 were all required for full virulence in immature pear; while Ea1189Δ*nlpI* and Ea1189Δ*ydcN* also exhibited alterations in swarming motility and biofilm formation. In addition, two genes, *hrpF* and *hrpQ* encode putative components of the T3SS. Both Ea1189Δ*hrpF* and Ea1189Δ*hrpQ*

were non-pathogenic when inoculated into immature pears, highlighting the important role of type III secretion.

Evaluation of commercial algaecides to mitigate *Phytophthora* spp. in naturally-infested water

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Phytopathology 101:S119

Few management options exist to mitigate *Phytophthora* species in water. Because Oomycetes are closely related to brown algae, application of commercial algaecides to infested water may prevent the dissemination of propagules of *Phytophthora* spp. We evaluated the efficacies of four commercial algaecides (three copper-based products and one hydrogen dioxide product) to *Phytophthora* spp. in naturally-infested water from two streams in western South Carolina. In each month of 2010, replicate aliquots of water (15 liters) from each stream were placed in 19-liter containers that remained in the stream to maintain ambient temperature. Algaecides were applied at the maximum label rate. Each month, one algaecide was tested at lower rates to identify minimum efficacy levels. Before and at 2 and 4 hours after treatment, 200-ml subsamples of water were filtered through polyvinylidine membranes (5 µm pores) and filters were inverted onto a selective medium to allow colonies of *Phytophthora* spp. to develop. *Phytophthora* spp. were detected each month in both streams in non-treated water. However, *Phytophthora* spp. were not detected in water after treatment with the three copper algaecides but were detected on occasion in water treated with the hydrogen dioxide product. Copper-based algaecides appear to be effective throughout the year, over a range of temperatures, and at several rates; therefore, they may prove to be an effective management strategy for *Phytophthora* spp. in some water systems.

Transmission of the opportunistic cotton (*Gossypium hirsutum* L.) boll pathogen *Pantoea agglomerans* by the brown stink bug (*Euschistus servus* Say)

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Damage to developing cotton bolls by piercing-sucking insects such as stink bugs has traditionally been attributed solely to pest feeding. Previously, we showed clear differences in severity of boll damage resulting from southern green stink bug (*Nezara viridula* L.) fed sterile food compared to those fed food contaminated with a rifampicin (Rif) resistant, opportunistic *Pantoea agglomerans* strain (Sc 1-R). Insects not exposed to Sc 1-R caused localized wounding at the feeding site, whereas seed and lint necrosis occurred in bolls pierced by bugs infected with Sc 1-R. *Euschistus servus* (Say), the brown stink bug (BSB), is another key pest of cotton. Here, we examined whether adult BSB could vector Sc 1-R. Sterilized green beans were dipped in either sterile H_20 or a suspension of Sc 1-R. Next, BSB were provided either of the food sources (2-d), and then sterile beans (5-d). BSB were then caged with a greenhouse boll at 2 weeks post-anthesis (5-d). Bolls were examined 2 weeks later. No disease was evident in bolls with wounds caused by control BSB; yet bacteria were detected from respective seed and lint tissue on non-selective media (10^4 cfu/g) and no growth on Rif amended media. Disease was observed in bolls probed by Sc 1-R contaminated BSB with concentration reaching 10^3 cfu/g tissue on Rif media. These data demonstrated that the BSB is a capable vector of *P. agglomerans* strain Sc 1-R.

***Tomato leaf curl Peru virus*: A locally evolved monopartite New World begomovirus**

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Tomato production in Peru has been affected by a leaf curl disease associated with whiteflies since 1995. A DNA-A component recently characterized from tomatoes with leaf curl symptoms in Peru was suggested to represent a new bipartite begomovirus named *Tomato leaf deformation virus* (ToLDV). Here, we report that ToLDV is actually a monopartite begomovirus tentatively named *Tomato leaf curl Peru virus* (ToLCPEV). DNA-A components were amplified by rolling circle amplification from symptomatic tomato tissue collected in 1998 and 2010, cloned and sequenced. Two clones from 1998 and one from 2010 were infectious in tomato plants and induced stunted growth and leaf curling and purple vein symptoms, which were indistinguishable from symptoms in the field. ToLCPEV was not sap-transmissible, and immunolocalization studies revealed that it is phloem-limited. Based on the absence of an AV2 open reading frame, the presence of an N-terminal PWRLMAGT motif in the capsid protein and phylogenetic analyses, ToLCPEV was more similar to DNA-A components of bipartite New World begomoviruses than to monopartite viruses from the Old World. Mutational analyses revealed that ToLCPEV-DNA-A ac4 mutants were infectious in tomato and *Nicotiana benthamiana* plants but did not induce symptoms, whereas av1 (capsid protein) and ac1 (Replication-associated protein) mutants were not infectious. Thus ToLCPEV is one of the first examples of a *bona fide* monopartite begomovirus from the New World.

Detection, diversity, and molecular characterization of closteroviruses infecting Hawaiian ti (*Cordyline fruticosa* L.)

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The ti plant (*Cordyline fruticosa* L.) is culturally important throughout most of Polynesia and has considerable economic importance in Hawaii where the foliage is commonly used in cultural ceremonies as well as ornamental and food industries. Ringspot symptoms were recently observed on leaves of the common green variety of ti, dramatically reducing commercial production of cut foliage. Sequencing of high-molecular weight double-stranded RNAs isolated from symptomatic tissues revealed the presence of four distinct closteroviruses, which were designated Cordyline virus 1 (CoV-1), CoV-2, CoV-3, and CoV-4. Phylogenetic analyses using the Heat Shock Protein 70 homolog could not assign any of these viruses to current genera within the family *Closteroviridae*. A reverse-transcription PCR assay was developed for the detection and discrimination of these viruses. Based on this assay, it appears unlikely that any of these viruses are the causal agent of ti ringspot, as they could be found in both symptomatic and asymptomatic plants. It was also found that individual ti plants often harbor multiple viruses, and the geographic distribution of these viruses in Hawaii is not uniform, suggesting vector transmission of these viruses. This is also supported by the detection of these viruses in ornamental ti varieties recently derived from seed. Further molecular characterization of these viruses and identification of a vector will contribute to our knowledge on the diversity of the family *Closteroviridae*.

Evolutionary ecology of invasion in the *omics* era: Examining inbreeding depression and invasion success of the common horsenettle, *Solanum carolinense*

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Solanum carolinense is a perennial, invasive weed found in cropfields and pastures. The ability of some families to self fertilize may aid the invasion of new environments. If individuals with contrasting breeding histories vary in their ability to respond to ecological stresses, it may reduce the evolutionary advantages of selfing potential. The project presented here focuses on the use of comparative metabolomics and functional genomics to analyze the differential expression of genes triggered by herbivore damage between plants with contrasting breeding history (selfed vs. outcrossed). Individual ramets belonging to five families that vary in selfing ability were assigned to each of two treatments: herbivore damage (damaged or undamaged) and breeding history (outcrossed or selfed). The herbivory treatment involved a 48-h feeding trial using *Manduca sexta* caterpillars. Total RNA was extracted from leaf samples collected 8 hours after the feeding trial. Total volatile profiles were measured in planta prior to collection of leaf samples for RNA analysis. Our combined results show the presence of interactions in the patterns of gene expression and volatile compound release between selfed and outcrossed progeny across herbivory treatments; these interactions will likely affect the extent of inbreeding depression. These results will be examined in the context of the evolution of mixed mating systems in plants, in terms of community ecology and the biology of invasive species.

An inhibitory effect of a novel *Bacillus* sp. strain against potato common scab

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A bacterial strain (BAC03) was isolated from a Michigan potato field, and characterized for suppression of *Streptomyces* spp., the cause of potato common scab. The antimicrobial activity of BAC03 was quantified on culture plates against six species of *Streptomyces*, including *S. scabies*, *S. acidiscabies*, *S. stelliscabiei*, *S. aureofaciens*, *S. lydicus*, and *Streptomyces* strain DS3024. BAC03 had significantly ($P < 0.05$) higher inhibitive effect (inhibition zone from 0.8 ± 0.1 cm to 3.5 ± 0.2 cm) compared to *Bacillus subtilis* strain QST713 (inhibition zone from 0.2 ± 0.1 cm to 1.7 cm), a commercial biocontrol agent. In a greenhouse, radish and potato were grown in potting soil infested with *S. scabies* (10^7 CFU/cm^3). Radish biomass was

significantly ($P < 0.05$) higher (337% increase of fresh roots and 365% increase of fresh leaves) in pots treated with *Bacillus* spp. BAC03 (10^6 CFU/cm^3) than the non-treated. Potato growth was promoted (157% fresh leaves) and disease severity was reduced 85% by BAC03, compared to non-treated controls. In order to identify the mechanisms associated with the antagonistic activity, non-ribosomal peptide synthetase were extracted with acid precipitation and ribosomally synthesized proteins were precipitated with ammonium sulfate, and both were tested against *Streptomyces* spp. isolates. A protein was derived in responding to antagonistic activity. Further identification and characterization of the compounds are ongoing.

Evaluation of systemic acquired resistance inducers for control of basil downy mildew

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Basil downy mildew (BDM), caused by *Peronospora belbahrii*, is a devastating foliar disease recently discovered in South Florida in 2007. BDM has spread to over 20 states and has become a major threat to sweet basil production in the U.S. Current management strategies including the use of fungicides are inadequate. In this study, five systemic acquired resistance (SAR) inducers i.e., Actigard® (ASM), 3-amino-butanoic acid (BABA), isonicotinic acid (INA), salicylic acid (SA), and sodium salicylate (NaSA) were evaluated in the greenhouse for their potential to control BDM. Foliar sprays of ASM applied as pre- (P), post- (PO) or pre + post (PP) inoculations at rates 25 to 400 mg/L significantly ($P < 0.05$) reduced BDM severity from 35.4 to 99.5% compared to the non-treated control (CK). Foliar spray of ASM at 50 mg/L 3 days after inoculation (DAI) resulted in a 93.8% reduction in BDM severity, but only a 48.9% reduction was achieved when applied 7 DAI. BABA sprayed at rates up to 100 mg/L failed to control BDM. However, BABA applied as PP at high rates showed a significantly improved efficacy against BDM from 81.1 to 91.8%. *In vitro* tests indicated that ASM and BABA at rates lower than 1.0 mM had no effect on sporangial germination of *P. belbahrii*. INA, SA and NaSA evaluated at various rates and timings varied in the effect on BDM. ASM or BABA at low rates followed by foliar sprays of Prophyt® and Quadris® significantly reduced BDM severity compared to either of the SAR inducers alone.

Effects of green manures on nematode population densities in an organic tomato field

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An increased demand for organic foods has resulted in a greater need for pest management strategies in organic vegetable production systems. To this end, cover crops incorporated as green manures in an organic tomato field were studied for effects on nematode population densities. Treatments were: 1) mixed species hay (*Festuca arundinacea*, tall fescue; *Dactylis glomerata*, orchard grass; *Phleum pretense*, timothy; *Trifolium pretense*, red clover; *Medicago sativa*, alfalfa); 2) *Vicia villosa* (hairy vetch); 3) *V. villosa* plus *Secale cereale* (rye); 4) *V. villosa* plus *Raphanus sativus* (forage radish); and 5) a bare ground control, six plots/treatment. Cover crops were incorporated with a chisel plow in April 2010. Plots were sampled before incorporation, <2 weeks later, midseason and harvest. *Meloidogyne* was found in only 3 plots, at the second sampling time. Total plant-parasitic nematode numbers/100 cc soil at that time were 21.3, 2.5, 33.8, 26.3, and 8.3 (means for treatments 1-5, respectively), and at harvest were 26.9, 40.8, 46.7, 43.8, and 40.8. No significant differences were found among treatments or dates, and no root galling was observed. Marketable tomato yield means ranged from 2.6 lbs (on two selected plants/plot) for hairy vetch+rye to 5.9 pounds for hairy vetch; no significant differences were found among treatments. The results indicate that under low nematode pressure, the cover crops did not result in any change in nematode populations.

Mating disruption for *Planococcus ficus* S.: How to successfully initiate a novel sustainable control tool

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Phytopathology 101:S120

Mating disruption has become an important tool in Integrated Pest Management programs, particularly for lepidopteran pests. Over a three year field trial we have investigated mating disruption for another pest, the vine mealybug, *Planococcus ficus* (Signoret) (*Hemiptera*, *Pseudococcidae*). The vine mealybug infests some vineyards in Mendoza, Argentina, and is one of the more important vineyard mealybugs in the world. This insect excretes honeydew, which promotes sooty molds, and vectors grape leafroll associated viruses (GLRaV). The possibility to use mating disruption for vine mealybug presents challenges but offers the potential ecological wine grape production in the affected areas. In field trials, a synthetic sex pheromone was released in plastic dispensers at a rate of 600 dispensers/ha. Trials were conducted at two mealybug infestation densities (Low/ High), and with or without pesticide applications. Results showed that at high infestation densities the pesticides must also be applied to control the mealybug in the first year, but a reduction was seen in the second year also without pesticides. In vineyards with low mealybug infestation densities, effective control was provided in the first year. Results also showed an initial reduction of the number of vines affected, and after the second year a reduction in the number of fruit clusters damaged. A recommendation is made to apply mating disruption for a minimum of three seasons to provide effective control.

Genetic structure and patogenicity of *Phytophthora infestans* sensu lato collected from Solanum betaceum in southwestern Colombia

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Late blight caused by *Phytophthora infestans* is a limiting and devastating disease in several solanaceous crops in the tropics. In South America, specific *P. infestans* populations have been characterized, associated with exotic tropical fruits. These populations are able to infect different wild and cultivated hosts causing important economic losses. In this study, *Phytophthora infestans* sensu lato isolates collected from *Solanum betaceum* in Nariño and Putumayo states were analyzed using phenotypic and genetic features. Aggressiveness tests were realized using a detached leaf bioassay. Isolates belonged to the A1 mating type, Ia mtDNA haplotype and EC-3 lineage. Results obtained with microsatellites markers (SSRs) showed that the population structure varied from clonal populations previously reported in Colombia, suggesting high levels of genetic diversity among all isolates. Our results also revealed high levels of variation in aggressiveness among the isolates when tested on susceptible and resistant cultivars. In detached leaf bioassays we observed that isolates did not infect *Solanum tuberosum*, suggesting host specificity. These results have significant implications for the understanding and characterization of the evolutionary history and epidemiology of the pathogen in the North Andean highlands.

Molecular and phenotypic variation of German populations of *Fusarium graminearum* causing head blight in wheat

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Fusarium graminearum sensu stricto (s.s.) causing head blight (FHB) in wheat is a destructive pathogen. In a survey of 12 populations with each of 30 heads from naturally infected wheat fields in Germany. *F. graminearum* s.s. dominated the *Fusarium* population with 64.9% out of 521 single-spore isolates. All three chemotypes were identified by PCR assays with a dominancy of 15-acetyldeoxynivalenol chemotype (92%). The twelve populations showed a high genetic diversity within the small scale sampling areas of 1-3 hectares resulting in 300 haplotypes out of 338 isolates revealed by 19 microsatellite primers. Genetic diversity within populations (72%) was considerably higher than among populations (28%) as shown by analysis of molecular variance (AMOVA). Three of the populations were additionally analyzed phenotypically for mean FHB rating and deoxynivalenol (DON) content on a moderately susceptible wheat genotype in two locations and two years. Data revealed significant ($P < 0.01$) genotypic variation within each population for both traits. Partitioning of genotypic variance revealed similar values like AMOVA. In conclusion, *F. graminearum* s.s. populations in Germany displayed a tremendous genetic variation on a local scale. Multiple resistance genes of different origin should be introgressed in breeding programs to obtain a long-term stable FHB resistance.

Antifungal compounds in ripe fruit from a resistant blueberry cultivar suppress infection by *Colletotrichum acutatum*

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Anthracnose fruit rot, caused by *Colletotrichum acutatum*, is among the most important diseases of blueberries. Most cultivars are susceptible but 'Elliott' is resistant. Our objective was to identify possible antifungal compounds that play a role in this resistant response. Initially, chemical fractions from lyophilized ripe fruit of 'Elliott' and the susceptible cultivar 'Jersey' were

extracted with water, methanol, and ethyl acetate. Extracts were screened on solid media for suppression of microconidiation of *C. acutatum*. The methanolic extract was further fractionated and the soluble methanolic fraction from 'Elliott' was the most biologically active. This fraction was dried, dissolved in water, and screened *in vivo* by pre-treating ripe 'Jersey' fruit using 0.5, 1, 2, and 4% solutions and subsequently inoculating the fruit with *C. acutatum*. An 88% reduction in infection incidence was observed after 12 days with the 4% solution. Anthocyanins and flavonols were then quantified in fruit of the two cultivars using HPLC-MS. 'Elliott' fruit contained more anthocyanins (5.38 mg/g of freeze-dried tissue) than 'Jersey' (3.75 mg/g of freeze-dried tissue); however, the same compounds were found in both cultivars. Additionally, several unique flavonols were present in 'Elliott' (four distinct peaks). Further purification and identification of these compounds will provide new insights into the role of anti-fungal compounds in the resistance response in 'Elliott' fruit.

The SA and ET signaling pathways mediate tomato resistance to bacterial wilt at cool temperatures

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Salicylic acid (SA) and ethylene (ET) signaling pathways play an important role in tomato defense responses against bacterial wilt disease caused by *Ralstonia solanaceraum*. Tomato plants infected with cool-tolerant Race 3 biovar 2 strain UW551 upregulated genes in both the ET and SA defense pathways. The speed and amount of defense gene induction predicted the degree of host resistance. Interestingly, temperature significantly affected host defense gene expression. Defense genes were more strongly expressed at 20°C than at 28°C in tomato plants with comparable pathogen populations. We measured wilt disease progress at 20°C and 28°C after transgenic salicylic acid-degrading *NahG* and ethylene-insensitive *Never Ripe* tomato plants were infected with strain UW551. *Never Ripe* was more susceptible to bacterial wilt than its wild-type parent, confirming the importance of ET for disease resistance. This effect was more pronounced at 20°C than at 28°C. Disease progress in *NahG* and wild-type tomatoes was comparable at 28°C, contrasting with the gene expression results that implied SA involvement in host resistance. At 20°C however, the *NahG*-transgenic line was significantly more susceptible to *R. solanacearum* than its wild-type parent. Collectively, these data suggest that the relevant tomato defense responses are inhibited at elevated temperatures, leading to increased susceptibility to infection by *R. solanacearum*.

Phenotyping *Yr17* resistance in wheat to stripe rust and *Yr17* virulence in *Puccinia striiformis*

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Yr17 has been used as an all-stage resistance gene to protect wheat from stripe rust caused by *Puccinia striiformis* f. sp. *tritici*. However, it has been difficult to accurately identify *Yr17* resistance in wheat seedlings and *Yr17* virulence for race identification. The objective of this study was to determine the effects of post-inoculation temperature on the host-pathogen interaction. Seedlings of 21 wheat lines with *Yr17* (based on a linked molecular marker) were inoculated with two avirulent races (PST-3, PST-78) and two virulent races (AR10-04, PST-127) and incubated at 10°C, 18°C, and a gradually-changing regime from 5–18°C that has been used for race identification. Infection types were recorded 20 dai using the 0–9 scale for stripe rust in which 0–4 is avirulent/resistant and 5–9 is virulent/susceptible. The two virulent races were similar and were incorrectly identified as avirulent 13, 12 and 36% of the time at 10, 5–18 and 18°C, respectively. PST-3 was incorrectly identified as virulent 10, 5 and 0% of the time at 10, 5–18 and 18°C, respectively. PST-78 was incorrectly identified as virulent 68, 44 and 6% of the time at 10, 5–18 and 18°C, respectively. *Yr17* resistance was most effective at18°C, but none of the temperatures promoted accurate phenotyping of wheat lines or *P. striiformis* isolates. It may be more accurate to designate *Yr17* as an adult-plant resistance gene. PST-78 may be heterozygous for virulence on *Yr17*.

Comparison of old and new strains of *Puccinia striiformis* f. sp. *tritici* for ability to initiate stripe rust epidemics in wheat

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Since 2000, a new strain (based on AFLP phenotype) of Puccinia striiformis f. sp. tritici has replaced the old strain in eastern United States, and stripe rust has been more severe than before 2000. Epidemics begin from overwintering infections that develop into discrete foci by spring. The objective of this research was to determine if the strains differed for ability to initiate epidemics from overwintering infections. Plants of a susceptible cultivar were grown outdoors, inoculated lightly with isolates AR90-01 and AR97-01 (representative of old strain) and AR00-05 and AR03-33 (representative of new strain), incubated in a dew chamber, and transplanted into field plots of the same cultivar at Fayetteville and Kibler, AR, during the falls of 2007 and 2008. Random pots were incubated in a growth chamber to determine the levels of initial infection for each isolate, and levels among isolates were either similar or higher for isolates of the old strain. To quantify the amount of disease in the spring, the average severity across all stems in 0.5-m lengths of the two rows adjacent to each transplant was determined, and data were analyzed using analysis of variance. Isolates of the new strain consistently caused significantly more stripe rust than isolates of the old strain, indicating that isolates of the new strain are more aggressive than isolates of the old strain for initiating epidemics, and this helps explain why stripe rust has been more severe since 2000.

Evaluating artificial microRNAs for engineering resistance against tospoviruses

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MicroRNAs (miRNAs) are a highly conserved class of small non–coding RNAs, which are both highly specific and effective to achieve silencing of genes. We are using artificial microRNAs (amiRNA) technology for introducing resistance to *Tomato spotted wilt virus* (TSWV). amiRNAs were developed targeting viral RNA sequences encoding the nucleocapsid protein (N) and the silencing suppressor (NSs) genes of TSWV. An Arabidopsis thaliana miR159 precursor was modified to express virus-specific amiRNAs. Transient expression of amiRNAs in *Nicotiana benthamiana* by agroinfiltration has confirmed expression of virus-specific amiRNAs by Northern blot analysis. The ability of the amiRNA constructs to confer resistance to TSWV has been confirmed in virus challenge experiments. Stable Arabidopsis and tobacco plants have been generated with selected constructs. We are investigating various construct design features to improve the efficiency of expression of the mature amiRNAs that would provide effective resistance against tospoviruses.

Application of multiplex PCR to mixed populations of tomato bacterial pathogens

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Diagnosis of foliar bacterial diseases of tomato has been enhanced with polymerase chain reaction (PCR) and primers for the detection of pathogens. Furthermore, specific primers have been developed to detect specific pathogens in mixed bacterial populations. Previous work in other laboratories has evaluated multiplex PCR reactions for simultaneous detection of multiple species of bacterial pathogens using pure cultures. The objective of this study was to optimize a multiplex PCR protocol for specific detection of *Xanthomonas* species, *Pseudomonas syringae* pv. *tomato*, and *Clavibacter michiganensis* subsp. *michiganensis* in field samples of tomato foliage. The primers selected were RST 65 and RST 69 (Xanthomonas spp.), MM5 and MM6 (*P. syringae* pv. *tomato*), and CMM5 and CMM6 (*C. michiganensis* subsp. *michiganensis*). Temperature parameters and concentrations of reaction components for single PCR reations with these pathogens and their respective primers were harmonized to allow for DNA amplification of the three pathogens in one reaction. The resulting multiplex PCR gave optimal results for all three primer pairs at an annealing temperature of 57.2 C. Pure cultures were used to develop the protocol. The sensitivity and specificity of the assay will be discussed. A sensitive multiplex PCR protocol for evaluation of field samples will allow for rapid identification of these pathogens, facilitate population studies, and provide a valuable diagnostic tool.

Morphological and Molecular diagnosis of *Corynespora cassiicola* and *Cercospora* sp. causal agents for hydrangea leaf spot diseases

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Hydrangea leaf spot disease often referred to as *Cercospora* leaf spot was associated with six pathogenic fungi with *C. cassiicola* and *Cercospora* sp. having the highest frequency of occurrence as the major pathogens. The two fungi, *C. cassiicola* and *Cercospora* sp. do not produce spores readily in culture and their morphological identification can be challenging. While *C. cassiicola* cause small discrete lesions less than 10 mm in diameter and larger marginal non-discrete lesions, the lesion similarity to those caused by

Cercospora complicate disease diagnosis. In addition, variability of symptoms caused by *C. cassiicola* in different hydrangea cultivars was observed to farther complicate the identification of *C. cassiicola* as the primary pathogen. Misdiagnosis of *C. cassiicola* may inflate the severity of *Cercospora* in hydrangea leaf spot diseases. To aide identification of *C. cassiicola* and *Cercospora* sp., morphological distinction between the two fungi in culture were evaluated, differential cultivar reactions to *C. cassiicola* and *Cercospora* sp., were identified and specific primers were developed as molecular diagnostic tools. DNA sequences for *C. hydrangea* has not previously been deposited at the GenBank, and the DNA sequence of *Cercospora* sp. pathogen of hydrangea matched to that of *C. beticola*, *C. fukushiana* and, *C. penzigii*.

Association of *Plum pox virus* M strain with plum fruit dropping in Iran
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Phytopathology 101:S122

Severe fruit dropping was recently observed in some plum orchards of Mazandaran province; in order to investigate possible involvement of *Plum pox virus* (PPV), 75 leaf and shoot samples collected during the summer late and autumn of 2010. Sampling was based on typical PPV symptoms. Serological diagnosis was made by DAS-ELISA using a commercial PPV polyclonal anti¬serum (Bioreba). Molecular detection was made by trapping virus particle with the above polyclonal antiserum and IC-RT-PCR was performed by using the general pair of primers P1/P2. Total RNA were extracted from dormant buds and barks using RNeasy Plant Mini Kit (Qiagen, Germany). The results showed that 48 out of 75 plum samples were found to be infected with PPV. One-step RT-PCR was performed using general primer pairs (P1/P2). The P1/P2 primers revealed and confirmed the presence of the virus by amplifying the expected 243 bp fragment located at the C-terminus of PPV CP gene. The results of RT-PCR analysis were in complete agreement with the DAS-ELISA and IC-RT-PCR results. The type of strain determined by RT-PCR target¬ing (Cter) CP, using P1/PD and P1/PM pair of primers that distinguish two major PPV-D and PPV-M strain, respectively; The RT-PCR analyses confirmed that all 48 samples were infected with PPV-M type. Subsequently, molecular strain typing was confirmed by RFLP of 243 bp amplicon using *Rsa*I and *Alu*I restriction endonucleases digestion. The pattern after enzyme digestion showed all of PCR products contained the *Alu*I site.

Studies on the mix infection of *Tomato yellow leaf curl virus* (TYLCV) and *Watermelon chlorotic stunt virus* (WmCSV) in south of Iran
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Phytopathology 101:S122

164 leaf samples of cucurbit specius including *Cucurbita pepo*, *Cucumis melo*, *Citrullus lanatus* and *Cucumis sativus* with sever curling and mottling symptoms were collected from Hormozgan and Sistan-Baloochestan provinces in Iran. The samples were tested antisera against three viruses: TYLCV-specific monoclonal antibody, CMV polyclonal antibody and ZYMV polyclonal antibody (Bioreba) applying DAS-ELISA. Based on the result, TYLCV was indicated in 112 out of 164 suspected samples from the both provinces. The similar disease symptoms observed in cucurbit plants might have been caused by infection with other viruses such as Watermelon chlorotic stunt virus (WmCSV). So some of the samples positive against TYLCV were used to nucleotic acids extraction for investigate the presence of the two viral agents by viruses-specific primers in cucurbit crops. Total nucleic acids were extracted from leaf tissues using the DNeasy Plant Mini Kit (Qiagene). Nested PCR using the primerpair TYSacv/TYCPc1023 and PTYCPv369/TYCPc1023 was performed for amplification of a 1379 bp fragment and a 671 bp fragment (respectively) of monopartite TYLCV genome and direct PCR using WmCSV-specific primers (WAI-XbaI-(v)/WAI-XbaI-(c) and WAI-SmaI-(v)/ WAI-SmaI-(c)) was conducted to amplify the full-length DNA-A and the full-length DNA-B (respectively). Specific fragments of viral genome were amplified in both assays. The serological and molecular results confirmed that all cucurbit plants surveyed in this study were mix infected with two viruses.

Vegetative Compatibility Group (VCG) characterization of *Fusarium oxysporum* f. sp. *cubense* in Asia
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Recent epidemics of Panama disease (Fusarium wilt) in China and the Philippines caused by the virulent Tropical Race 4 (TR4) of *Fusarium oxysporum* f. sp. *cubense* (Foc) have posed a serious threat to the banana industry in Asia, and beyond. This race, belonging to the Vegetative Compatibility Group (VCG) 1213/16, is extremely important because it attacks the widely grown and traded Cavendish varieties, and many local cultivars grown by smallholder farmers. A study to determine the geographic distribution of the various pathogenic VCGs of Foc in Asia was carried out as a key step towards designing policies and measures to prevent further spread of TR4. Samples were collected from diseased banana plants between 2006 and 2009 from 12 countries in tropical Asia. Foc was isolated from these samples, single-spored, and nit-1 and nit-M mutants generated in laboratories in Australia and South Africa. These mutants were then paired with an international VCG-tester set for Foc using the technique described by Puhalla in 1985. Nine VCGs (1213/16, 0120/15, 0121, 0123, 0124/5, 0126, 0128, 01218, 01220) were identified in Asia. VCG1213/16 (TR4), was the dominant VCG from samples collected in China, Indonesia, Malaysia, Philippines, and Taiwan but not found in samples from the other countries. VCG 0124/5, a VCG associated to Foc Race 1, was the dominant VCG in samples from India, Bangladesh, Cambodia, Sri Lanka, Vietnam, and Thailand. No Foc infection of banana was found in Papua New Guinea during these surveys.

Field resistance of selected banana cultivars against Tropical Race 4 of *Fusarium oxysporum* f. sp. *cubense* in the Philippines
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Recent epidemics of Fusarium wilt caused by virulent Tropical Race 4 (TR4) of *Fusarium oxysporum* f. sp. *cubense* (Foc) posed serious threat to the Philippine banana industry. Based on monoculture cropping of TR-4 susceptible Cavendish varieties, the nation's multi-million US$ export banana industry is at risk. To develop disease management tactics, selected introduced and local cultivars were studied for their reaction to Foc in a field previously severely affected by TR4. Evaluated were two commercially grown Cavendish, and 2 variants from Taiwan, 3 local cultivars and 1 improved hybrid from Honduras. The experimental plot comprised of 10 tissue-culture derived plants spaced 2.5 × 3 m, replicated 10 times, and arranged in completely randomized block design. Disease incidence was assessed weekly by monitoring any symptom including leaf chlorosis, wilting and/or pseudostem splitting. The 2 commercially grown Cavendish, Grand Naine and Williams, showed susceptible reactions, with incidence of more than 90% before shooting. *Lakatan*, a popular local cultivar was most susceptible with 100% incidence. GCTCV119, and Formosana, Cavendish variants did not show any infected plants in the first crop, although some symptoms were appearing in the ratoon crop. *Saba*, an important cooking banana was highly resistant with no disease incidence, even in the ratoon crop. Vegetative Compatibility Group (VCG) analyses confirmed that Foc TR4 VCG1213/16 was associated with the infections.

***Agrobacterium*-mediated transformation of sugarcane with the anti-apoptotic gene CED-9 confers abiotic stress tolerance**
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Phytopathology 101:S122

Embryogenic calli of sugarcane (Sacharum officinarum L.) genotypes TCP87-3388 and CP72-1210 were transformed with the anti-apoptotic gene CED-9, a *C. elegans* homolog of the mammalian Bcl-2 cytoprotective gene family. Transformed plants were selected on culture medium containing Geneticin, and characterized by ELISA for the presence of *nptII* protein. T2 transgenic lines were evaluated for drought tolerance at two different developmental stages; 40 and 90 days post germination, with water deprivation periods of 10 and 20 days, respectively. Candidate drought tolerant plants were recovered in both tests. The selected deprivation water periods represent the minimum amount of time after which wild type plants were unable to recover (even after rehydration). Selected transgenic lines remained viable and were not impaired in development. These results suggest that the anti-apoptotic gene CED-9 integrated into the genome of sugarcane may confer drought tolerance. Further experiments are underway to investigate the role of CED-9 in other abiotic stresses, including salt, cold and d heat.

Development of an *in vitro* multiplication method of sugarcane transgenic lines to improve stress tolerance screening
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Phytopathology 101:S122

Because of the high number of transgenic sugarcane lines needed for the efficient screening of candidate plants tolerant to abiotic stress, the development of a robust propagation system is required. With traditional vegetative propagation methods, each sugarcane plant under greenhouse condition will be able to generate 6 to 10 T2 plants in a 6–8 month period. In order to improve this rate-limiting step, a protocol for *in vitro* propagation of transgenic lines was developed. Plants are regenerated via organogenesis from the meristematic region of the leaf rolls. Preliminary results show that using this novel method, a 10 fold increase in the number of plants generated was achieved compared to traditional methods (6–10 plants with traditional method versus ~60 plants with *in vitro* propagation). Additionally, using this method, we were able to use younger plants (4 months in greenhouse after the tissue culture process) instead of mature plants (6–8 months in greenhouse after the tissue culture process), thus significantly reducing the propagation timeline. Taken together, this method increases the number of propagated plants, reduces the propagation time by half, substantially decreases the amount of space needed, and dramatically increases the number of repetitions per treatment. Transgenic lines propagated by this method are being used in preliminary *in vitro* screens for abiotic stress tolerance, such as heat, cold, salt and drought.

Application of a real-time PCR assay for detection of eastern filbert blight in hazelnut breeding

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Eastern filbert blight (EFB) is a devastating disease of European hazelnut, Corylus avellana L., which causes economic losses in Oregon, where 99% of the U.S. crop is produced. The incitant, Anisogramma anomala (Peck) E. Müller, is native east of the Rocky Mountains, where it is harbored by the American hazelnut (C. americana Marshall). While C. americana is tolerant, EFB causes dieback and death of C. avellana. Detection and identification of A. anomala in nursery stock and breeding populations is challenging and time-consuming using conventional methods, because disease symptoms show only after 16 months from infection, and the fungus can only be cultured from sporulating perithecia. In this study, a culture-independent TaqMan real-time PCR assay was developed that enables pathogen detection from field samples within a few hours. The assay was validated with the target pathogen, closely related fungal species, and a number of other microorganisms that inhabit C. avellana. The detection limit of the assay is 0.1 pg A. anomala genomic DNA, which enables EFB diagnosis many months before disease symptoms develop. Compared with traditional diagnostic methods, we found that the TaqMan real-time PCR assay is more sensitive, efficient, and rapid. This assay can expedite breeding for disease resistance by early and accurate diagnosis of EFB. It also has applications for disease management, especially in assessing nursery stock for the presence of the pathogen.

Characterizing *in planta* expression of G_N-S, a soluble form of *Tomato spotted wilt virus* G_N glycoprotein

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Tomato spotted wilt virus (TSWV) is an economically important disease of vegetable and ornamental crops worldwide. The virus is transmitted by thrips (Thysanoptera) in a circulative-propagative manner. Previous *in vitro* feeding experiments documented that the soluble form of TSWV G_N, G_N-S, interferes with virus acquisition and transmission by thrips. We expressed G_N-S transiently in *Nicotiana benthamiana* to characterize G_N-S behavior *in planta* and to determine if G_N-S has potential for controlling virus transmission by thrips. The localization pattern of green and red fluorescent protein fusions (GFP or RFP) of G_N-S were compared against the wild type G_N (G_N-Wt) and nucleocapsid (N) proteins. G_N-S was distributed throughout the cytoplasm in 72% of cells examined and the distribution was similar to the GFP control. In contrast, G_N-Wt displayed a distinct punctate pattern in 72% of cells examined. Co-localization experiments revealed that G_N-Wt targets to the Golgi (96%) and G_N-S and Golgi marker co-localization was observed in only 44% of cells evaluated. N displayed a complex localization pattern two days after agro-infiltration with protein accumulating in cytoplasmic foci of varying sizes and in small foci associated with the cell periphery suggesting that some N may localize to the plasmodesmata. We documented the localization of G_N-S in plant cells and our findings indicate that generation of G_N-S transgenic plants may be a viable TSWV control strategy.

Evaluation of the effects of soil moisture on the damage potential of *Rotylenchulus reniformis* on cotton

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Phytopathology 101:S123

A trial to reduce the risk of damage to cotton by *Rotylenchulus reniformis* was conducted in a 26 ha field in south Alabama in 2009 and 2010. The field was delineated into three management zones using apparent soil electrical conductivity (EC) and elevation, and the nematicides 1, 3-dichloropropene, aldicarb, oxamyl and abamectin were applied alone and in various combinations within each zone with an untreated control. Population densities of *R. reniformis* prior to nematicide treatment were 535, 1096 and 71 vermiform life stages/150cm3 of soil for zones 1, 2 and 3, respectively. Zones 1, 2 and 3 averaged increasing volumetric water content ($P < 0.1$) of 0.138, 0.150 and 0.184 cm3/cm3 throughout the season. Evaluation of the interaction of *R. reniformis* population and soil moisture on cotton yields indicate that the driest zone, zone 1, was at the highest risk of yield loss and benefitted with yield gains ($P < 0.1$) from higher rates of nematicides. Although zone 1 supported only half the initial *R. reniformis* population compared with zone 2, the combination of soil moisture and nematode stress in zone 1 resulted in a significant ($P < 0.1$) yield increase over the untreated control. The factor of water availability throughout the growing season should be considered in risk assessment when creating site-specific management zones for *Rotylenchulus reniformis*.

Pattern recognition favorability of temporal dynamics of asian soybean rust using backpropagation neural network

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The presence of free water on leaf surface and the average temperature during the wetness are the main factors for the occurrence and intensity of the progress of asian soybean rust. The aim of this study was to develop a neural network to characterize the weather favorability for the development of soybean rust in major soybean producing regions of Brazil. During the seasons 2007, 2008 and 2009 were carried out 22 experiments, which were collected meteorological data, plant development and disease severity. Data from 22 outbreaks were collected over three years. To quantify disease severity it was used the digital image processing by the software QUANT. For development of neural networks were used as input: the duration of leaf wetness (hours), the average temperature during leaf wetness, the first day that the disease was observed in each experiment and as output of the networks neural had foliar severity. The neural networks were developed in the Matlab Neural Network Toolbox, version 2009, using the backpropagation algorithm for training the networks, with 60% of the data was used for training, 20% of the data to test and 20% for validation. Choosing the best combinations of neurons was based on lower mean square error, mean prediction error and highest coefficient of multiple determination. The best combination of neurons showed the mean square deviation equal to 6.891 and the mean prediction error equal to 21.78% and determination coefficient of 0.842 in the validation scenarios.

Distribution and genetic variation of *Thecaphora amaranthi* in amaranth crop regions in Mexico

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Phytopathology 101:S123

Thecaphora amaranthi was reported in the amaranth crop in Tlaxcala State some time ago. Due to the importance of this amaranth smut for infecting the inflorescence ovaries and can replace the seeds, and besides the farmers behavior of each year exchanging amaranth seed, it was advisable to know the distribution of the disease and the smut genetic variation. Six production lots of *Amaranthus hypochondriacus* located in regions of four States and Distrito Federal were sampled. From each lot nine apparently smut panicles were collected for sori examination, morphological identification, characterization by PCR: ITS-rRNA, sequencing, and genetic variation analyzed with *Alu*, *Alu+Hae*II, *Hpa*II and *Hae*III restriction enzymes by RFLP. *T. amaranthi* was identified at grain physiological maturity stage. Its distribution was only in tree production lots in regions of two States; Ozumba and Montecillo in Mexico State, and San Miguel del Milagro, Tlaxcala (this last one was the same region and State where was previously reported). Genetic variation was showed for two isolates, each of them, from each of the two regions in Mexico State. Even though the movement of farmers seed, the amaranth smut was identified in only one more State although showed genetic variation in the new registered regions.

Primary postharvest evaluation of chemicals as inducers of resistance against *Penicillium digitatum* and *Penicillium italicum* on citrus fruits

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Various synthetic or natural compounds have been described as capable of controlling a large variety of plant diseases without showing a direct antimicrobial activity. Eight of these chemical inducers (potassium silicate (PSi), acetyl salicylic acid (ASA), β-aminobutyric acid (BABA), 2,6-dichloro-isonicotinic acid (INA), sodium silicate (SS), harpin (H), benzothiadiazole (BTH), and salicylic acid (SA)) were evaluated as postharvest treatments to induce resistance to citrus green and blue molds, caused by *Penicillium digitatum* and *Penicillium italicum*, respectively. For each pathogen, 30 µL of the chemical solution at least at three concentrations of active ingredient were placed, using a micropipet, in a rind wound. About 24 h later, 30 µL of conidial suspension were inoculated in an adjacent new wound. For each combination of chemical, concentration, and pathogen, 4 replicates of 5 oranges each were used per treatment. Treated fruit were incubated at 20°C and 90% RH for 7 days before determination of disease incidence and severity and pathogen sporulation. Four of the eight chemicals somewhat induced resistance to molds. On 'Valencia' oranges, PSi (300 mM) and BABA (0.3 mM) significantly reduced blue mold incidence by 50 and 37%, respectively, and INA (0.03 mM) reduced green and blue mold incidence by 26 and 58%, respectively. On 'Lanelate' oranges, BTH (0.9 mM) reduced green mold incidence by 20%. No significant effect was observed on disease severity and pathogen sporulation.

Detection of *Ralstonia solanacearum* in Hawaiian field soils and evaluation of composts for suppressing pathogen populations

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Phytopathology 101:S124

Bacterial wilt caused by *Ralstonia solanacearum* is the most important disease affecting edible ginger (*Zingiber officinale*) in Hawaii. Serious outbreaks began occurring in 1993 and large losses continue every year. *R. solanacearum* is persistent in soil and following crop failure, fields are abandoned and left unsuitable for ginger production for many years. While PCR detection of *R. solanacearum* in ginger tissue and water is straightforward, detection in soil has been problematic. DNA extracted from field soil rarely produced a *R. solanacearum*-specific PCR product even when collected from a field affected by bacterial wilt. We evaluated several enrichment-PCR methods and found them useful in determining the presence, viability and relative abundance of the pathogen. Soil sample extracts allowed to enrich in selective media over a period of 96 hours routinely yielded *R. solanacearum*-specific PCR products after 24 or 48 hours, even from fields not planted with ginger for several years. In several cases, fields that tested positive using enrichment PCR were planted with ginger, with large losses in the ensuing crop. In one case, after initial disease onset, the grower applied high rates of a bran-enriched compost and avoided significant losses. We are performing controlled greenhouse studies, using enrichment PCR for evaluating various soil amendments, including compost and vermicompost preparations, for their ability to reduce pathogen populations in naturally infested field soil.

Corn yield components affected by controlling needle nematodes

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Phytopathology 101:S124

A field known to have needle nematodes present was selected as the site to determine plant responses that may relate to yield improvement (stand, plant height, tasseling date, ear length, ear weight, or number of rows of kernels per ear) in response to products that reduce nematode feeding damage. All plants in the middle two rows of four row plots were evaluated for leaf collar numbers when the plants were in the four to seven collar stages, and marked with a colored stake near the base of each plant. Evaluations from that point were recorded by stake color (leaf collar stage). Competitive seed applied nematicide resulted in significantly lower ($P = 0.015$) stands at emergence, but all treatments resulted in similar final stands. No significant differences were observed among treatments for plant height, tasseling date or the number of rows of kernels per ear. Ear length increased when the treatment had a product that protects from early season nematode damage compared to Poncho 250 or Poncho 1250 for plants at V5 ($P = 0.02$), V6 ($P = 0.081$), and V7 ($P = 0.069$) and ear weight followed the same trend ($P = 0.132$ to $P = 0.231$). The ears of the smaller plants (V5 and V6) were closer in length and weight to the larger plants (V7) when a nematode control product was part of the treatment. Poncho/VOTiVO and Poncho 1250/VOTiVO had the highest total yield although it was not significant.

Control of *Fusarium virguliforme* (sudden death syndrome) with a seed treatment

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Field results from 2010 testing in a soybean trial with severe symptoms of sudden death syndrome (SDS), causal pathogen *Fusarium virguliforme*, demonstrated that plots grown from seed treated with L1940-A exhibited little or no visual symptoms of SDS while adjacent plots without L1940-A as a seed treatment had severe symptoms. Later in 2010, green house experiments and a paper towel assay were completed to verify field observations. The paper towel assay resulted in the untreated control seed having 55% of the seeds showing *F. virguliforme* mycelial growth, while L1940-A at 0.05 mg ai/seed had 2.5%, Poncho/VOTiVO + L1940-A at 0.1 mg ai/seed had 7.5% and L1940-A at the 0.15 mg ai/seed application rate had no seeds with mycelial growth. The green house trial resulted in significantly lower incidence and severity of SDS with treatments that contained L1940-A compared to the untreated control.

Integration of balanced crop nutrition and Chlorpyrifos in management of Coffee Berry Borer, *Hypothenemus hampei* (Coleoptera: Scolytidae) in Kenya

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Phytopathology 101:S124

The control of Coffee Berry Borer, *Hypothenemus hampei* (Ferrari) using cultural, chemical and biological strategies has remained a challenge. The cultural control is tedious and expensive, chemical control is ineffective when not applied on time because of unique life cycle of the borer while biological control has remained not very promising. To address the issue of Berry Borer control, use of balanced crop nutrition integrated with chlorpyrifos as foliar sprayed insecticide was assessed. Three different soil fertilizers were used as source of balanced crop nutrition. A field trial was laid out on a main coffee block planted with Ruiru 11, a coffee cultivar resistant to both the Coffee Berry Disease and Leaf Rust caused by *Colletotrichum kahawae* Waller and Bridge and *Hemileia vastatrix* Berkeley and Broome, respectively. Soil fertilizers; NPK 17:17:17, NPK 22:6:12 and Organic Compost (NPK 0.8:0.2: 1.0) were applied in three different coffee sub blocks each with two plots, one sprayed with chlorpyrifos and unsprayed. The mean Berry Borer infestation for three years ranged between 1.16% and 19.39%. Except in one year, the Berry Borer infestation remained below 10% under any treatment, a level that was below the economical injury level. The study indicated that the three balanced soil applied fertilizers assessed, when integrated with Chlorpyrifos or applied alone controlled the Berry Borer.

Inheritance of resistance to Fusarium root rot in common bean

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Phytopathology 101:S124

Fusarium root rot (FRR) is a major disease of common bean. Knowledge of inheritance of resistance is important in developing resistant varieties. A 12 × 12 full diallel mating scheme generated 132 F1 progenies that were advanced to F3. Progenies were evaluated for resistance to FRR in a screen house. GCA effects were significant ($P \leq 0.01$) for disease scores. SCA effects were not significant ($P \leq 0.05$) in the F1, but were significant ($P < 0.01$) in the F3 indicating that resistance was governed by both additive and non-additive gene effects. Reciprocal differences were significant ($P \leq 0.01$) reflecting influence of maternal effects. Non-maternal effects were strong in the F3, suggesting a complex form of cytoplasmic-genetic interaction. Average heterosis in most crosses were equal in each of the generations, indicating that epistasis was probably more influential than dominance of individual genes. Bi-modal distributions were characteristic of F3 distributions, and fit expected ratios for 2 or 3 segregating loci. Parent-offspring heritability estimates were moderate. Results indicated that resistant parents contain a number of different resistance genes that can be combined to produce strong and durable resistance. Lines MLB-49-89A, MLB-48-89A, RWR719 and Vuninkingi, with large and negative GCA effects contributed high levels of resistance in crosses and would be recommended for use in breeding programs.

The implications of non-crop hosts in the epidemiology of *Tomato spotted wilt virus* in the *Solanaceae* of Georgia

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Phytopathology 101:S125

The southern Georgia farmscape offers an environment highly conducive to disease and insect pressure. Although first reported in Georgia in 1986, *Tomato spotted wilt virus* (TSWV) was not considered a problem until 1989. Since that time, farm gate losses in agricultural community have been devastating. The cultivation of several susceptible crops in close proximity to each other, the year-round availability of numerous, non-crop host species, the widespread presence of thrips vectors such as *Frankliniella occidentalis* and *F. fusca*, and the temperate environment conducive to TSWV infection cycles makes its control difficult. Observations focused on the farmscape have opened up new insights into the epidemiology of TSWV. The role non-crop hosts play in the epidemiology is crucial in understanding the overall disease cycle since inoculum sources of TSWV from these hosts could be a major part of the dynamic. A wide ranging screening of non-crop hosts of TSWV was begun in 2002 and continued for 9 years. During the study, over 100,000 weed samples were collected and screened for TSWV. Overall, approximately 5% were infected with TSWV. Seasonal infection levels and potential keystone species were identified and correlated with the associated crop's infection levels. While the farmscape's level of potential virus inoculum explained a portion of the disease dynamic, there is still much that is not known about the epidemiology of TSWV.

Detection of *Fusarium oxysporum* f. sp. *canariensis* and *F. proliferatum* from palms in southern Nevada

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Phytopathology 101:S125

Fusarium wilt is a serious vascular wilt disease on palms. *Fusarium oxysporum* f. sp. *canariensis* (FOC), primarily infecting Canary Island date palm (*Phoenix canariensis*), was identified from only one location in Las Vegas in 1999. The extent of the disease occurrence in southern Nevada is unknown. During 2009 and 2010, we received symptomatic frond samples collected from 10 *P. canariensis* and 1 California fan palm (*Washingtonia filifera*) in Las Vegas and Henderson. Samples when plated on PDA media rendered only growth of *Fusarium* species. Molecular identification was employed to determine their identities by amplifying, cloning, and sequencing a portion of genomic sequence diagnostic for FOC (GenBank Accession No. AF118442). Ten isolates obtained from *P. canariensis* were identified as FOC, while the isolate from *W. filifera* as *F. proliferatum*. Of the 9 FOC isolates, 5 had a DNA sequence 100% identical to that of FOC isolate 703C (Accession No. FJ895295.1) and remaining 4 had 100% identical to FOC isolate 2675A (Accession No. FJ895298.1). The two groups of FOC had significant sequence gap and nucleotide variations. Compared to FOC sequences, *F. proliferatum* had a 33bp-long gap and 24 nucleotide differences. To our knowledge, this is the first report confirming FOC in Henderson, NV and documenting its occurrence in two urban areas of southern Nevada. Our study suggests that 2 types of FOC have been introduced and spread to more urban palm trees.

Fluopyram products for the control of diseases of horticultural crops

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Fluopyram is a new fungicide active ingredient in development worldwide by Bayer CropScience. Application for registration is pending with the Environmental Protection Agency with an expected registration summer or fall of 2011. Luna products are providing excellent crop quality at-harvest and in storage / transportation from both early season and late season applications. Disease control in several perennial crops has been excellent and has resulted in healthier trees with reduced disease in the following year. Fluopyram is root systemic and providing excellent foliar disease control from preventative applications to the soil. Multiyear trial results will be presented.

The participatory training of farmers in integrated production and pest management using the Farmers's Field School approach in Burkina Faso, 2001–2010

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Phytopathology 101:S125

The Burkina Faso participatory integrated production and pest management training program was launched in 2001. The first two phases of the program took place respectively from 2001 to 2005 and from 2006 to 2010. The program is funded by both the Burkinabé government and the Dutch government and is implemented by FAO. The program trained some 15,000 farmers. These training activities employed the farmer's field schools methodology and were completed on rice, vegetable and cotton-based cropping systems. The effects of the training were evaluated through a follow-up system. The average yield of rice of trained farmers' fields was 26% higher than the yield of rice of the same farmers before the training. The same trend was observed for vegetables. The training on cotton-based cropping systems was done in the perspective of intensification and diversification. The average gross margin with IPPM was 2.5 times that of farmers' practices while dealing with cotton. With corn, the IPPM average gross margin represented 3 times of farmers' practices. Other results of the IPPM program included institutionalization, promotion and sustainability and, the training of graduate students. The main constraints to the development of the program included illiteracy of the majority of farmers the weakness of farmers' organizations, poor equipment and low supply of agricultural inputs as well as low market of rice and vegetables and the instability of the price of cotton on the international market.

Development and validation of Citrus leprosis virus-C (CiLV-C) molecular detection and identification methods for use in regulatory diagnostic assays

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In the last decade Citrus leprosis virus-C (CiLV-C) spread fast from South America to Central America and recently recorded in Mexico. This proximity and the potential damage cause concerns in the United States, where the disease has not been present since the 1960s. We developed two multiplex one-step conventional RT-PCR protocols for the detection and identification of CiLV-C in citrus plant samples. One of these protocols is based on the CiLV-C-specific published primers MPF and MPR that amplify a 339 bp fragment from the movement protein gene in RNA 2. The other protocol is based on CiLV-C published primers K565 and K568 that amplify a 414 bp fragment of ORF 2 in RNA1. To develop a multiplex one-step RT-PCR we amplified each of these assays together with a Nad5 internal control assay targeting plant RNA. We also developed a multiplex one-step TaqMan real-time RT-PCR protocol for the detection and identification of CiLV-C in citrus plant samples. This assay targets a segment of the movement protein gene on RNA2 using CiLV-specific primers (CiLVf & CiLVr) and TaqMan FAM labeled probe (CiLVp). The reverse primer CiLVr and the probe CiLVp were designed by NPGBL, while the forward primer CiLVf, based on the published primer MPF, was modified by NPGBL. The one-step real-time RT-PCR was similarly multiplexed with a Nad5 internal control assay targeting plant RNA. These new protocols were used to detect CiLV-C in citrus samples from Costa Rica and Panama.

Development and validation of a multiplex one-step RT-PCR for the improved detection of potyviruses infecting imported germplasm

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Phytopathology 101:S125

Foreign plant germplasm is a valuable source of novel genes not present in the U.S. plant gene pool. Potyviruses infecting imported plant germplasm can cause serious problems in agricultural, rural and native plant systems. The previously developed universal potyvirus NIb primer pair (NIb2F and NIb3R) was reported to detect all tested 40 potyvirus isolates representing at least 23 species. To increase speed and accuracy a multiplex one-step RT-PCR assay was developed using the NIb primer pair and an internal plant RNA control (Nad5). The assay was validated and head-to-head compared with two other primer pairs (HP and CI) routinely used for potyvirus detection in diagnostic laboratories. Twelve different host species suspected to be infected with potyviruses were tested. The one-step multiplex RT-PCR assay produced the expected potyviruses amplicons of 350 bp as well as the host RNA internal quality control amplicon of 180 bp from all tested samples. The previously published potyvirus primer pairs produced inconclusive results for six (HP) or three (CI) of the virus isolates. PCR-amplified DNA fragments produced using the NIb primers were cloned and sequenced to verify the specificity of the assay. Eleven known potyvirus species and five previously uncharacterized potyviruses were identified. This multiplex one-step RT-PCR assay is well suited for the detection of known and unknown potyviruses in samples.

Proteome reference map for the soybean cyst nematode

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Phytopathology 101:S126

Soybean cyst nematode is the most destructive pathogen of soybean worldwide causing an estimated $2 billion in losses annually. Proteomic technologies are powerful tools to examine protein expression profiles as well as modification of proteins. We adapted these tools to investigate pathogenesis of SCN. We investigated and optimized protein extraction protocols and resolved several SCN proteins by two-dimensional gel electrophoresis. Three different protein extraction methods including phenol/ammonium acetate, thiourea/urea solublization (lysis method) and trichloroacetic acid/acetone were evaluated to determine their efficacy in separating SCN proteins by 2-DE. The phenol method showed higher protein resolution and spot intensity of all proteins compared with the other two methods. In addition, within the high-pI region, proteins resulting from phenol based extraction were well resolved and strongly detected. Protein spots obtained from the phenol method were subjected to matrix-assisted laser desorption/ionization time of flight mass spectrometry or liquid chromatography mass spectrometry to test their quality. In continuation of this project, we are also investigating differentially regulated proteins of infected root among resistant and susceptible soybeans. This information will help us to have a greater ability to identify the pathogen, understand its biology, host-pathogen interactions, and ultimately, to formulate improved disease management practices.

Virus-like particles of *Maize rayado fino virus*, *Cucumber mosaic virus*, and *Lolium latent virus* as chemical bio-conjugate substrates

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Phytopathology 101:S126

Plant viruses and virus-like particles (VLPs) are highly organized structures with unique chemical and physical properties. These properties can be exploited to generate nano-materials which have multiple uses. Novel VLPs can be produced by either genetically modifying the viral genome or by chemically attaching *in vitro* a variety of ligands, including fluorescent dyes, polymers, peptides and carbohydrates, to reactive groups on the viral capsids. Each of the subunits forming the VLPs potentially represents a platform to display functional groups, with control over spacing and orientation. Combining molecular biology, chemistry, and nanotechnology techniques, we explored the possibility to perform chemical modifications on the isometric VLPs of *Maize rayado fino virus* and *Cucumber mosaic virus* and the flexuous *Lolium latent virus*. The orthogonal reactivity and the suitability to serve as chemical bio-conjugate substrates was tested using cysteine side chain thiol-reactive probes including fluorescein-5-maleimide and lysine side chain amine-labeling reagents, including NHS-Fluorescein. Fluorescently labelled particles were analyzed by SDS-PAGE and by biotinylation assays demonstrating the feasibility of these reactive amino acids to be used in more advanced conjugation chemistries.

Effects of *Coniothyrium minitans* strains on viability of sclerotia of soybean white mold fungus

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Phytopathology 101:S126

Soybean white mold fungus (*Sclerotinia sclerotiorum*), preserves its sclerotia viable from three to several years, and degeneration of sclerotia are difficult. Therefore, alternatively antagonistic fungi have role mycoparasitising sclerotia. We compared the effectiveness of *Coniothyrium minitans* (CM) of Contans® WG and a newly isolated strain CMN09 from sclerotia of white mold infected plants, northeast research and demonstration farm, Nashua, IA in 2009. Freshly grown sclerotia on PDA were aseptically spread in 9-cm glass Petri dishes (20 per dish) containing sterilized (SS) and unsterilized (USS) wet soil (80 g soil + 20 ml DSW). There were 4 treatments of each of (a) Contans-CM spray, (b) CMN09 spray and (c) unsprayed controls. In aseptic conditions, 10 plates each of SS and USS were spray-inoculated with pycnidiospores suspension of Contans and another 10 plates each of SS and USS with pycnidiospores of CMN09. The spray suspension of each strain carried 2.2×10^8 pycnidiospores/m^2. Five plates each from SS and USS were un-inoculated controls. Inoculated and un-inoculated plates were sealed with Parafilm, and a set of five plates from (a), (b) and (c) were incubated at 23°C, and another set at 3°C in 12 h fluorescent light. At 15 d interval for 90 days, 10 sclerotia from each treatment were sampled, surface sterilized and plated on PDA. Our observations showed that the sclerotia inoculated with CMN09 had low to 0% viability in SS compared with Contans and controls, indicating CMN09 may be more aggressive than Contans.

Elucidation of negative interactions between glyphosate and azoxystrobin and effects on *Rhizoctonia solani* severity under field conditions

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Roundup Ready sugar beet varieties and their wide use has led to an increase in glyphosate (GLY)-containing herbicides. Recent studies showed a reduction in efficacy of azoxystrobin (AS) when applied with GLY. Azoxystrobin is used to control *Rhizoctonia solani* AG 2-2 crown and root rot (RCRR) which can cause losses up to 50%. Interactions between GLY and AS might be of great importance since a combined application would be desirable to decrease application costs. The objective of this study was to test tank-mix applications of AS and GLY for their ability to control RCRR. The factorial experiment consisted of conventional and GLY based weed management practices combined with different AS applications timings. Tank-mix applications were applied as a banded or broadcast application. Reduced AS efficacy resulting from negative interactions between products was not observed. Data showed highly significant differences between conventional and GLY-based weed management practices with GLY increasing final stand and reducing RCRR. Weed competition in conventional plots led to a decrease in vigor resulting in smaller beets that could have been more susceptible to infections with RCRR. Comparing infected control plots of each weed management practice showed no significant differences for disease severity, but GLY treated plots showed a slight reduction (6%) in disease severity, verifying the absence of negative GLY effects.

A commercial extract of the brown seaweed Ascophyllum nodosum suppresses thrips in peppers, cucumbers and Hass avocados

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Phytopathology 101:S126

Thrips are found worldwide and cause damage to vegetables, fruits, and flowers. Some thrips are vectors of diseases such as Tomato Spotted Wilt Virus. A proliferation of thrips may also cause respiratory and skin irritation to workers. Effectively managing thrips with non-toxic materials has proven to be one of the most challenging aspects of natural pest control. An extract from the brown seaweed, Ascophyllum nodosum, harvested sustainably in Nova Scotia, reduced leaf deformation from Western Flower Thrips (Frankliniella occidentalis) based on leaf area measurements by 158% compared to the control on greenhouse-grown jalapeno peppers. Trials on greenhouse-grown cucumbers demonstrated a 54% reduction in the amount of leaf area damaged by thrips when plants were treated with A. nodosum extract compared to the water-treated control. Field-grown Hass avocado trees had 68% fewer Avocado Thrips (Scirtothrips perseae) per leaf compared to the control. This reduction in thrip numbers was not significantly different from abamectin; the most common chemical control for this insect in avocados. In addition, there were 87% less colonies of Persea mites (Oligonychus perseae) per leaf in the A. nodosum-treated trees compared to the control, which was also not significantly different from the abamectin standard. A. nodosum extract applications result in significantly less feeding damage by thrips on greenhouse-grown peppers and cucumbers and field-grown avocados.

Functional analysis of the Cucumber mosaic virus 2b protein

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Phytopathology 101:S126

Cucumber mosaic virus (CMV) is an economically important plant virus with a broad host range. The genome of CMV consists of three single-stranded, positive-sense RNA molecules, which encodes five proteins. The smallest one (110 aa) is the multifunctional 2b protein encoded by the RNA2. The 2b protein has a function in symptom induction, in viral movement, suppression of RNA silencing and as an antagonist of the salicylic acid mediated defense response. In our work the alanine scanning mutagenesis of the 2b protein was carried out. In the infectious clone of Rs-CMV the three-three consecutive amino acids of the 2b protein were replaced with alanine. The infectivity of the 37 mutant clones was tested on *Nicotiana clevelandii* plants in the presence of RNA 1 and 3. The infection was monitored by Northern analysis of the inoculated, and the systematically infected leaves, and the stability of the mutants was verified by nucleic acid sequence determination after RT/PCR. The majority of the mutant viruses caused similar symptoms as the

original Rs-CMV. In these cases the sequence analysis confirmed the stability of the mutations. In the case of six mutants symptoms were not observed and the presence of viral RNA was not detected in the non-infected leaves. In two cases the symptoms developed later, and in two further cases the test plants recovered. Our results will be discussed in relation with the known structure of the 2b protein. The project was supported by OTKA K75168 grant.

North Dakota populations of *Leptosphaeria maculans* are becoming more diverse

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Phytopathology 101:S127

Canola (*Brassica napus*) was introduced to North Dakota more than 25 years ago. Currently, >90% of the canola produced in the U.S. is grown in North Dakota. Blackleg, caused by the fungus *Leptosphaeria maculans*, became endemic in canola growing areas in mid 1980s. This disease is capable of reducing yield by >50%. In 1991, *L. maculans* isolates retrieved from infected plant tissues were determined to belong to pathogenicity groups (PG), 1 and 2. This classification was made using a set of three *B. napus* differentials, Quinta, Glacier and Westar. We collected isolates in 2004, 2007, and 2009 and phenotyped using the same differentials. Each of 195 isolates were inoculated on three sets of six plants from each differential by depositing 10 µl of a 10^7 pycnidiospores ml^{-1} suspension on tiny wounds made with sterile needles on the cotyledons leaves. Reaction to inoculation was recorded 10 days later using a 0–9 severity scale. Prevalence of each pathogenicity group was estimated for each year and across all counties and Simpson's diversity index was calculated. The index values of 0.2, 0.25, 0.3 and 0.46 in 1984–2001, 2003, 2007, and 2009 respectively indicated that only few PGs were dominant earlier but now it is more diverse and high proportion of new PGs (PGT, PG3 and PG4) have been introduced in ND. Presence of highly aggressive strain of *L. maculans* in high percentage and appearance of unknown groups poses a serious threat of this disease to canola industry in North Dakota.

Population genetic structure of the fungus *Leptosphaeria maculans* in commercial canola (*Brassica napus*) fields in North Dakota

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Leptosphaeria maculans, is a fungal pathogen that causes blackleg or phoma stem canker of canola (*Brassica napus*) worldwide. In North Dakota, blackleg is one of the most devastating diseases of canola and the severity of the disease is increasing with occurrence of new pathogenicity groups. To study the genetic structure of the blackleg population in North Dakota, 276 isolates of *L. maculans* were collected from infected stubbles from commercial canola fields in ten North Dakota counties over a period of two years. Isolates within a county were considered a population. Populations were analyzed using six microsatellite markers. A total of 229 haplotypes were identified and high gene diversity (H = 0.454 to 0.682) was observed in the populations. High level of population differentiation (G''_{ST} = 0.149 to 0.683, $p < 0.001$) was observed among most of the pair-wise comparison between the populations. Analysis of molecular variance (AMOVA) also indicated that 84% of the genetic variation was found within the population, while the remaining 16% was found among the populations. Further analysis with more number of samples per location and additional microsatellite and minisatellite markers will help us better understand the genetic structure of *L. maculans* from North Dakota.

Interactive effects of temperature and wetness duration on infection parameters of *Pseudoperonospora cubensis* in cucurbit varieties

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Downy mildew of cucurbits caused by the oomycete *Pseudoperonospora cubensis*, is one of the most important diseases affecting cucurbits. A model based on a modified Weibull function was recently developed to quantify the effects of temperature and leaf wetness duration on sporangia germination and host infection. However, differences in host resistance on the predictive ability of the model have not been determined. Three cucurbit host types namely, cucumber (cv. Straight 8), cantaloupe (cv. Kermit) and squash (cv. Table Queen), were inoculated with *P. cubensis* and exposed to constant temperatures of 5 to 30°C during leaf wetness durations of 2 to 24 h in growth chamber experiments. Germination was assessed after each wetness period, while leaf area infected was assessed 5 days after inoculation. Germination and infection data were fitted to the model using nonlinear regression. Cultivar, temperature, wetness duration and their interactions significantly ($P < 0.0001$) affected germination and disease severity. For example, at 20°C, 15% leaf area infected was expected following 2, 4 and 8 h of wetness for Straight 8, Table Queen and Kermit, respectively. When temperature was increased to 25°C, 15% disease severity was expected following 3, 7 and 15 h of wetness for Straight 8, Table Queen and Kermit, respectively. Based on model parameters, host based nomograms were developed to predict infection risks based on combinations of temperature and leaf wetness duration.

Microsatellite profile of Puccinia psidii in Hawaii and South America

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Phytopathology 101:S127

Rapidly mutable microsatellite markers were used to assess the genetic relationships among rust populations from South America and Hawaii. Such genetic markers provide reliable genetic information, allowing inferences about potential sources of pathogen introduction. The hypothesis is that Puccinia psidii populations from South America are distinct from the rust populations that became established in Hawaii. The eight microsatellite loci analyzed revealed 14 multilocus genotypes (MGs) within the 221 P. psidii isolates. Isolates collected on different hosts in South America (Eucalyptus spp., Psidium guajava, P. araca, Syzigium jambos, S. cumini, Myrciaria cauliflora, and Eugenia uniflora) presented distinct MGs. In constrast, all rust isolates collected on nine myrtaceous hosts in the Hawaiian Islands (Metrosideros polymorpha, M. excelsa, Eugenia koolauensis, Rodomyrthus tomentosa, Myrtle communis, S. samarangense, M. quinquinervia, S. cumini, and S. jambos) were composed of only a single unique MG. The MG comprising all isolates from Hawaii is distinct from the MGs found in South America so far, suggesting that the Hawaiian isolates did not come directly from South America. Isolates from California, Florida, Central America and Caribbean must be analyzed to better understand potential relationships with pathogen dispersion to Hawaii.

Multilocus genotypes indicate selection by host in Puccinia psidii populations from Brazil

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Population genetic approaches were used to determine the genetic structure of the rust fungus Puccinia psidii in Brazil, believed to be the pathogen putative center of diversity. Ten microsatellite markers were used to examine the amount and distribution of genetic variability within 148 rust isolates collected on seven Myrtaceae hosts across a wide geographic area. Analysis of molecular variance indicated no genetic differentiation among isolates from different geographic locations, and high differentiation between isolates from different hosts (97%, $P > 0.001$). Principal coordinate plots, also indicated high degree of genetic differentiation among isolates collected on different host species, revealing five major groups. The Neighbor Joining tree also clustered the rust isolates on five groups, based on host of origin. The high proportion of repeated multilocus genotypes within each host, combined with high values of IA and rD, and low values of FIS, indicates high rate of clonal reproduction. The haplotype MJ-Network also supported the hypothesis of host selection and clonal reproduction. Microsatellite data indicates potential selection by host and high rate of clonal reproduction on P. psidii population from Brazil. Phylogenetic studies are underway to check the possible existence of cryptic rust species.

Resistance to the stem rust 'Ug99' race group in spring wheat landrace accessions from the USDA-ARS National Small Grains Collection (NSGC)

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Phytopathology 101:S127

Germplasm collections of crop landraces conserve genetic diversity and may contain novel sources of disease resistance. New sources of stem rust resistance are needed to help manage the *Puccinia graminis* f. sp. *tritici* 'Ug99' race group (TTKS lineage). From 2007 to 2011, nearly 3000 spring common wheat landraces from the NSGC were screened for resistance in eight field seasons at the Kenya Agricultural Research Institute, Njoro, where the Ug99 race group is endemic. Most accessions were selected for screening based on resistance to U.S. races, but approximately one-third were chosen randomly or by geographic origin. Accessions showing resistance in one season were re-tested in Njoro and screened as seedlings against race TTKSK. Resistant accessions were also screened with molecular markers diagnostic for *Sr2*, *Sr24*, and *Sr36*. To date, field results are available for seven of eight seasons and 165 accessions have shown resistance in more than one season. With 57% of the marker screening completed to date, 8 and 11 of the resistant accessions were positive for markers associated with *Sr36* (wmc477) and *Sr2* (csSr2), respectively. Less than 5% of the field-resistant accessions that were negative for *Sr2* were susceptible to TTKSK as seedlings, suggesting potential new sources of adult plant resistance (APR). To genetically characterize the resistant accessions, association mapping studies are being conducted and bi-parental mapping populations are being developed.

Evidence for multiple fungicide resistance in field populations of *Venturia inaequalis*

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Phytopathology 101:S128

Apple scab caused by *Ventuaria inaequalis* is the most economically important fungal disease of apple in the eastern United States. Site-specific fungicides remain the most effective tool for scab management in commercial apple production but they are prone to development of resistance. All scab management programs in the region therefore recommend rotating fungicides with different chemistries to mitigate resistance development. Analysis of a large sample of *V. inaequalis* population (4841 isolates from 131 orchards in 14 states) tested for resistance to dodine, the sterol demythelation inhibitor (DMI) myclobutanil, and the quinone outside inhibitor (QoI) tryfloxystrobin indicated a highly significant ($P < 0.0001$) incidence of isolates with resistance to the three fungicides. The odds of an isolate resistant to myclobutanil also being resistant to dodine were more than twice (odds ratio = 2.31; 95% confidence interval [CI] = 1.95 to 2.75) those of a sensitive isolate, and a DMI-resistant isolate was >2.5 times as likely to be quantitatively resistant to the QoI fungicide (odds ratio = 2.69; 95% CI = 2.30 to 3.16) compared to a DMI-sensitive one. Moreover, the incidence of isolates with resistance to at least two of the three fungicides was significantly higher ($P < 0.0001$) in orchards subjected to a total of ≥20 or ≥15 applications of a DMI or a QoI fungicide, respectively, by the time of the survey. These results suggest a reassessment of the current strategies for resistance management is required.

Spatial and temporal patterns of insect damage and aflatoxin contamination in corn at pre-harvest

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Ear-feeding insect damage and aflatoxin contamination are the key impediments to corn yield and quality under warm climatic conditions worldwide. A series of experiments have been conducted to examine the contribution of insect damage to aflatoxin contamination. To assess the spatial and temporal patterns, aflatoxin contamination and insect damage was sampled twice with a 4-wk interval before harvest in 2008 and 2009 using a grid-sampling method. The feeding damage by each of the ear/kernel-feeding insects (i.e., corn earworm/fall armyworm damage on the silk/cob, and discoloration of corn kernels by stink bugs), and maize weevil population were assessed at each grid point with five ears. The spatial distribution pattern of aflatoxin contamination was also assessed using the harvested corn samples from each sampling point. The aflatoxin level was not correlated to the number of maize weevils, but correlated to stink bug-discolored kernels in the corn fields in 2008, whereas the 2009 data showed the opposite. The maize weevil infestation, stink bug-damaged kernels, and aflatoxin levels also showed a clustered distribution pattern with a strong edge effect across the fields. The comparison of the results from the two-sampling dates showed that temporal pattern of aflatoxin levels was only changed in 2009, but not in 2008. The separation of silk- and cob-feeding from kernel-feeding insects and their damage in relation to aflatoxin accumulation and its management strategies will also be discussed.

Effect glucorafano isolated of broccoli florets on the germination of *Rhizopus stolonifer* spores

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Phytopathology 101:S128

Rot caused by *Rhizopus stolonifer*, is one of the most severe postharvest diseases of strawberry, the strategy most used to control disease, is the pre-and post-harvest treatment with fungicides, but their use is increasingly restricted due to public awareness of hazardous waste in the fruits. Glucosinolates are natural products containing nitrogen and sulfur and its antimicrobial activity has been shown in other research. For this work, we collected fruits of strawberry, with symptoms of rot: from them it was isolated and identified the fungus *Rhizopus stolonifer*. The spores of the pathogen were placed on PDA with different glucoraphane concentrations (1.54, 0.92, 0.46, 0.15, 0.02 y 0 mug muL^{-1}) isolated from broccoli florets. We measured spore germination until the control treatment show the highest percentage of germination. The median lethal concentration was 1.01 mug muL^{-1} and the concentration that completely inhibited the germination of spores was 2.16 mug muL^{-1}.

The effect of imazalil on Colletotrichum gloeosporioides isolated from avocado (Persea americana) fruit

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Colletotrichum gloeosporioides is the causal agent of anthracnose in avocado, leading to production losses of almost 20%. Nowadays, the most useful strategy for the control of the disease is the fungicide treatment; however, in Mexico there are only a few compounds authorized for postharvest use. For this reason, the effect of imazalil on the mycelium growth and spore germination in vitro was evaluated in this study. The fungus was isolated from avocado fruits from Uruapan, Michoacan. Twelve concentrations of imazalil (0.01, 0.1, 1.0, 5.0, 10, 50, 100, 200, 400, 600, 800 and 1000 ppm) were evaluated in vitro. The fungicide doses corresponding to each treatment were previously dissolved in the culture medium. A completely random experimental design was used. For the evaluation of the mycelial growth, 5 plates per treatment were used, and for the spore germination 100 spores were evaluated for each treatment. The variance analysis showed that the factors under study had a statistically significant effect (Pr> F = 0.0001) on the mycelial growth and spore germination. The effect of imazalil was observed from the 5 ppm dose, which provided an efficacy of 79.86%, while from 10 ppm up to 1000 ppm the efficacy reached 100%. In addition, it was found that the LC 50 of imazalil for the control of C. gloeosporioides is 0.79 ppm. Imazalil proved to be excellent for controlling C. gloeosporioides in vitro. However, in Mexico it has not been authorized for its use on avocado.

Survey of *Erwinia amylovora*, causal agent of fire blight, from apple and pear orchards in Utah for streptomycin resistance

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Fire blight caused by the bacterium *Erwinia amylovora* results in millions of dollar in losses worldwide. It is the most important disease problem for apple and pear growers in Utah. Currently the only effective management strategy is the application of streptomycin. In 2006, resistant isolates were detected in an apple orchard for the first time in one Utah county. To determine the distribution of resistant isolates and the level of resistance, isolates collected in 2006, 2007, 2010 and 2011 from apple trees across the state were tested for resistance to streptomycin. Each isolate was initially screened at 0, 100 and 1000 ppm of streptomycin. Bacteria were quantified with a spectrophotometer and 100 microliters were spread on LB agar. A whole was punched from the agar and the streptomycin solution was pipetted into the well. The plates were evaluated after 24 hours for bacterial growth. A bacteria-free zone around the well was observed for sensitive isolates that was not seen with resistant isolates. Isolates sensitive at 100 ppm were tested at lower concentrations and isolates resistant at 1,000 ppm were exposed to higher concentrations. The majority of resistant isolates were found in Utah County where most apple and pear orchards are located. Resistant Isolates tolerated at least 100,000 ppm of streptomycin.

Susceptibility of mesquite species to powdery mildew in Arizona

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Mesquite (*Prosopis* sp.) is a popular tree in landscapes in Arizona because of its drought tolerance and attractive growth habit. Powdery mildew has been observed from late summer until early spring on mesquite leaves. It has been identified as *Pleochaeta polychaeta* based morphological descriptions and comparison to herbarium specimens. To determine the susceptibility of different mesquite species to powdery mildew, 175 mesquite trees from eight species were surveyed for the presence of powdery mildew from fall 2008 until early 2009 on The University of Arizona campus in Tucson, AZ. Leaves were inspected under a dissecting scope for the presence of powdery mildew. Only the North American mesquite species *P. glandulosa* var. *glandulosa* and *P. velutina* were infected with powdery mildew. No powdery mildew was observed on *P. alba*, *P. nigrum*, *P. chilensis*, *P. pubescens* and *P. chilensis* × *flexuosa* in the vicinity of infected trees. The powdery mildew affects the aesthetic value of severely infected trees but seems to have little effect on long term tree health.

Root-knot nematode species in golf course greens in the Western U.S.A.

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Root-knot nematodes (*Meloidogyne* sp.) have been a problem in golf course greens in the Western U.S.A. for several years. Symptoms include irregular yellow patches in the turf grass that may eventually die. The problem with managing populations of Meloidogyne sp. was exacerbated after the only chemical product available for control on living turf was removed from the market in 2009. To develop alternative management strategies, it is important to determine which species of Meloidogyne are present in golf courses. Root-knot nematodes collected from over 100 golf courses in the western states were extracted from the soil using a mist extractor. Juvenile (J2) root-knot nematodes were identified using a dissecting scope. Individual J2s were cut in half in extraction buffer and lysed. PCR was conducted using primers amplifying the D2-D3 region of the 28S gene as well as the ITS region. Thus far we have detected, five known and two unidentified species. The known species identified are *M. naasi*, *M. minor*, *M. chitwoodi*, *M. marylandi* and *M. graminis*.

Microorganisms and antifungal properties associated with noni (*Morinda citrifolia*) fruit and fermented juice in Hawaii

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Noni (*Morinda citrifolia*), a medicinal plant grown in Hawaii and other Polynesian regions, is reportedly therapeutic for diabetes, high blood pressure, and certain types of cancer. Noni fruit often produce fermented juice that differs in chemical, physical and microbial properties. To determine how storage factors affect juice quality we studied the occurrence of microorganisms during noni fermentation and also report on antifungal properties of pasteurized juice exudates in *in vitro* bioassays. Firm, yellow, mature noni fruit were held in sealed glass jars for up to 42 days at 22°C. Juice exudates were analyzed weekly for microbial populations and chemical properties. Puree of fresh soft fruit was also analyzed. Bioassays consisted of PDA plates spread with spore suspensions of fungal pathogens and spotted with juice, puree, or sterile distilled water. Bacterial populations did not differ from 0 to 35 days, but were highest at 42 days. *Mucor circinelloides* f. sp. *circinelloides*, a fungus consistently isolated from fermented juice samples, had populations that peaked at 14 days. Fresh noni puree was microbe-free or low in microbial populations. Total soluble solids (% TSS) were highest in fresh noni (9.8), then significantly decreased after 14 days (5.7) storage. In bioassays, the TSS range of noni puree or juice with antifungal activity against several pathogens of tropical fruit crops was determined: activity was absent at 4 to 5% TSS; intermediate at 6% TSS; and highest at 7% TSS or greater.

The plant growth-promoting rhizobacterium Bacillus cereus AR156 induces resistance in Arabidopsis thaliana and tomato

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Phytopathology 101:S129

We previously isolated a rhizobacterium Bacillus cereus AR156. It was shown to significantly protect tomato against bacterial wilt caused by Ralstonia solanacearum and root-knot disease caused by Meloidogyne incognita. Here, we investigate the ability of AR156 to promote plant growth and induce the systemic protection of Arabidopsis thaliana and tomatoes in greenhouses against bacterial speck disease caused by Pseudomonas syringae pv. tomato DC3000. Compared to mock-treated plants, AR156-treated ones showed an increase in biomass and reductions in disease severity and pathogen density in the leaves of Arabidopsis. The defense-related genes PR1, PR2, PR5, and PDF1.2 were concurrently expressed in the leaves of AR156-treated plants, suggesting simultaneous activation of the salicylic acid (SA)- and jasmonic acid (JA)/ethylene (ET)-dependent signaling pathways by AR156. The above gene expression was faster and stronger in plants treated with AR156 and inoculated with DC3000 than that in plants only inoculated with DC3000. Similarly, AR156 also increased the average biomass of the tomato and elicited induced systemic resistance (ISR) against DC3000. In the further study, we will investigate how AR156 can simultaneous activation of these pathways, which will be instrumental in improving the application of AR156 to plant protection. Guo JH is the corresponding author.

Development of microsatellite markers for population genetic analysis of *Waitea circinata* var. *circinata*

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Waitea circinata var. *circinata* is an emerging pathogen of turfgrass in North America. It causes brown ring patch of turfgrass in golf courses and amenity areas. Recent attention is on its almost countrywide distribution despite having been reported for the first time in U.S.A. in 2007. To better understand the population biology of *W. circinata* var. *circinata*, we have isolated eight promising microsatellite markers from an enriched genomic library. Seventy clones were arbitrarily selected from the library and sequenced. Of these, 48 (68%) contained microsatellites. Twenty-seven of the 48 candidate microsatellite loci shared sequence similarity with one another or had their repeats too close to the end of the sequence. Primers flanking the repeat region were designed for the remaining microsatellite loci and were initially screened on five isolates of *W. circinata* var. *circinata* from different geographic regions. A total of 10 (62%) microsatellites were polymorphic. Two of these had many null alleles when subjected to a larger sample size. The eight candidate microsatellite loci are further being characterized with additional 35 isolates of *W. circinata* var. *circinata*, two *W. circinata* var. *zeae* and three of *Rhizoctonia solani*.

Industry-wide assessment of methyl bromide alternatives and sting nematode management in Florida strawberry

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Florida field research continues to focus on a co-application approach of different fumigants, herbicides, and other alternative tactics to achieve pest control efficacy and crop growth response similar to that of methyl bromide soil fumigation. With increasing cost and reduced availability, growers are increasingly transitioning to a diverse array of methyl bromide alternative tactics, many of which have not been adequately evaluated in field research. Since 2009, over 50 commercial strawberry fields with recurring histories of nematode problems have been studied to characterize differential responses of fumigant alternatives using estimates of relative strawberry yield. Relative strawberry yields were determined from ground truth survey of plant size categories and with NDVI (Normalized Difference Vegetation Index) using GreenSeeker® (NTech Industries; Ukiah, Ca) optical sensors. Ground truth surveying of plant size distribution repeatedly demonstrated the accuracy of in-field, remotely sensed NDVI. Relative strawberry yields determined from ground truth survey of plant size categories was well correlated with NDVI estimates of canopy cover. Overall, the methodology is being used to provide growers guidance and quantitative performance data on alternatives to methyl bromide soil fumigation for nematode management on a farm by farm and industry-wide basis.

Large scale demonstration trialing of drip fumigants in Florida strawberry

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Phytopathology 101:S129

Three drip applied soil fumigants were evaluated as alternatives to methyl bromide for pest control efficacy and strawberry yield enhancement in five grower field trials. Soil treatments included chisel applied methyl bromide (50%) chloropicrin (50%) (288 – 320 lb/a), compared with the drip applied

fumigants including, metam sodium (75 gpa), Pic Clor 60EC (300 lb/a) and Telone Inline (35- 48 gpa) applied and evaluated with either one or two drip tapes per bed at either of two farm locations. Assessments of plant growth included differences in plant size, health, vigor, and yield with treatments arranged as a completely randomized block design with at least 4 replications per treatment. In general, a significant drip tape effect (1 vs 2) was observed with strawberry yield and plant growth improvement with methyl bromide chloropicrin (a horticultural effect). Strawberry plant growth and yield was significantly improved 10 to 15% when drip fumigants were delivered with two drip tapes per bed compared to one. With two drip tapes per bed, improvements in strawberry plant growth occurred as a result of a horticultural effect (improved water and nutrition) and also as a fumigation effect (improved movement and bed distribution of the fumigant). Additional research is required to validate the fumigant, horticultural, and economic benefits of the drip fumigants and additional drip tape.

Endospore forming bacteria indigenous to landscape planting beds and their inhibition of *Rhizoctonia solani*

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Indigenous endospore forming bacteria were collected from root samples of bedding plants grown in established commercial landscape beds. One hundred twenty-nine bacterial strains associated with 14 species were identified using FAME analysis. All strains were evaluated for in vitro inhibition of damping off disease caused by the fungus *Rhizoctonia solani*. The strongest in vitro inhibition of *Rhizoctonia* by soluble exudates in cocultivation was observed with strains belonging to 6 species: *Bacillus cereus*, *Bacillus. pumilus*, *Bacillus thuringiensis*, *Lysinibacillus sphaericus*, *Bacillus amyloliquefaciens*, and *Bacillus subtilis*. Most strains that inhibited *Rhizoctonia* growth in cocultivation also inhibited growth via volatile compounds. All 129 strains were evaluated for ability to protect impatiens plants from subsequent challenge infection by *Rhizoctonia solani*. Certain strains of endospore forming bacteria also enhanced plant growth. It is apparent from this study that this bacterial community associated with roots of plants within established planting beds produces soluble antifungal compounds, volatile antifungal compounds, and enhance plant growth. In developing biological control, it may be a more practical approach to promote or enhance a natural, multifaceted community of *Bacillus* strains within our planting beds.

Characterization of a novel satellite RNA associated with natural population of *Cucumber mosaic virus* (CMV) in Wisconsin snap bean fields

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Selected strains of *Cucumber mosaic virus* (CMV), in addition to three viral genomic RNAs, contain a small linear, single stranded RNA called satellite RNA. Previously, CMV sat-RNAs have been reported to have dramatic influences on symptom expression, ranging from symptom attenuation to increased symptom severity. Further, the appearance of CMV sat-RNA is very common under greenhouse conditions, but is considered less common in agricultural and natural ecosystems. We have identified a sat-RNA naturally occurring in fields of CMV-infected snap beans in Wisconsin. A specific product of approximately 350 bp was amplified by RT-PCR with specific primer pairs. The amplified product was cloned and transformed into the pGEM-T Easy vector and *E. coli* DH5α cells, respectively. Three colonies were selected and sequenced bi-directionally and analyzed. BLAST analysis confirmed these small RNAs as CMV sat-RNAs with 339 nucleotides. Sequence comparison showed that WI-satRNA shares the highest nucleotide sequence identity (95%) with a Spanish sat-RNA, but they differed by 15 nucleotide substitutions, three deletions and one insertion. Therefore, it appears this newly emerged WI-sat-RNA is a unique CMV sat-RNA. Field-collected, CMV-positive snap bean plants often showed severe CMV symptoms when co-associated with sat-RNA. Emergence of this CMV outbreak beginning in 2000 in the upper Midwest and Northeastern U.S., is associated with the emergence of the WI-sat-RNA in the CMV population.

Optimization of RNA isolation and qRT-PCR strategies to monitor microbial gene expression in soil

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The use of quantitative reverse transcriptase real-time PCR can lead to a better understanding of the microbial processes occuring in the rhizosphere and in situ action of biocontrol mechanisms. However, it is hindered by various technical factors when analyzing environmental soil samples. In this study, the efficiency of RNA isolation protocols, soil parameters such as clay content, and quantification approaches (absolute or relative) were investigated for their effects on microbial gene transcript quantification. *Pseudomonas* sp. LBUM300, a biocontrol agent of interest producing 2,4-diacetylphloroglucinol (DAPG) and hydrogen cyanide (HCN), was used as a model organism to target *phlD* and *hcnC*, involved in DAPG and HCN production, respectively. Time course experiments were conducted by inoculating soils with different quantities of LBUM300, and for the first time, a recently developed artificial exogenous spike-in RNA control (myIC) was used for relative quantification. When comparing the RNA isolation protocols, the quantity of isolated RNA and detected gene transcripts were significantly affected by clay content, RNA isolation protocol and the interaction between both factors. Absolute and relative quantifcation lead to similar transcriptional trends for both genes, however the relative method proved more reliable for detection of low transcript numbers. In conclusion, recommendations are made as to which technical approaches seem well adapted for quantifying microbial gene transcripts in soil.

Use of plastic and spray mulches to manage insects vectoring plant viruses

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An investigation was conducted looking into ways to repel aphids and thrips from pepper plants to prevent virus infection has been carried on since 2008 in Kern. Silver reflective mulch has been used as a method to repel aphids and thrips from various crops to prevent virus transmission. It is often used on tomatoes, melons, and peppers to prevent virus infection such as tomato spotted wilt virus (TSWV), tobacco mosaic virus (TMV), CMV and others. Other colored plastic mulches have been shown to increase plant size and yield. The main objective of this study was to determine which plastics mulches besides silver reflective mulch could repel aphids and thrips to prevent virus transmission. Another objective was to determine if a more cost effective spray on mulch could be used to repel aphids and thrips. Lastly determine what effect these different mulches have on plant growth and yield.

Homologous recombination and the invasion of a new plant host by the pathogenic bacterium, Xylella fastidiosa

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Homologous recombination plays an important role in the structuring of genetic variation of many bacteria; however its importance in pathogen evolution has yet to be established. We investigated the involvement of recombination in the shift to a novel host (mulberry) by the plant pathogenic bacterium Xylella fastidiosa. X. fastidiosa infects xylem and causes leaf scorch diseases in many economically important plant species, including Pierce's disease of grapevines. Mulberry leaf scorch was identified about 25 years ago in the eastern U.S. and since that time it has spread to California. Previous genetic analysis separated the mulberry isolates from the 4 recognized subspecies. Comparison of a newly sequenced genome of a mulberry strain with pre-existing genome data showed that this form originated by massive recombination between two of the subspecies, Xylella fastidiosa subsp. fastidiosa and Xylella fastidiosa subsp. multiplex, resulting in a genome consisting of roughly an equal mix of the two subspecies. The extensive recombination involved in the origin of the mulberry type, combined with a very low level of within-type genetic variation, suggests the host shift was achieved after strong selection acted on genetic variants created by inter-subspecific homologous recombination. These data show that the invasion of mulberry by X. fastidiosa provides a compelling example of the importance of recombination in the shift of a pathogen to a new plant host.

Identification and differentiation of gall midge species from West Africa

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The African rice gall midge (AfRGM), *Orseolia oryzivora* Harris and Gagné (Diptera: Cecidomyiidae), is a major biotic constraint to lowland rice production in West Africa. Studies have shown that resistance of rice varieties

to AfRGM differs markedly from one location to another. This is probably due to genetic differences between the gall midge populations at different locations. An understanding of the genetic variation amongst the population of the *Orseolia* species is necessary for breeding programmes aimed at effective development of cultivars with durable resistance to AfRGM in West Africa. Gall midge larvae and pupae were collected at random from various localities in lowland and irrigated rice ecologies in Nigeria, Mali, Burkina Faso, Cameroon and Sierra Leone. The insects were then processed for DNA analyses. PCR analysis of genomic DNA from the insects was carried out using sequence characterized amplified regions (SCAR) primers developed by Nwilene *et al*., 2006. Cluster analysis revealed two major insect genotypes (*OSG-1* and *OSG-2*). *OSG-1* was further divided into two subgroups (*OSG-1a* and *OSG-1b*). All the three reference insects (*Orseolia bonzii*, *Orseolia nwanzei* and *Orseolia oryzivora*) were genetically distinct. While *Orseolia bonzii* and *Orseolia oryzivora* were genotyped as *OSG-1b* along with other twelve insects, only *Orseolia nwanzei* was genotyped as *OSG-2*. The study revealed the population structure of gall midge species in different rice ecologies of West Africa. Key words: Rice varieties, *Orseolia oryzivora*, *Orseolia bonzii*, *Orseolia nwanzei*, PCR analysis, SCAR markers, population structure, differentiation.

An in vitro baiting assay for recovery of *Phytophthora ramorum* from waterways

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The current protocol used by the USDA Forest Service for recovering *Phytophthora ramorum* from potentially infested waterways is by in situ baiting with non-wounded rhododendron leaves for 1 to 2 weeks. Filtration also has been used for recovery by passing aliquots from a 1-liter sample of water through membrane filters with 3- or 5-µm pores. We compared these recovery methods to an in vitro baiting assay that used both intact leaves and leaf pieces of rhododendron. For all three assays, pathogen detection was attempted by nested PCR and isolation on selective medium. For the in vitro assay, 800 ml of stream water was collected in 100-ml aliquots and placed in a 1-liter Nalgene screw-top bottle; the water was baited with 20 leaf pieces and one whole, non-wounded leaf. The bottle was held at 18 to 20°C in the dark for 3 days; baits then were removed, rinsed in distilled water, and blotted dry. Pathogen detection was initiated immediately for the leaf pieces, but whole leaves were incubated in a moist chamber for up to 7 days to allow lesion development. *P. ramorum* was recovered by the in vitro assay in 12 of 18 samples collected from streams where *P. ramorum* had been recovered by the current protocol and by filtration in 10 of the 18 samples; inoculum densities in these samples ranged from 1 to 130 cfu/liter. The in vitro assay showed promise as an alternative to the current protocol, but additional evaluation is needed before recommending this new recovery method.

Bacterial and fungal pathogens associated with diseased oil palm (Elaeis guineensis) plants in Pamol Plantations, Cameroon, Central Africa

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Oil palm is an important crop in Cameroon because of income generated from palm oil and palm kernel oil. Pests and diseases are important production constraints to this crop. In April 2010, ten oil palm fields and two nurseries in Lipenja and Kokundu divisions of Pamol were surveyed for fungal and bacterial pathogens infecting them. In each field, stem, root and leaf samples of 4 symptomatic plants having chlorosis, stem rot, brown leaf spots and 1 asymptomatic sample were collected randomly and their tissues placed on Peptone-pentachloronitrobenzene, Potato Dextrose Agar and Nutrient Agar. Pure cultures raised from single colonies were identified based on morphology and biochemical assays. Out of 110 samples collected, 38.2% had fungi which include Fusarium oxysporium, F. verticilliodes, Botryodiplodia theobromae, Curvularia lunata, Collectotricum gleospoiriodes, Trichoderma sp., Coryne-spora sp., Cercospora sp., Cladosporium sp. and 21.8% bacteria; Bacillus cereus, Bacillus substilis, Penicillium sp. and Xanthosomas sp. Their frequency of occurrence were 63.0%, 13.6%, 4.5%, 27.2%, 40.9%, 9%, 4.5%, 18.0%, 2.0%; 54.5%, 45.4%, 4.5% and 4.5%. Mixed occurrence of microorganisms was noted in all diseased samples, with 21.0% having 4, 33.0% with 3 and 45.5% with 2. Latent infection with BT, Curvularia sp. and Bacillus substilis was noticed in 5 out of 22 asymptomatic plants. Purified culture of each microorganism was inoculated singly and in varied combinations onto 4 months old asymptomatic plants to determine their pathogenic effects.

Temporal and spatial spread of Cucurbit downy mildew in the eastern United States

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Dynamics of cucurbit downy mildew, caused by *Pseudoperonospora cubensis*, in the eastern U.S. in 2008 and 2009 were investigated based on disease records collected in 24 states as part of the Cucurbit downy mildew ipmPIPE monitoring program. The mean season-long rate of temporal disease progress was 1.4 new cases per day across the two years. Disease progress was slow during the spring and early summer and did not enter its exponential phase until mid June. The median nearest-neighbor distance of spread of new disease cases was approximately 110 km, with about 15% of the distances being >240 km. Considering all cucurbits, the epidemic expanded at a rate of 10 km per day in both years. Disease outbreaks were spatially aggregated and the extent of spatial dependence was up to 1,000 km. Results suggests that disease out-breaks in the Great Lakes and mid-Atlantic regions may be due to the spread of *P. cubensis* sporangia from outbreaks of the disease near the GA/SC/NC border rather than from overwintering sites in southern Florida. Space-time point pattern analysis indicated strong ($P < 0.001$) evidence for a space-time interaction and a space-time risk window of about 3 to 5 months after first disease outbreak and 300 to 600 km was detected across the two years. Results support the hypothesis that infection of cucurbits by *P. cubensis* appears to be an outcome of a contagion process and factors occurring on a large spatial scale (~ 1,000 km) facilitate the spread of the disease in eastern United States.

Systemic nematicidal activity of fluensulfone against the root-knot nematode *Meloidogyne incognita* on pepper

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Phytopathology 101:S131

Fluensulfone, a new nematicide belonging to fluoroalkenyl group, has proved to be very effective in controlling root-knot nematodes *Meloidogyne* spp. by soil application. We evaluated the systemic activity of the compound on *M. incognita* on peppers. Root application of fluensulfone via soil-drenching showed only slight nematode control activity when applied 4 days but not 10 days after inoculation. A single foliar spray of peppers with a fluensulfone solution at 3.0 g/l prior to inoculation reduced the galling index by 80% and the number of nematode eggs by 73–82% of the control. The reduction in these parameters by fluensulfone was much higher than those obtained by oxamyl or fenamiphos at the same concentration. This activity was also observed when the plants were sprayed 21 days prior to inoculation. A series of experiments suggested that foliar spray with fluensulfone prior to inoculation reduced the nematode invasion. In fact, the number of invading juveniles in the roots of seedlings sprayed with fluensulfone was lower than that in the roots of the control plants. However, foliar spray after inoculation did not inhibit the nematode development inside roots. These results suggest that fluensulfone applied to the foliage may translocate to the roots and affect the nematode invasion into roots. Fluensulfone may be used as foliar application for root-knot nematode control.

***Myrothecium roridum* tode and its toxin shows potential for management of water lettuce**

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Phytopathology 101:S131

The management of aquatic weeds with synthetic chemical herbicides is not only expensive but is beset with myriads of environmental and health implications. The current trend in weed management includes the use of host-specific mycoherbicides and phytotoxins. The effects of *Myrothecium roridum* (IMI 394934) and its phytotoxin on water lettuce (*Pistia stratioles*) were examined. The fungus isolated from diseased water hyacinth plants in Badagry Creeks, Lagos, Nigeria was made into a suspension (1 × 106 spores/ml) with 0.01% tween 80 in sterile distilled water and the pathogenicity on fresh non-diseased *P. stratioles* was investigated. The pathogen was re-isolated from the dead inoculated test plant in conformity with Koch's postulates. Detached leaves of *P. stratioles* were infiltrated with 10 µL (30%) crude fungal toxin produced in potato sucrose broth and monitored for 7 days for foliar symptom. Visual examination of disease development revealed a necrotic symptom in the *P. stratioles* leaf on day 5 and 100% mortality was also observed on day 37 post fungal inoculation. The phytotoxin caused foliar symptom on the leaves with an average severity index (ASI) of 3.0 (< 15% vein discoloration) on day 3 and 6.0 (above 75% vein discoloration) on day 7 post toxin infiltration. These results indicate that *M. roridum* and its phytotoxin are effective in causing lethal effects on *P. stratioles* hence can be considered as potential herbicidal agents, suitable for use in the management of *P. stratioles*.

Survival potential of *Phytophthora infestans* sporangia in relation to meteorological factors

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Assessment of meteorological factors coupled with sporangia survival curves may enhance effective management of potato late blight, caused by Phytophthora infestans. We utilized a non-parametric density estimation approach to evaluate the cumulative probability of occurrence of temperature and relative humidity conducive for late blight outbreak at a potato production field site at Presque Isle, ME. Sporangia survival probabilities were also computed based on microclimatic data. The influence of solar radiation on sporangia survival potential was also computed, based on the modified survival model. The joint distribution of temperature and relative humidity were similar among years, and favorable for late blight outbreak. Sporangia survival duration and frequency coincided with the potential period for pathogen infection during the cropping cycle. Sporangia survival probability (SSP) ranged from 0–64%, but had variable frequency and temporal changes during the cropping cycle. Analyses of SSP showed that 5–10% of cropping cycle is associated with 48–64% survival probabilities. High humidity and low temperatures were correlated with low solar radiation and increased risk for disease outbreak. By modeling periods of elevated survival potential in diverse locations and production regions, precise forecasts and disease controls can be optimized. P. infestans survival curves and climatic variables can be utilized in predictions of late blight on potato tubers and for better disease controls.

Corn and soybean yield responses using sedaxane, a new seed treatment experimental fungicide from Syngenta

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Rhizoctonia root rot is one of the most common soil-borne diseases of soybean (*Glycine maxx* L.) and corn (*Zea mays* L.). *Rhizoctonia solani* causes pre- and post-emergence damping-off and seedling blight, resulting in stand and yield reduction. The use of seed applied fungicides like azoxystrobin and fludioxonil are a common way to manage the disease to get optimum stand establishment and increase yield. Sedaxane is a new experimental fungicide active ingredient being developed by Syngenta Crop Protection for seed treatment use. Sedaxane belongs to the succinate dehydrogenase class of fungicides (SDHI) FRAC group 7. The mode of action of sedaxane is the inhibition of fungal respiration. Documented field studies are presented to demonstrate the benefits of sedaxane on corn and soybean yield resulting from the control of *R. solani* and by improving the root health of the plants over several growing seasons.

Evaluation of *Cercospora sojina* isolates sensitive and resistant to azoxystrobin using a mycelial growth inhibition assay

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Phytopathology 101:S132

Azoxystrobin resistant isolates of *Cercospora sojina*, the causal agent of frog eye leaf spot disease of soybean, were detected for the first time in 2010 in Tennessee (Zhang, Bradley and Newman) (http://bulletin.ipm.illinois.edu/article.php?id=1434; http://agriculture.tennessee.edu/news/releases/2010/10-10-FrogeyeLS.html). The method commonly used to determine the sensitivity of *C. sojina* isolates is a conidia germination assay conducted in agar plates amended with azoxystrobin and salylhydroxamid acid (SHAM). In this study, a mycelial growth inhibition method was evaluated to determine sensitivity levels of isolates to azoxystrobin and to discriminate resistant and sensitive isolates. The mycelial growth inhibition of 3 azoxystrobin sensitive and 3 resistant isolates was evaluated on potato dextrose agar amended with azoxystrobin (0, 0.001, 0.01, 0.1, 1 and 10 mg/L) and SHAM (50 mg/L) to establish the sensitivity of the isolates (ED_{50} values). Mycelial inhibition was correlated with the sensitivity response obtained from the conidia germination assay. The mycelial growth inhibition method was effective in determining the azoxystrobin sensitivity of *C. sojina* isolates and confirmed the results obtained using the conidia germination assay. The mycelia growth inhibition method can be used in future azoxystrobin resistant studies to discriminate resistant and sensitive isolates. This method is also less labor intensive than the standard conidia germination assay.

Usefulness of a high-throughput transient expression system to test virus-derived genetic constructs for resistance against *Grapevine fanleaf virus*

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Phytopathology 101:S132

Grapevine fanleaf virus (GFLV) causes fanleaf degeneration disease of grapevines. Since resistance to GFLV has not been identified in wild or cultivated grapevines, varied genetic constructs derived from GFLV have been generated with the aim of conferring resistance in transgenic grapevine rootstocks. To reduce the time and expense involved in the production and testing of transgenic grapevines for resistance to GFLV, a high-throughput approach has been developed for evaluating the anti-viral potential of candidate constructs by utilizing an *Agrobacterium tumefaciens*-mediated delivery system to achieve transient expression in *Nicotiana benthamiana*, a systemic host of GFLV. This approach allows for screening putative resistance constructs over a considerably shorter time frame than testing transgenic grapevines. Replicated experiments have indicated that many of the genetic constructs can reduce virus titres in agroinfiltrated plant tissues, as shown by enzyme-linked immunosorbent assays and semiquatitative RT-PCR, with differential levels of anti-viral activity observed among constructs. In order to test whether the transient approach is an accurate predictor of the anti-viral competency of constructs, transgenic *N. benthamiana* have been produced and utilized in resistance screening assays. Results from comparative resistance evaluations using the transient expression system and stable *N. benthamiana* transformants will be discussed.

Resistance to race TTKSK of *Puccinia graminis* f. sp. *tritici* in tetraploid wheat

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A group of races of *Puccinia graminis* f. sp. *tritici* in the TTKS (or Ug99) lineage possess broad virulence to wheat cultivars worldwide, and only a few genes in adapted cultivars have resistance to these races. In attempts to identify new stem rust resistance genes effective against race TTKSK, we evaluated cultivated and wild tetraploid wheat (*T. turgidum* ssp.) for resistance to TTKSK and other races with broad virulence. A high frequency of TTKSK resistance at the seedling stage was observed, as 395 (21% of 1882) accessions exhibited low infection types. Studies to determine the genetic basis of TTKSK resistance at the seedling stage revealed that resistance in tetraploid wheat is conferred mostly by single genes. Additional resistance genes effective against races TRTTF and TTTTF were also identified. Three hundred seventy tetraploid accessions were evaluated for resistance in the field screening nurseries of Debre Zeit (Ethiopia) and St. Paul (MN). One hundred fourteen accessions exhibited resistant to moderately resistant responses to stem rust in both nurseries. Fifty-two accessions susceptible to TTKSK, TRTTF, and TTTTF at the seedling stage were resistant at the adult stage. These accessions may possess adult plant resistance. Since all these tetraploid species share the same genome as durum wheat, resistance genes could be easily transferred to durum wheat by conventional breeding approaches.

Pesticidal activities of Hyptis suaveolens in pest management

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Fresh, mature and healthy plants of *Hyptis suaveolens* were collected in Akungba Akoko, Ondo State, Nigeria. The essential oil of the plant was extracted sequentially through solvent extraction methods using n-hexane, di-ethyl ether and methanol. The Methanolic extract was further prepared to obtain concentrations of 100%, 75%, 50%, 25%, 10%, 5%, 4%, 3%, 2%, and 1% which were tested for pesticidal activity against cultures of selected pest species of storage crops and mosquitoes. The experiment revealed the high insecticidal capability of *H. suaveolens* in the control of mosquitoes through fumigant application and 100% mortality of *Sitophilus oryzae*, *Sitophilus zeamais* and *Callosobruchus maculatus* within 10 seconds at the 50% methanolic extract in the contact treatment.

Fungicide application on disease resistant wheat: Is the response what you would expect?

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Phytopathology 101:S132

Foliar fungicides are often applied to susceptible winter wheat varieties in Oklahoma to protect yield potential when disease pressure is high. Typically fungicides are not applied to disease resistant wheat varieties. Here we analyze disease rating and yield data from five variety trials planted in 2005, 2008, 2009 and 2010 at two locations in Oklahoma. Trials were planted in a

randomized complete block design (four replications) with a split plot arrangement of treatments with wheat varieties as the main plot and fungicide treatment (sprayed or non-sprayed) as the subplot. Varieties ranged from susceptible to resistant to leaf rust, which was the predominate disease. Four of five trials had increased mean yields when all varieties were combined. The mean percent yield (bu/ac) increase with all trials and varieties pooled was 6.0 ($p = .09$). Twenty-four of twenty-eight varieties had mean yield increases when trials were combined, with variety 2174 having the greatest positive yield response to fungicide (19.4%) and TAM 401 having the greatest negative response (–18.4%). These observations suggest that fungicide applications on disease resistant varieties may benefit yield and indicate that yield increases may be variety dependent.

Sedaxane, a new experimental active ingredient from Syngenta for seed treatment use

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Sedaxane is a new experimental fungicide active ingredient developed by Syngenta Crop Protection for seed treatment use. It belongs to the chemical class of Pyrazole-Carboxamides and inhibits Succinate-Dehydrogenase, a central enzyme of the fungal respiration chain. Due to its activity spectrum and biokinetic properties, the AI is especially suited for seed treatment use. It is active against many important soil- and seed-borne diseases like *Ustilago* spp., *Tilletia* spp., *Monographella nivale*, and *Rhizoctonia* spp. Examples from greenhouse and field tests are presented to exemplify the level and spectrum of activity of Sedaxane.

The development of a specific Real-Time TaqMan for the detection of Clavibacter michiganensis supsp. michiganensis

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Phytopathology 101:S133

The detection of *Clavibacter michiganensis supsp. michiganensis* is of great economical importance. This seed born pathogen, with quarantine status, is the causative agent of bacterial cancer in tomato and causes economic losses in commercial tomato and seed production. The currently used detection methods all have drawbacks. The development of a new detection method was therefore still needed. ISHI-NL used AFLP® to find specific fragments present in all selected *Clavibacter michiganensis supsp. michiganensis* isolates. Only one single fragment of interest was found. The fragment was partial coding for a gene producing a protein two-component system sensor kinase. This gene was used to develop the specific RZ_Ptssk MGB based TaqMan®. The developed RZ_Ptssk TaqMan® was tested and compared with existing Real-Time PCR assay's on a collection of 67 isolates. The RZ_Ptssk TaqMan was the only assay correlating 100% with a pathogenicity test on tomato plants. Several ISHI_NL and international labs are using the RZ_Ptssk TaqMan® for their own specific purpose. Until today no false negative or false positive isolates have been reported.

Effect of fungicides on the control of postharvest diseases in papaya fruits

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Phytopathology 101:S133

In the state of Colima, Mexico there are about 1,500 hectares of papaya (*Carica papaya* L.). This crop is affected by different fungal pathogens. Postharvest fruit diseases are the most important problem affecting fruit quality and causing economical losses during its commercialization. The fungus *Colletotrichum gloeosporioides* causes the major disease in postharvest. Others fungi affecting the fruits are *Rhizopus*, *Lasiodiplodia*, *Alternaria*, *Phomopsis* and, *Botrytis.* Chemical control is commonly used in the packing houses. Different fungicides were evaluated to control postharvest diseases: Trifloxistrobin, Azoxistrobin, Thiabendazole, Benomyl, Imazalil, Prochloraz, Mancozeb, extract of *Malaleuca alternifolia* (tea tree) and, one control. All the products were applied at 1,000 ppm. One fruit was used as the experimental unit and 10 replications were included. Each fruit was submerged during five minutes in a solution of water mixed with the fungicide to be evaluated. After treatment, the fruits were stored at room temperature. After six days, Trifloxistrobin and Imazalil showed the best control, registering 0.3 and 0.5% of diseased area of the fruit (DAF), respectively. At 10 days, 70 to 80% of edible fruits (EF) were registered. Also, Benomyl and Azoxistrobin had a good control of the diseases with 2.3 to 3.7% DAF and 50 to 70% of EF. Thiabendazole, Prochloraz, Mancozeb and, *M. alternifolia* showed a deficient control with 5.5 to 8.1% DAF and 0 to 50% EF. The control fruit showed 10.3% DAF and 10% EF.

Management of diseased leaves with black sigatoka to reduce the disease severity in banana Grand Nain

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Phytopathology 101:S133

Black sigatoka (*Mycosphaerella fijiensis*) is the most important foliar disease affecting banana crop in Mexico. The control of this disease is carried out by a continuous use of fungicide sprays and cultural practices. The cultural control reduces the inoculum level of the pathogen and the favorable environmental conditions for its development. The main cultural practice to reduce the inoculum level is the removal of the whole affected leaves or only the diseased portions of them. In this study, different types of management of the removed leaves were evaluated to determine the effect on black sigatoka severity in banana Grand Nain (*Musa* AAA). The treatments were: 1) leaves placed over the ground and dispersed at random (control), 2) leaves placed over the ground and suited in rows, 3) leaves placed over the ground and suited in mini-composting (forming small heaps) and, 4) leaves placed in rows over the ground and applied monthly with urea at 10%. Each treatment consisted in plots of one hectare. Disease severity was evaluated weekly according Stover scale modified by Gauhl. All the treatments with management of leaves and suited in rows o mini-composting, as well as leaves treated with urea showed less disease severity in relation to the control. Also, the application of urea reduced the weight of leaf biomass during two weeks, accelerating its decomposition. In some samples, the production of pseudothecia and the expulsion of ascospores were 50 and 10%, respectively in comparison with the control.

Characterization of microbial populations from *P. infestans* suppressive Andean soils in Ecuador

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Phytopathology 101:S133

Late blight, caused by *Phytophthora infestans*, is a constant threat to potato production in developing countries and its management requires a large number of fungicide applications. In Ecuador, there is a low incidence of tuber blight, possibly due to the presence of natural suppressive soils in potato producing regions. These soils represent a source of microorganisms with potential for biological control activities. In this study a culture collection was generated and characterized from soils with known suppressive activity. Isolates from three microbial groups (*Pseudomonas*, *Bacillus* and Actinomycetes) were screened for *in-vitro* inhibition activity against *P. infestans* and *Rhizoctonia* sp. Amplified rDNA restriction analysis (ARDRA) was used for molecular characterization of isolates. Groups segregated according to sampling time or inhibition activity, but not according to sampling site. The percentage of isolates showing inhibition activity towards both pathogens varied according to group, being higher for the Actinomycetes, followed by the *Pseudomonas*. Our results will contribute to the development of alternative management practices for potato production in the Andes.

Aflatoxin-producing fungi in maize fields of Sonora, Mexico at varying elevations: A three year study

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Aflatoxin contamination of maize, a critical staple of billions, by *Aspergillus flavus* is a recurrent problem in the tropics and subtropics. Maize is produced across a broad range of elevations in the state of Sonora, Mexico. The current study evaluated the influence of elevation on the composition of aflatoxin-producing fungal communities associated with maize and the stability of those communities over time. Fungal isolates (1,230) belonging to *Aspergillus* section *Flavi* were recovered from field soil previously cropped to maize in 27 locations across 300 km at elevations ranging from 6 m to 2,100 m in the summers of 2006, 2007 and 2008. Fungal community structure was characterized for the *A. flavus* L strain isolates (846) with vegetative compatibility analyses utilizing nitrate non-utilizing auxotrophs. In total, 125 vegetative compatibility groups (VCGs) were detected; VCG composition varied greatly from year to year. Many VCGs that were very common in one year were rare or not detected in other years. Only 10% of VCGs were detected in each of the three years studied while 63% of VCGs were detected

only in a single year. These results suggest that dynamics of communities of aflatoxin-producing fungi resident in agricultural fields are complex resulting in rapid shifts in composition.

Potential organic substrates for soil application of *Microsphaeropsis amaranthi* and *Phomopsis amaranthicola* bioherbicides

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Microsphaeropsis amaranthi and *Phomopsis amaranthicola*, applied as bioherbicides, have shown utility controlling common waterhemp (*Amaranthus rudis*) and redroot pigweed (*Amaranthus retroflexus*), two weeds having resistance to multiple herbicides. However, unless specially formulated, the survival, infectivity, and efficacy of these fungal pathogens as foliar sprays is constrained by environmental factors. Field and growth chamber trials, conducted in Western Illinois, examined BioAPT Granular Peat-Based Microbe Carrier and corn stover as potential substrates for these organisms as soil-applied PRE- or POST-emergent products. BioApt or corn stover infested substrates were distributed evenly over the soil surface of 8.9 cm^2 pots at a rate of 1.5 g per pot after planting 50 weed seeds (PRE) or when weed seedlings reached the 1-2 true leaf stage (POST). Microplot trials, at two field locations, evaluated BioApt applied at 40 g per 30 cm^2 microplot. Bioherbicide PRE- and POST-emergence effectiveness in trials was determined by counting weed emergence, rating disease incidence, and measuring plant biomass (10 to 14 DAT). Our results indicate that PRE or early POST granular applications of these bioherbicide organisms have some activity but more work is needed to develop effective application parameters. Determining an economical and effective substrate, allowing these organisms to be soil-applied as PRE or POST emergent products could benefit the possible commercialization of these bioherbicide organisms.

A preliminary account of the sanitary status of *Prunus* species in the National Clonal Germplasm Repository

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The USDA National Clonal Germplasm Repository (NCGR) at the University of California, Davis is recognized as one of the richest sources of stone fruit material in the U.S. The repository maintains more than 1600 *Prunus* accessions representing 94 species collected from around the world. However, the phytosanitary status of the NCGR *Prunus* collection has never been systematically evaluated. We have now completed the first comprehensive testing for virus and virus-like diseases for a small part of the collection. A total of 223 trees representing 185 different cultivars of Cherry, Almond, Peach, Apricot and Plum were sampled. qRT-PCR and/or RT-PCR analysis was used to test for 13 different viruses, 2 virods, and a phytoplasma. Though the majority of these trees were asymptomatic, all tested pathogens were detected in at least one tree. This included *Apple chlorotic leafspot virus*, which had never previously been reported in California. The infection rate of the trees ranged from 66% infection with *Prunus necrotic ringspot virus*, to 0.5% phytoplasma infection. The PCR amplicons from positive samples were sequenced to analyze the molecular variability between isolates.

The effect of seasonal changes on grapevine leafroll associated viruses' titer and distribution in the vine

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A quantitative virus survey was performed for two years with three months interval to monitor the seasonal virus titer profile of Grapevine leafroll associated viruses (GLRaV) in infected grapevines. The viruses included in the study were GLRaV-1, -2, -3, -4, -5, and -9 and GLRaV-2 Redglobe strain (GLRaV-2RG). A time course experiment was performed in which sixty five grapevines varieties previously determined to be multiply infected with different grapevine viruses were selected as the starting material. The samples were collected in three month intervals in May August November and February over two years. From May to November, leaf petioles and in February dormant grapevine cuttings were collected. The samples were tested by using One-Step qRT-PCR. The data showed that in general the titer of viruses associated with leafroll disease was higher in the period from November to February. The lowest titer was observed in the period from May to August. In addition a distribution study was performed in which samples from 5 different locations of each GLRaV-infected vines were selected and tested. The results showed that these viruses were not equally distributed within the same grapevine plant.

Proposed guidelines for sample processing and downstream detection of grapevine viruses

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This study sets up guidelines for efficient sample processing and downstream detection of grapevine viruses. Different methods for homogenization, RNA extraction and qPCR based detection of grapevine RNA viruses have been evaluated. Semi-automated and automated homogenization techniques were compared to process samples from grapevine petioles and cambial tissue. Four different high throughput automated nucleic acid extraction platforms were compared with the RNeasy Plant Extraction kit for their capacity and efficiency for extracting RNA from grapevine infected tissues. The RNAs prepared from each extraction platform was then used as template for a comparative analysis of quantitative PCR (qPCR) by One-Step qRT-PCR, Two-Step qRT-PCR and low density arrays (LDA) detection. This study showed that a thorough homogenization of grapevine tissues using the Tissue Lyser as well as DNase digestion of the purified RNA prior to cDNA synthesis improved the virus detection and yielded the lowest Cq values in qRT-PCR. Comparison of different RNA extraction methods showed that methods implementing the magnetic bead-based technology were superior to other methods compared. Comparing different qPCR detection methods, One-Step qRT-PCR showed the lowest Cq values for the same sample tested and was able to detect higher number of positive samples compared to Two-Step qRT-PCR and LDA.

Comparative expression analysis of genes encoding pectin methylesterase enzymes in *Phytophthora infestans* during infection of *Solanum tuberosum*

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Phytophthora infestans is the causal agent of late blight disease of potato, which causes billions of dollars of crop loses annually. Current research efforts on late blight and P. infestans focus on understanding the mechanism of pathogen infection. While it is accepted that the pathogen infects hosts through the use of specialized structures, most importantly the appressorium, it is also known that the force of this structure alone is not sufficient to penetrate the host's rigid cell wall. As a result, investigations are now being conducted to examine the specific role played by cell wall degrading enzymes (CWDE), which are thought to be involved in *P. infestans* infection development. Previous research showing variability in the relative fold expression (RFE) of several CWDE in P. infestans grown in vitro has prompted this study. Here we report on the expression profiles of all P. infestans genes previously identified by bioinformatic approaches as belonging to a gene family with known pectin methylesterase (PME) activity. The expression patterns of these genes were analyzed by quantitative real-time PCR, using total RNA samples obtained at six different time points during the infection process on potato plants. Data from the in planta assays indicate that there are significant differences in the expression levels of the targeted genes at different times during the infection process, suggesting that PMEs might play a key role in P. infestans pathogenicity.

First report of *Phytophthora ramorum* infecting *Trachelospermum jasminoides* in Oregon

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Phytopathology 101:S134

Phytophthora ramorum can reportedly infect >125 plant species, many of which move through the nursery trade. In April 2010, *P. ramorum* was detected infecting *Camellia* plants growing in a nursery in Washington County, Oregon. During the delimitation survey to determine the extent of the infestation, necrotic leaf spots and premature leaf abscission were observed on several plants of star jasmine (*Trachelospermum jasminoides*). Five symptomatic leaves were collected for testing using a commercial *Phytophthora* DAS-ELISA kit (AgDia Inc., Elkhart, IN). DNA was extracted from ELISA-positive tissue using a Plant DNeasy kit (Qiagen Inc., Valencia, CA) and then tested for *P. ramorum* using the federally approved ITS and Elicitin qPCR protocols (USDA/APHIS/PPQ/CPHST Work Instructions WI-B-T-1-6 and WI-B-T-1-7). Isolations were made from the symptomatic leaves

onto the semi-selective medium PARP. One *Phytophthora* isolate was obtained from the symptomatic leaves; this isolate was morphologically identical to *P. ramorum*. Results from the ITS and Elicitin qPCR tests verified the presence of *P. ramorum* DNA in the leaf tissue. As required by federal protocol, a subsample of DNA was sent to the USDA/APHIS/PPQ Molecular Diagnostic Laboratory for official confirmation. In June 2010, USDA/APHIS/PPQ/MDL confirmed our identification of *P. ramorum*. To our knowledge, this is the first report of *P. ramorum* infecting star jasmine in Oregon.

Blackstain root disease effects on foliar nutrients, chlorophyll content, and internodal growth in ponderosa pine

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We excavated root systems from four codominant non-symptomatic (based upon crown characteristics) and seven symptomatic mature ponderosa pine in the Lassen National Forest in NE California. Trees were approximately 100 years old. Data were obtained on foliar nutrients N, P, Ca, Mg, Fe, Mn, B, and Zn. Chlorophyll a and b concentrations were also determined, along with fascicle and needle length, needle and fascicle fresh and dry weights, and fascicle number and flush length. Root infection was quantified by measuring stain circumference on major lateral roots, expressed as percentage of total sampled root circumference. Non-symptomatic trees had two times greater flush length and more retained fascicles per flush than symptomatic trees. Chlorophyll a and b concentrations peaked during the second year after needle appearance in all trees and were only slightly higher overall in non-symptomatic trees. Foliar Zn, Ca, and Mn concentrations were highest in non-symptomatic trees while no such trends were evident for N, P, K, and Mg. Foliar Ca and Mn increased with needle age, which was up to seven years at this location. Symptomatic trees had a range of 23 to 66 percent of sampled root circumference involved with blackstain vs 0-4 percent for non-symptomatic trees. Given the amount of root infection observed in symptomatic trees, foliar nutrient and chlorophyll contents are conserved, although infection effects are most striking when expressed as flush lengths and amount of retained needles and fascicles.

Sensitive detection and discrimination of *Xyllela fastidiosa* subsp. *pauca*, causal agent of citrus variegated chlorosis

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Xylella fastidiosa subsp. *pauca* (*Xfp*), a xylem-limited bacterium and select agent, causes citrus variegated chlorosis (CVC). The pathogen has spread through South and Central America, but is not in the U.S. CVC could significantly impact citrus producing U.S. states. A method for early, accurate and sensitive detection of *Xfp* in plant tissues is needed by plant health officials for inspection of products from quarantined locations, and by extension specialists for detection, identification and management of disease outbreaks and reservoir hosts. Two sets of specific PCR primers and probes, targeting *Xfp* genes for fimbrillin and periplasmic iron-binding protein, were designed. A pair of conserved primers targeting the cobalamin synthesis protein gene was designed to detect all possible *X. fastidiosa* strains. All three primer pairs and probes were validated *in silico* against published sequences. PCR products were cloned and sequenced for confirmation. Primer sets Xf.CVC_fim1 (110 bp product), Xf.CVC_pib4 (82 bp product) and Xf_csp6 (92 bp product) detected as little as 1 fg of plasmid DNA carrying *X. fastidiosa* target sequences at cycle threshold (Ct) values of 27.92, 30.19 and 29.55, respectively. These PCR assays are useful for *X. fastidiosa* detection, discrimination, diagnosis and quantification, and for applications in breeding programs, biosecurity and microbial forensics.

Population structure and mating system of the faba bean pathogen, *Didymella fabae* (anamorph: *Ascochyta fabae*), in Syria

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Ascochyta blight of faba bean (*Vicia faba*) is caused by the fungal pathogen *Didymella fabae*. The fungus has a bipolar mating system and the sexual stage has been reported from artificially infected experimental plots in Syria. Eighteen sequence-tagged microsatellite (STMS) markers were developed to investigate the genetic structure among a sample of 96 isolates from three geographic locations (sub-populations) in Syria. Genetic linkage analysis of the markers detected 8 linkage groups and clone-corrected data set of 63 isolates based on a randomly-chosen set of 8 unlinked markers were analyzed. AMOVA indicated small (10%) but statistically significant (PhiPT = 0.105, $P = 0.005$) genetic differentiation among sub-populations but the entire sample of isolates was assigned to a single genetic population using a Bayesian clustering algorithm. A PCR-based mating type assay was used to determine mating type and a 1:1 ratio of mating types could not be rejected in each sub-population and among all sampled isolates. Multilocus gametic disequilibrium was estimated with index of association within sub-populations and among all isolates using 8 STMS markers and the mating type marker. The null hypothesis of random mating was rejected within each sub-population and among all isolates. These results question the role of ascospores as a significant source of inoculum for this disease in Syria.

Population genetic structure of *Phytophthora cinnamomi* associated with Phytophthora root rot of avocado (*Persea americana*) within California

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Phytophthora root rot (PRR) of avocado (*Persea americana*), caused by *Phytophthora cinnamomi* (Pc), is the most serious disease of avocado worldwide. The pathogen population genetic structure of Pc is usually associated with low genotypic diversity and the dominance of a single mating-type, consistent with asexual reproduction. However, no population level studies have specifically been conducted in the avocado growing regions of California (Ca). Therefore, we used AFLP markers to investigate pathogen diversity of Pc from 16 groves from the Northern and Southern avocado growing regions. Additional isolates from other countries and hosts were also used for comparative purposes. Three distinct clades were found based on UPGMA analysis of 22 polymorphic loci; one clade contained only isolates from Southern Ca, one clade contained isolates from both Northern and Southern Ca, and the last clade contained mostly non-Ca isolates from additional hosts. From the Ca avocado populations, a total of 16 genotypes out of 169 isolates were found. The results indicate significant population structure in the Ca avocado Pc population, low genotypic diversity consistent with asexual reproduction, and potential evidence of movement of clonal genotypes between the two growing regions. Since two main clades were found among Ca isolates of Pc, these results may have implications for rootstock breeding against Pc if differences in virulence or aggressiveness occur within these two clades.

Endophytic associations and production of mycotoxins by the *Aspergillus* section *Nigri* species

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The filamentous fungi of the *Aspergillus* section *Nigri* (black aspergilli) are considered plant pathogens of maize (*Zea mays*) and peanuts (*Arachis hypogaea*) where they can cause similar disease symptoms as *Fusarium verticillioides*, such as seedling blight. However, the main concern with black aspergilli is their ability to produce carcinogenic mycotoxins such as ochratoxin A (OTA) and the fumonisins (FB1, FB2, and FB3). Our preliminary work indicated that these fungi were endophytes of maize. The first aim of our research was to provide evidence of endophytic associations between maize using yellow and red fluorescent protein-labeled strains of *A. niger* var *niger* (*yfp*) and *A. carbonarius* (*rfp*). The identities of the fungi were determined using a repetitive-sequence-based DNA method. This study revealed that both *Aspergillus* species had similar host colonization patterns in maize as endophytes. The second aim was to determine mycotoxin production by these species. We determined the ability of 167 field isolates to synthesize OTA, FB1, FB2, and FB3 in maize kernels as natural substrate and that of the species isolated, only 10% of *A. niger* var niger strains produced OTA. However, almost 60% of the field isolates were able to produce the fumonisins. Our results are the first to indicate that black aspergilli associated with field maize in the United States are able to produce carcinogenic compounds, and are potential threats to human and domestic animal health.

PVX-M3 – A deviant pepper isolate of Potato virus X

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Potato virus X (PVX) and cucumber mosaic virus (CMV) co-infection was determined on pepper showing typical mosaic symptom in Hungary in 1976. PVX was separated from CMV by thermal treatment of crude extract of the virus infected tobacco passed to healthy plant. This PVX-M3 isolate did not differ from common PVX strains based on the symptoms caused on different test plants. A polyclonal antiserum prepared against this isolate reacted similarly to the known isolates of PVX (PVX-G, PVX-NyH). The purified virion of PVX-M3 and PVX-NyH isolated from potato has been stored. In the autumn of 2009 proved to be both isolate infectious. Similarly of the previous investigation of polyclonal antibodies (Loewe) PVX-M3 and PVX-NyH isolates gave a strong reaction, but ADGENE monoclonal antibody only reacted with PVX-NyH isolate. Negative results with monoclonal antibody indicated an unusual property of the coat protein. Coat protein gene of this isolate was cloned and sequenced. We observed 4 amino acid changes in the N-terminal variable part of the protein compared to other isolates. The second two of which are separated by one amino acid induced changing in the protein structure resulting loosing hydrophobity and an alpha-helix disappearing. Thus, the epitope recognized by the monoclonal antibody has been changed. This may explain why that monoclonal antibody failed to detect this virus isolate. The project was supported by NKTH-TECH-09-A3-2009-0210 and TAMOP–4.2.1./B-09/1KMR-2010-0005 grants.

Morphological-molecular characterization of *Phytophthora*, *Pythium* and *Phytopythium* on intensive crops in Buenos Aires – Argentina

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Surveys of Oomycetes associated to diseases of ornamental, vegetable, soybean and fruit crops were carried out from 2006 to 2010 in cropping areas of Buenos Aires Metropolitan Area. Seventy five isolates were obtained from symptomatic roots, stems and leaves plated in selective media and transferred to CMA, PDA and V8. Isolates of *Phytophthora*, *Pythium* and *Phytopythium* were identified to species levels based on morphological and molecular characteristics. Colony morphology, cardinal growth temperatures, and characters of the sexual and asexual stages were documented. The ITS rDNA region was amplified (ITS5/ITS4), sequenced and BLAST aligned with the NCBI and the *Phytophthora* Database. For each host-pathogen relation Koch's postulates were fulfilled. Update 6 *Phytophthora* spp. and 8 *Pythium* spp. were identified including *Phytophthora capsici*, *P. cinnamomi*, *P. cryptogea* 'complex, *P. nicotianae*, *P. sojae* and P. taxon *kelmania*, *Pythium aphanidermatum*, *P. cylindrosporum*, *P. intermedium*, *P. irregulare*, *P. spinosum*, *P. splendens*, *P. sylvaticum*, *P. ultimum* var. *ultimun* and *P. ultimum* var. *sporangiferum* and *Phytopythium chamaehyphon.* Four putative *Pythium* sp. nov, and one *Phytopythium* sp. nov. were found. *Phytophthora* taxon *kelmania*, *P. sylvaticum*, *P. cylindrosporum*, *and P. chamaehyphon* are new reports for Argentina.

Molecular analysis of fumonisin biosynthetic genes in non-toxigenic *Aspergillus niger* strains

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Aspergillus niger and other black *Aspergillus* species are common residents of table and wine grapes and raisins throughout the world. Recently, *A. niger* was shown to produce fumonisin B_2 (FB_2), a carcinogenic and cytotoxic polyketide mycotoxin. In our laboratory, a recent survey of black *Aspergillus* strains isolated from California raisins showed that 66% of the *A. niger* isolates produce FB_2. To determine why the non-toxigenic *A. niger* strains do not produce FB_2, primer sets were designed for multiplex PCR analysis of eight genes within the fumonisin biosynthetic gene cluster. Out of 65 non-toxigenic strains, 29 strains yielded PCR products only for *fum1*, encoding a polyketide synthase, and *fum19*, encoding an ABC transporter. In 10 other strains, only *fum6*, encoding a cytochrome P450 monooxygenase, was absent. In contrast, PCR products for all eight gene targets were detected in 20 of the 65 non-toxigenic strains, suggesting that there may be structural or regulatory defects in one or more genes essential for FB_2 biosynthesis in those strains. The occurrence of multiple genotypes among non-toxigenic *A. niger* strains raises questions regarding the ecological significance of FB_2 production, and may be useful in designing biocontrol intervention strategies for reduction of fumonisin contamination of grapes and other fruits.

Quorum sensing directly controls the Hrp regulatory cascade and the Gac/Rsm signal transduction pathway in the gall forming *Pantoea agglomerans*

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Gall induction by *Pantoea agglomerans* pv. *gypsophilae* (*Pag*) on gypsophila is *hrp*-dependent. Disruption of the quorum sensing (QS) genes *pagI* and *pagR* significantly reduced gall size and expression of the *hrp* regulatory genes *hrpXY*, *hrpS* and *hrpL in planta* as determined by qRT-PCR. Transcription of the QS and *hrp* regulatory genes was reduced by inactivation of IAA or cytokinin biosynthetic pathways. The interrelationship between the QS, *hrp*/*hrc* cluster, the Gac/Rsm cascade and virulence has been investigated by gel shift experiments and the effects of mutants on gene expression using qRT-PCR *in planta*, colonization and gall formation in gypsophila. Gel shift electrophoresis indicated that PagR directly binds to the *hrp* regulatory genes in a C_4-HSL-dependent manner to putative *lux* box in their promoters. Moreover, PagR also binds to a putative *lux* box located in the *gacA* promoter, which encodes the response regulator of the GacS/GacA two component system. The Gac/Rsm cascade, which controls the activity of RsmA, was investigated by studying the effects of *gacS*, *gacA*, *rsmB* and *csrD* mutants as well as overexpression of *rsmB* and *rsmA* for all the above-mentioned parameters. Overexpression of RsmA reduced virulence whereas its elimination abolished gall formation. Results presented suggest that PagR is a central regulatory factor that controls virulence through direct activation of the Hrp regulon and control of RsmA activity through the Gac/Rsm cascade.

Protection of cucumber diseases by using hot water extract from spent substrate of edible mushrooms

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Since the impact of agrochemicals on environmental contamination, human health and pesticide resistance, alternative technologies for pest management are being investigated. One technology is the use of elicitors that are released from the mycelia of fungi. In this study, the mycelia of edible mushrooms that are prevalent in the spent substrate were used to induce defense mechanism in cucumbers. Plants were treated with autoclaved water extract from spent mushroom substrate (AWESMS) of *Lyophyllum decastes* or *Pleurotus eryngii* by dipping the first true leaf, and inoculated with the target pathogen after 1 week. Results showed that AWESMS of *L. decastes* significantly reduced diseases by *Colletotrichum orbiculare* and *Podosphaera xanthii* in more than 80%, but this effective result was not observed with *Corynespora cassiicola*. The AWESMS of *P. eryngii* was effective against *C. orbiculare* but not against *P. xanthii*. When cucumber plants were grown in pots containing a mixture of autoclaved spent substrate of *L. decastes* and soil (1:2, v/v), a disease reduction of over 70% was observed with *C. orbiculare*. The AWESMS of *L. decastes* showed no antifungal activity against *C. orbiculare* and a significant increase of expressions of chitinase and β-1,3-glucanase 24 h after pathogen inoculation was observed. The use of spent substrate for disease control may offer a new technology for the recycling and management of waste from mushroom cultivation.

Grafting of a commercially important but bacterial wilt susceptible tomato variety with disease resistant rootstocks for open field production

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Bacterial wilt of tomato caused by *Ralstonia solanacearum* is a serious production issue worldwide including Florida and Virginia. Grafting bacterial wilt susceptible scions with new and traditional resistant rootstocks is a

possible approach for managing the disease in open field production. Field and greenhouse studies were conducted at Quincy, FL, and Painter, VA during 2009-2010 that evaluated seven new tomato rootstocks (Jjak kkung, Cheong gang, RST 105, RST 106, BHN 998, BHN 1053, BHN 1054) and a traditional rootstock (Hawaii 7998) as possible resistant sources for grafting a commercially popular, but bacterial wilt susceptible tomato variety BHN 602. Greenhouse studies in *R. solanacearum* inoculated potting medium confirmed that all the rootstocks except RST 105 were moderately or highly resistant to the pathogen. Field studies showed that the plants grafted on to Cheong gang, RST 106, BHN 998, BHN 1054, and Hawaii 7998 exhibited the highest level of disease resistance in FL and VA. Field studies also indicated that all these rootstocks had significantly higher yield compared to un-grafted and the self-grafted entries under high disease pressure. The findings from these trials illustrate that bacterial wilt can be effectively managed by grafting with resistant rootstocks. Although grafting will increase the cost of transplants, it will be economically beneficial in fields with a history of bacterial wilt, ensuring the sustainable production of the crop.

Marker-assisted selection improves the efficiency of bioprospecting and results in the recovery of novel biocontrol bacteria

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In the search for natural products, large collections of microorganisms are screened for novel and effective isolates. In order to more efficiently recover and exploit, a greater variety of plant-associated bacteria as biopesticides, we developed a multivariate sampling and marker-assisted selection strategy. In doing so, we quantified the relative effects of different sampling and selection factors on the diversity of recovered bacteria, showing that variation in all factors could result in the recovery of distinct genotypes as defined by amplified ribosomal DNA restriction analyses (ARDRA). The efficiency of bioprospecting was improved by focusing phenotypic characterization solely on representative ARDRA-defined genotypes. Subsequent sequencing and phenotypic analyses revealed that our marker-assisted selection strategy led to the recovery several rare and, to date, poorly characterized genera of plant-associated bacteria with significant biocontrol activities. The modes of action of several of these strains is currently being investigated.

The *Nicotiana benthamiana* Hsp-alpha protein (NbHsp-α) interacts with the movement protein of the bipartite begomovirus *Bean dwarf mosaic virus*

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Geminiviruses are plant-infecting single-stranded DNA viruses. Bipartite members (*genus Begomovirus*) encode a nuclear shuttle (NSP) and movement protein (MP) to facilitate trafficking of viral DNA across nuclear and cell wall boundaries, respectively. A yeast two-hybrid screen was performed to identify *Nicotiana benthamiana* host proteins that interact with the MP of the begomovirus *Bean dwarf mosaic virus* (BDMV). One potential MP-interacting protein identified in the screen was a small heat-shock protein (Hsp), NbHsp-alpha. NbHsp-alpha is a plant specific Hsp with homologues in *Arabidopsis thaliana* and other plant species. There are several subfamily groups of small Hsps in general, some of which function as molecular chaperones. We obtained the full-length *NbHsp-alpha* gene from *N. benthamiana* and confirmed that NbHsp-alpha interacts with the BDMV MP *in vivo* by yeast two-hybrid and co-immunoprecipitation assays. Transient expression of a red fluorescent protein (RFP)-NbHsp-alpha fusion protein in *N. benthamiana* leaves revealed localization to punctuate structures in the cell membrane and wall. Evidence these were plasmodesmata came from co-localization of RFP-Hsp-alpha with a green fluorescent protein (GFP)-*Tobacco mosaic virus* (TMV) MP fusion protein. The role of NbHsp-alpha in BDMV infection was assessed in plants overexpressing the protein or in which the gene was silenced.

Nematicidal activity of plant essential oils and components from *Gaultheria fragrantissima* and *Zanthoxylum alatum* against pine wood nematode

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Pine wilt disease, caused by the pine wood nematode, *Bursaphelenchus xylophilus*, was firstly reported in Busan, a city located in south-eastern coast of Korea. Since then, it has spread to several areas of the peninsula. In this study, we investigated the nematicidal activity of 29 commercial plant essential oils and some of their components which have not been tested before against *B. xylophilus*. Good nematicidal activity against *B. xylophilus* was achieved with essential oils of *Gaultheria fragrantissima* and *Zanthoxylum alatum*. GC-MS analysis of the corresponding oils led to the identification of 2 and 10 major compounds, respectively. Four compounds such as methyl salicylate, ethyl salicylate, methyl trans-cinnamate and ethyl trans-cinnamate were tested individually for their nematicidal activities against the pine wood nematode. Methyl and ethyl salicylates showed strong nematicidal activity at concentration of 2.0 mg/mL. Concentrations of 1.0 mg/mL as well as lower concentrations showed only minor effects. Another compound, methyl trans-cinnamate, showed 100% activity at concentrations of 0.0625-2.0 mg/mL. In case of ethyl trans-cinnamate, 100% mortality was observed at concentrations of 0.25-2.0 mg/mL. The essential oils and their components described herein merit further study as potential nematicides against the pine wood nematode.

Co-packaging of genomic RNAs and virion accumulation are controlled by the N-terminus of the *Red clover necrotic mosaic virus* capsid protein

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The RCNMV genome is composed of two ssRNAs, RNA-1 and RNA-2, that are packaged into two distinct virion populations containing either one copy of each genomic RNA or 4 copies of RNA-2 only. The N-terminal 50 amino acids of the RCNMV CP are rich in basic residues and are essential for RNA binding. Here, we constructed a series of alanine substitution mutations in the N-terminal region of the CP and investigated the biological significance of these basic residues during encapsidation and infection. Our results revealed that triple alanine substitutions for lysine residues at either positions 4, 7, and 8 (R1mt) or 25, 33, 38 (R2mt) affected either the ratio of packaged genomic RNAs or virion accumulation levels, respectively. Mutant R1mt induced more pronounced symptoms than a wild-type infection while the mutant R2mt exhibited wild-type symptoms. Furthermore, we found that mutant R1mt exhibited decreased packaging of RNA-1, but not RNA-2, suggesting that this mutation may interfere with specific recognition of RNA-1 and co-packaging of both genomic RNAs during encapsidation. In the case of mutant R2mt, the mutation did not affect systemic infection and symptom expression but exhibited significantly reduced virion accumulation levels. Taken together, these results suggest that lysine residues at positions 4, 7 and 8 within the N-terminal 10 residues play an important role in specific recognition of RNA-1 and/or the complex of RNA-1 and RNA-2 for co-packaging of both genomic RNAs.

Forest Phytophthoras of the World website

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Phytophthora diseases threaten the biodiversity and sustainability of forests globally. Researchers around the world are working to better understand *Phytophthora* organisms, to recognize newly emerging diseases they cause, and to develop management practices that minimize the spread and severity of disease. Information on forest Phytophthoras is scattered, making it difficult for professionals to stay abreast of new species, disease epidemics, scientific publications, and educational materials. Our website aims to provide scientific and educational resources to aid in the international understanding and management of forest Phytophthoras. The website includes descriptions of each forest *Phytophthora* species including morphology, growth characteristics, phylogeny, host range, ecology, and disease symptoms. Citable articles on individual species will be published as the online journal, *Forest Phytophthoras*. Other website features include educational and management resources; a searchable photo gallery; an easy-to-use synoptic key; a disease finder to identify potential causal organisms based on known hosts, location and symptoms; links to a global mapping function; an illustrated glossary; and key references in a searchable database with easy export options. Access the website at www.ForestPhytophthoras.org.

Historical pathways of introduction for non-indigenous forest pathogens

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Non-indigenous pathogens introduced since the late 1800s have caused sixteen damaging forest epidemics that have reduced or now threaten the diversity and sustainability of U.S. forests. Evidence for the most likely pathway of introduction is provided for these invasive forest pathogens based

on historical records and knowledge of pathogen biology. Eight pathogens were likely introduced on live plants: *Cronartium ribicola*, *Cryphonectria parasitica*, *Discula destructiva*, *Gremmeniella abietina* var. *abietina*, *Lachnellula willkcomii*, *Melampsora larici-populina*, *Phytophthora lateralis*, and *P. ramorum*. Two insect-vectored pathogens, *Ophiostoma ulmi* and *O. novo-ulmi*, were imported on logs, and another, *Raffaelea lauricola*, was likely introduced on solid wood packaging. The entry pathway could not be determined for five pathogens: *Ceratocystis fagacearum*, *Cryptodiaporthe populea*, *Phytophthora cinnamomi*, *Sirococcus clavigignenti-juglandacearum*, and the *Venturia saliciperda-Glomerella miyabeana* complex. Identification of pathways is critical to preventing new pathogen introductions. Findings emphasize the importance of improved mitigations for pathogens on live plants as global plant trade escalates.

Seasonal variation of *Candidatus* Liberibacter asiaticus in citrus branches and in vector, *Diaphorina citri*, in Central Florida sweet orange groves
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Candidatus Liberibacter asiaticus (Las) causes the insect vectored disease Huanglongbing (HLB) in Citrus spp. In Florida, the disease rate has increased two-fold every year since 2005. Significant knowledge gaps exist in Las transmission and epidemiology. Sweet orange groves in central Florida were selected to study seasonal dynamics of Las over a 3-yr period in host and vector (Asian citrus psyllid; ACP). Two to three hundred trees were selected in each grove and 1 leaf/tree was collected randomly every fortnight. Sample collection began in June 2010 with collections ongoing. For qPCR detection of Las, the midribs of 2–5 leaves were randomly pooled to obtain 30–40 samples/date. An estimated Las prevalence in the branches was calculated from the pools with PooledInfRate v3. Simultaneously, ACP collected from the same location were pooled at 1–6 ACP/sample for qPCR. Las prevalence increased in the moderately infected grove from summer to fall (25 to 36%) and decreased in winter (21%), while the highly infected grove had 42, 44, and 59% infection in summer, fall and winter respectively. Las prevalence in ACP also increased from summer to fall in moderately (16 to 43%) and highly (37 to 53%) infected groves. Seasonal dynamics of Las in ACP and citrus followed a similar pattern over summer and fall. If the trends are consistent for other seasons, it is likely that host and vector have similar seasonality, and that could be exploited to manage HLB.

Using phenotypic markers to identify common beans with two and three rust resistance genes
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Two new races of the rust pathogen (Uromyces appendiculatus) of common bean (Phaseolus vulgaris) appeared in Michigan and North Dakota in 2007 and 2008, respectively. These races rendered susceptible many previously rust resistant dry bean varieties carrying the widely used Ur-3 rust resistance gene. Among the susceptible was the recently released pinto bean cultivar Stampede. However, one of the parents of Stampede, the bean germplasm line BelDakMi-RMR-14 was resistant to the new races of the rust pathogen. This line has the Ur-3, Ur-6, and Ur-11 rust resistance genes and had previously been evaluated as resistant to some 90 other races of the bean rust pathogen. In the process of evaluating Stampede with the new races from Michigan and North Dakota, resistant Stampede plants were noticed. To determine the presence of rust resistance genes in the resistant Stampede plants, we evaluated their reaction to eight races of the rust pathogen that reliably identifies the Ur-3, Ur-4, Ur-6 and Ur-11 genes. These races provide phenotypic markers to identify bean plants with any of these genes individually and plants with different combinations of these genes. We identified many Stampede plants with the Ur-3 and Ur-11 rust resistance genes and few plants with Ur-3, Ur-6, and Ur-11. Both groups of Stampede plants were also resistant to the new races from Michigan and North Dakota as well as well as to many others races.

A multifunctional role for the type IV pilus in the bacterial biological control agent *Lysobacter enzymogenes*
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Fungal antagonism resulting from production of lytic enzymes and antibiotics has been linked to biological control activity by *Lysobacter enzymogenes*. Recent observations indicate that in addition to outward antagonism, the bacterium is also capable of establishing pathogenic interactions with fungal hosts. Using the genome sequence of *L. enzymogenes* strain C3, we have taken a forward genetics approach to investigate the molecular basis of pathogenesis and determine its role in biocontrol. Type IV pili (T4P) contribute various roles in bacteria, including gliding motility, extracellular polysaccharide (EPS) secretion, host attachment and pathogenesis. Genome analysis indicates that genes encoding for T4P biogenesis reside in six unlinked loci in *L. enzymogenes*. To evaluate functional roles for T4P in the bacterium, deletion mutations within major pil genes in each of the six loci were constructed. Mutational effects on EPS production and gliding motility varied depending on the specific pil gene mutated. In contrast, all mutant strains were affected in polar attachment to hyphal cells of the fungal host, *Cryphonectria parastica*. Furthermore, mutation within the secretin protein gene *pilQ* resulted in reduced virulence toward fungal host cells. These results support a multifunctional role for T4P in *L. enzymogenes*, including a significant role in pathogenesis of fungal hosts.

Management of charcoal rot of sweet potato in India
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Altogether 14 isolates of *Macrophomina phseolina (*Tassi) Goid. from different states of India were screened for their sensitivity against carbendazim both *in vitro* and *in vivo.* The effect of passage on most carbendazim sensitive isolate was tested by exposing to carbendazim continuously, alternately with captan, zineb and mancozeb and in mixture with them. This was studied both *in vitro* and *in vivo.* The most carbendazim resistant isolate was grown on medium containing different concentrations of various agrochemicals for its management. It was observed that carbendazim with captan, zineb, mancozeb, methomyl, endosulphan, monocrotophos, 2, 4-D, excel mera 71, zepclav 500, griseofulvin, oflaxacin 400, potassium chloride, sodium chloride, manganese chloride, urea, muriate of potash, iron, molybdenum, cobalt, copper and manganese completely inhibited the growth of pathogen both *in vitro* and *in vivo*.

Comparison of pecan scab predictions in Oklahoma using weather inputs from the National Weather Service, the Oklahoma Mesonet, and onsite-monitoring
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Inputs from networks of weather stations are often used in disease models to assist growers in timing fungicide sprays. A standardized national weather station network does not exist, despite disease epidemics affecting crops across the U.S. The National Weather Service (NWS) has a network of non-standardized stations, which can be used to predict scab (caused by *Fusicladium effusum*) epidemics across the U.S. pecan belt. In Oklahoma, the Mesonet is used for collecting weather inputs used by a pecan scab advisory where scab hours (SH; an hour where average T≥21.1°C and average RH ≥90%) are accumulated over a 14-d period. In 2010, SH was compared between 15 NWS and Mesonet stations in closest proximity to one another. On-site weather data were collected to determine SH at two sites in 2009 and one site in 2010. Regression analysis and T-tests were used to determine quantitative differences in daily SH for each comparison. Five NWS stations significantly (P >0.05) under-predicted SH, while six NWS stations significantly (P >0.05) over-predicted SH, compared to the Mesonet. Both the NWS and Mesonet significantly under-predicted SH compared to on-site SH at one location in 2009 and 2010. No significant differences were identified in SH measured by the on-site weather station and Mesonet in 2009. While NWS stations can be used to determine SH across the pecan belt, accuracy of SH measurements might be improved through site-specific interpolation of weather data.

Persistence of the walnut twig beetle in black walnut logs as influenced by chemical and cultural treatments
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The walnut twig beetle (*Pityophthorus juglandis*) is the vector of *Geosmithia morbida*, the cause of thousand cankers disease of black walnut (*Juglans nigra*). We studied whether clear plastic tarping or applications of bifenthrin, permethrin, and biodiesel to felled logs influenced the persistence of the walnut twig beetle in bark or altered the suitability of logs for future breeding by the insect. Logs from trees with thousand cankers disease were treated and arranged in a randomized complete block design at two Colorado locations. At periodic intervals sections of the logs were removed and placed indoors in insect emergence boxes. Beetle emergence from all logs at the two sites was variable six months after trees were felled; no beetle emergence was recorded

in a portion of both treated and untreated logs. Nevertheless, only logs treated with bifenthrin were consistently devoid of beetles. Walnut twig beetles emerged from a small proportion of the treated and untreated logs after 18 months and even from some logs in which the beetles previously had not been detected. *Geosmithia morbida* was isolated from the walnut twig beetle at each sampling date. These results indicate that logs can remain a source for both the beetle and fungus for at least 18 months.

Witch's broom phytoplasma infecting *Echinacea pallida* in Australia

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Purple coneflower (*Echinacea pallida*), is commercially cultivated in Australia for its medicinal properties. In 2005, coneflower fields in Tasmania, Australia began exhibiting symptoms typical of phytoplasma infection, including virescence, phyllody and chlorosis. Surveys of fields in 2011 indicated the presence of symptoms within one field only. Disease distribution in this field was assessed by hierarchical sampling, incorporating 10 spatially referenced transects. Along each transect, 20 individual plants were assessed at 1 m intervals for the proportion of symptomatic flower heads. Overall, incidence of infected plants was estimated at 32%, while the mean percentage of symptomatic flower heads was 12%. Spatial analysis indicated a random distribution of symptoms across the field. Phytoplasma infection was confirmed by DNA sequencing of a 1.2 kb region of the 16S RNA gene, obtained by amplifying total DNA extractions from symptomatic coneflower tissue. Individual sequences shared greater than 99% homology with 'Candidatus Phytoplasma australasiae'. Comparison of virtual restriction fragment profiles from the same genetic region confirmed that the pathogen belonged to the Witch's Broom (16SrII-D) group of phytoplasmas. All previous reports of phytoplasma infection of coneflower have indicated Aster Yellows (16SrI-C) group phytoplasmas as the causal agents.

Functional analysis of Asian soybean rust resistance pathways using virus-induced gene silencing

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Asian soybean rust is an aggressive foliar disease caused by the obligate biotrophic fungus *Phakopsora pachyrhizi*. Outbreaks of the disease have the potential to severely affect overall yields, and all commercially grown elite cultivars of soybean are susceptible to the pathogen. Germplasm screening efforts have identified five different genes (*Rpp1 – Rpp5*) that confer varying levels of resistance to select isolates of *P. pachyrhizi*. We are using virus-induced gene silencing (VIGS) in an effort to identify and characterize these resistance genes and the gene networks through which they operate. In recent years, VIGS has become an important molecular tool for studying the function of specific plant genes, including those involved in defense. A set of DNA-based VIGS vectors has been developed for use in soybean, and when combined with the genomic sequence, transcriptome analysis, gene mapping studies, and metabolomic data, VIGS is a power reverse genetic tool to assess functionality. To date, we have screened over 150 soybean genes and have identified several that compromise resistance when silenced. Here we provide an overview of the approach and a summary of genes that contribute to rust resistance.

Comparing the efficiency of visual scouting, spore trapping systems and a bioindicator for early detection of *Erysiphe necator* in California vineyards

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Powdery mildew (PM) caused by *Erysiphe necator* is the most serious disease of grapevines in California. Ascospores constitute the primary inoculum in vineyards where chasmothecia have overwintered. Air currents disperse spores, and airborne concentrations are largely a function of the local population of the pathogen. Early detection at low density is a key tactic in the management of the disease. In this work, three different detection methods were compared during two consecutive seasons in five vineyards in multiple locations in California. Rotorod and ionic spore trapping systems, both coupled with quantitative PCR using specific probes and primers, were used to monitor ascospore release and conidia dispersal throughout the season. Efficiency was compared to that of visual/manual scouting and bioindication by a native mycophagous beetle, *Psyllobora vigintimaculata*, an obligate consumer of *E. necator*. Initial insect incidence was positively correlated with initial PM incidence. Moreover, the two spore trapping systems were significantly different in terms of detection efficiency.

Microbial ecology of soils and strawberry roots in non-treated soils that appear to enhance plant growth compared to fumigated soils

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Soil fumigation using methyl bromide (MB) is used as a pre plant treatment to control a range of pathogens in crop production systems. A strawberry grower MB suppressed strawberry plant growth in one field compared to adjacent non-fumigated areas, an unusual phenomena and opposite of expectations. Planting occurred over 4 weeks after application suggesting chemical toxicity was not a factor. We hypothesized this was a biologically mediated process. Treated (MB) or non-treated (NT) soils were collected in a pair-wise sampling design in the spring at peak harvest. Strawberry leaf dry weights were 10.4 g/plant from MB treated rows and 29.8 g/plant in non-treated rows ($P < 0.001$). Soil and strawberry roots were tested for the presence or absence of pathogenic and beneficial microbes. Soil microbial numbers were counted using grow-out dilution assays. The strawberry root rot pathogens, *Pythium* and *Fusarium* populations were dramatically suppressed to 1175 and 1096 colony forming units per gram of dry soil (cfu/gds) in MB treated soils compared to 3255 and 10,058 cfu/gds in NT soils, respectively (P-value < 0.001). Therefore, MB treatment effectively reduced pathogen populations. Soil bioasssays with cross inoculations of soils were conducted to further determine if the NT soil harbored beneficial communities or if MB treatment negatively affected plant growth. Follow-up field studies have been implemented to further clarify the unique phenomena experienced is this particular field site.

Curtovirus quantification and species differentiation within mixed infections through real-time PCR

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Curly top is an important viral disease found throughout the United States. Viral infection causes chlorosis, malformation of leaves, stunting, and a reduction in yield. In chile, Beet Severe Curly Top Virus (BSCTV), Beet Mild Curly Top Virus (BMCTV), Beet Curly Top Virus (BCTV), Pepper Curly Top Virus (PeCTV), and Pepper yellow dwarf virus (PeYDV) are the species most commonly found. Current detection methods are qualitative and unable to differentiate between different curly top species. Plants in the field may be infected with more than one species at a time, and little is known of the quantity of virus species within a mixed infection. Genetic differences between species has allowed for the development of primers, which can differentiate among species commonly infecting chile. Utilizing Real-time PCR (Q-PCR), a novel system was developed to quantify and differentiate Curly top species within a mixed infection.

The occurrence of Cucurbit chlorotic yellows virus disease in Taiwan and evaluation of the virus infected fruit quality and yield

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Melon (Cucumis melo L.) and watermelon [Citrullus lanatus (Thunb.) Matsum & Nakai] are two high-value cucurbit crops and occupy plantation areas of more than 5,000 ha and 13,000 ha in Taiwan, respectively. The Cucurbit chlorotic yellows virus (CCYV), the members of the genus Crinivirus, is of the major threats for the cultivation of cucurbits in Taiwan. This virus is whitefly-transmitted crinivirus. From 2009–2011, we survey 538 melon field. Based on the locations, townships of Yunlin, Chiayi, and Tainan are regarded as southern Taiwan. The melon infected percentage of Cucurbit chlorotic yellows virus (CCYV) was only 0–5% on early growth stage, 15–20% on medium growth stage, 60–100% on late growth stage. The disease incidence upward 75% and losses were 32.8%, and decrease the Brix 5.2 and the lowest Brix was 7.2. The disease incidence from 25% to 50% and losses were 26.4%, and decrease the Brix 3.9. The disease incidence from 5% to 25% and losses were 13.9%, and decrease the Brix 2.3. The disease incidence from 3% to 5% and losses were 12.4%, and decrease the Brix 1.9. Although this virus was first reported to infect cucurbits in Japan in 2009 (2), in Taiwan

in 2010. The Cucurbit chlorotic yellows virus was widespread and often epidemic in cucurbit crops. The cucurbit crops infected by CCYV have been demonstrated reducing yield by 12.4–32.8%.

Generation of broad-spectrum resistance in transgenic tobacco and tomato plants against distinct tospovirus species of different serogroups

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Thrips-borne tospoviruses cause severe damages in important crops worldwide. In this investigation, a conserved region containing the RNA polymerase motifs within the L gene of *Watermelon silver mottle virus* was used to generate sense-translatable, sense-untranslatable, sense-frameshift, antisense and double-stranded constructs for *Agrobacterium*-mediated transformation of *Nicotiana benthamiana* plants. A total of 46.7–70.0% transgenic tobacco lines derived from the five constructs, each with 30 lines, showed complete resistance to WSMoV (the type member of WSMoV serogroup), and 35.7–100% plants of them exhibited broad-spectrum resistance against nine other unrelated tospoviruses from different serogroups, including individual type members of *Tomato spotted wilt virus*, *Impatiens necrotic spot virus*, *Iris yellow spot virus* and Peanut chlorotic fan-spot virus. The double-stranded construct conferred highest percentages for the broad-spectrum resistance. Northern analyses indicated that the broad-spectrum resistance is mediated by gene silencing. Transgenic tomato lines with individual constructs were also generated. High levels of broad-spectrum resistance against WSMoV and other different tospovirus serogroup species were also noticed. We conclude that using a single nucleotide fragment corresponding to the L gene conserved region triggers broad-spectrum resistance in tobacco and tomato plants against different serogroup tospoviruses at the genus level.

PacC mediated adaptation to alkaline pH is critical for developing infection hyphae in penetrated plant cells in *Magnaporthe oryzae*

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Plant cells are known to increase pH as a basal defense response to attacks by fungal pathogens. However, it is not clear how the pathogens successfully adapt to pH changes in the host. In this study, we identified five genes of the PacC pH-signaling pathway in the rice blast fungus *Magnaporthe oryzae* by insertion mutagenesis. Deleting any of the five genes resulted in sensitivity to alkaline pH and defects in infection hyphal development. MoPacC is expressed in conidia, appressoria, primary and secondary infection hyphae but its localization to the nucleus was only observed in infection hyphae formed *in planta*. In plant cells penetrated by *M. oryzae*, pH was increased to 7.2 or higher. Microarray analysis revealed that about 300 genes were directly up- or down-regulated in the *ΔmopacC* mutant under the alkaline pH. Most of them encode proteins involved in cell wall biogenesis, membrane transporters, electron transport and oxidoreductases, metabolisms of carbohydrates, lipids and amino acids, and cleavage and digestion of proteins. MoPacC has four forms, full-length $MoPacC^{60}$ and three truncated $MoPacC^{30}$, $MoPacC^{25}$ and $MoPacC^{9}$. Their localization to the nucleus could be induced by alkaline pH. In sum, our study indicated that activation of MoPacC is an adaptation to host cell alkalinization and its distinct forms function as transcriptional repressor or activator to regulate expression of genes for nutrient uptake and assimilation for infection hyphal development.

The *Strawberry Advisory System*: A forecast system for control of anthracnose and Botrytis fruit rots

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Anthracnose fruit rot (AFR), caused by *Colletotrichum acutatum*, and Botrytis fruit rot (BFR), caused by *Botrytis cinerea*, are the main diseases affecting strawberries in Florida. Fungicides are applied on a weekly schedule throughout the season to control these diseases. Calendar-based control programs were compared to applications based on previously published models that related disease incidence to weather variables. These models utilized leaf wetness and temperature during the wet period to predict disease outbreaks. Different thresholds for predicted AFR and BFR incidence were evaluated to trigger fungicide applications. Field trials were conducted for three seasons on two cultivars. The most effective model-based treatments reduced the number of sprays by about 50% without affecting disease control or yield. Selected models and thresholds were used to develop a web-based tool to advise growers of the current disease risk level and the need for fungicide application. The web-based forecasting system, named the Strawberry Advisory System (SAS), was implemented on the AgroClimate website (http://agroclimate.org/tools/strawberry/). Users can also be provided warnings of the need for fungicide applications via email and/or text messages. In preliminary grower trials, the SAS has been successful in eliminating many unnecessary fungicide applications and has proven user friendly.

Multiple gene genealogy analysis reveals Mycosphaerellaceae species known to be specific to *Eucalyptus* associated to native Myrtaceae in Uruguay

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Mycosphaerella species are among the most important pathogens affecting *Eucalyptus* worldwide. In Uruguay, a relatively large number of *Mycosphaerella* species are found on *Eucalyptus* but their occurrence on native Myrtaceae is unknown. Due to the close relationship between introduced *Eucalyptus* species and Myrtaceae native to Uruguay, the aim of this study was to identify Mycosphaerellaceae associated with leaf diseases on native Myrtaceae, and to determine their relationship with those affecting *Eucalyptus* plantations. Several surveys were conducted in native forests throughout the country. Diseased leaves were collected from native host species. Following fungal isolation, cultures were identified based on morphology and comparisons of partial DNA sequences for the ITS, EF1-α and Actin genes. Results revealed the occurrence of *Mycosphaerella aurantia*, *M. heimii*, *M. yunnanensis* and *Pseudocercospora norchiensis*, all known to be *Eucalyptus* pathogens. These results not only suggest the epidemiological importance of native Myrtaceae trees as hosts of exotic *Eucalyptus* diseases but also raise serious concerns about the movement of pathogens from *Eucalyptus* plantations to native trees and vice versa. Since most of these species occur on *Eucalyptus* in countries other than Uruguay, it seems likely that they were introduced into the country and have adapted to infect native Myrtaceae; however, further investigations are needed to test this hypothesis.

Virulence and molecular genotyping studies of *Sporisorium relianum* isolates in sorghum

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Head smut, caused by *Sporisorium relianum*, has been reported with increasing frequency in the grain sorghum growing regions of Texas. To analyze changes in pathogen virulence, four inoculation techniques were examined: soil and teliospore mixture, seed coating, media placement, and syringe injection. Of the four, syringe injection was determined to be the most effective. Inoculations of sorghum host differentials BTx643, BTx7078, BTx635, SC170-6-17 (TAM2571), SA281 (Early Hegari), and Tx414 showed 23 of 32 Texas isolates were race 4. Two isolates from College Station, Texas, were classified as race 1, but no race 2 or 3 isolates were found. New virulent races 5 and 6 were identified among isolates from south Texas. Using 16 AFLP primer combinations, genetic diversity was asessed in DNA samples from 49 *S. relianum* isolates, including 44 sorghum isolates from Texas, U.S.A., two from Uganda, and one from Mali; and two maize isolates from Mexico. Single-base extensions with EcoRI and MseI primers in the selective amplification increased the number of informative polymorphic bands. High genetic dissimilarity (50%) was observed between isolates originating from maize and those originating from sorghum. The resultant dendrogram, made using cluster analysis, grouped the Texas *S. relianum* isolates into four small clusters with >82% similarity. Other than for two race 6 isolates from Weslaco, Texas, no evidence for geographical or other restrictions on gene flow was evident.

Genetic diversity and pathotype determination of *Colletotrichum sublineolum* isolated causing anthracnose disease in sorghum

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Amplified fragment length polymorphism (AFLP) based genetic diversity analysis was conducted among 232 *Colletotrichum sublineolum* isolates collected from three distinct regions of Texas, and from Arkansas, Georgia, and Puerto Rico. AFLP analysis revealed significant levels of polymorphism (59%) among *C. sublineolum* isolates and high levels of genetic similarity, ranging from 0.78 to 1.00. Four UPGMA clusters grouped isolates irrespective of geographical origin or collection year. Pathotype analysis was conducted in the greenhouse for 20 of the *C. sublineolum* isolates selected at random from within and between groups using two inoculation methods. The study included 18 sorghum differentials [14 (SC326-6, SC414-12E, BTx378, TAM428, Tx2536, SC328C, QL3, BTx398, SC283, Brandes, SC112-14, Theis, BTx623, SC748-5) from previous studies conducted in Brazil and the U.S. and 4 (PI570841, PI570726, PI569979, IS18760) from Sudan]. Two of the 20 isolates (AMP55, AMP104) producing inconsistent symptoms were excluded from the pathotype analysis. Seventeen new pathotypes were established among the 18 isolates. BTx378 and QL3 were resistant and BTx623 and TAM428 were susceptible to all 18 isolates. These results show that resistance breakdown can occur within short periods of time under different environmental situations. The use of a common set of differentials and standard nomenclature is essential for better understanding variability in the global *C. sublineolum* population.

Host modification of *Penicillium solitum* during postharvest decay of apple fruit

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Penicillium solitum and *P. expansum* are closely related postharvest fungal pathogens, causing blue mold on pome fruits in storage. *P. expansum* is a more aggressive pathogen; however, the mechanisms governing disparities in fungal virulence are unknown. Modulating the pH of the host environment has been suggested as an important factor that contributes to fungal virulence. *P. expansum* has been shown to modulate its environment via secretion of organic acids into the host tissue with concomitant ammonia uptake during decay development. To determine if the less aggressive nature of *P. solitum* is due its inability to adequately modify its host environment, we examined the aggressiveness of *P. solitum* on two different apple cultivars, 'Golden Delicious' and 'Winesap.' Wounded apples were inoculated with a *P. solitum* conidial suspension (1×10^6) and evaluated 3, 7, 14 and 21 days after inoculation. Lesion diameter, decay depth, pH, ammonium concentration, and organic acid production in the decayed and healthy tissue were evaluated. On both cultivars, at each time point, *P. solitum* caused similar lesion size and reduced pH inside the lesion, compared to surrounding non-decayed tissue. The observed change in pH is mostly likely caused by ammonium depletion in the host and organic acid secretion by the fungus within the decayed tissue. Therefore, we hypothesize other factors may explain the observed differences in fungal virulence between these two *Penicillium* species.

Susceptibility of select U.S. winter wheat cultivars to wheat blast (*Magnaporthe oryzae*)

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Wheat blast, caused by a pathotype of *Magnaporthe oryzae*, is an emerging disease in South America. Countries reporting the disease are Brazil, Bolivia, Paraguay and Argentina. Field losses of 30 to 100% have been observed under favorable environmental conditions. The establishment potential of wheat blast in other regions of the world has not been determined, but the spread of this seed transmissible disease is likely. In anticipation of its arrival in the U.S., studies were initiated to assess U.S. wheat cultivars for disease resistance. Reported here are preliminary results of a biological safety level-3 greenhouse screening of 200 U.S. winter wheat cultivars for head blast resistance using a single Brazilian isolate (T-25). For each cultivar, 7 to 20 spikes at growth stage 50 were spray-inoculated with a *M. oryzae* conidia suspension at a rate of 1×10^5 conidia per ml applied until run-off. Individual spikes were enclosed in small a plastic bag to maintain high humidity for 24 h at 23–25°C. After 21 days, spikes were evaluated based in the number of infected florets per spike. Cultivars with less than 10% infection were retested. Test results showed a broad range of susceptibility. Soft red wheat cultivars ARS05-00443, GA00067-8E35 and GA011493-8E18 averaged less than 4% infection, while hard red wheats, KS0603A-57-1, Jackpot, and CO050173 averaged less that 10%. Identification of resistant germplasm will be essential for a U.S. disease recovery plan.

Effects of seedborne and overwintering inoculum on ray blight severity in pyrethrum

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Ray blight, caused by *Stagonosporopsis ligulicola* var. *inoxydablis* is an important disease affecting pyrethrum in Australia. Previous studies demonstrated the pathogen can be found within and on seed, although the contribution of this inoculum to epidemic severity is relatively unknown. Logistic regression models were constructed using defoliation severity data collected from 72 commercial fields over nine years. Models based on overwintering inoculum, expressed as the isolation frequency of the pathogen during autumn and winter, or seed contamination incidence and autumn-winter temperature and rain variables were highly predictive of ray blight epidemics. Path analysis was used to model the direct and indirect factors of weather variables and inoculum factors on disease intensity. This analysis indicated that seedborne inoculum of *S. ligulicola* var. *inoxydablis* contributed indirectly to defoliation severity through directly increasing pathogen overwintering frequency. Autumn and winter weather variables had indirect effects on defoliation severity through increasing overwintering success of the pathogen, but also direct effects on defoliation severity. These findings suggest that losses from ray blight may be minimized through the use of clean seed and/or reducing overwintering inoculum. This could be achieved by growing seed in drier, isolated locations, in combination with other inputs to minimize the probability of disease development.

Characterization of orthologs of Ax21 and two, two-component regulatory systems, phoPQ and colRS, in *Xylella fastidiosa*

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Xylella fastidiosa (*Xf*) is a gram-negative, xylem-limited plant pathogenic bacterium and the causal agent of Pierce's Disease of grapevine. Biofilms play a key role in early colonization and pathogenicity of *Xf* by providing a protected niche and enhanced survival in the xylem. In *Xf* and *Xanthomonas oryzae* pv. *oryzae* (*Xoo*), biofilm formation is induced by density-dependent gene expression mediated by diffusible signal factors (DSF). Recently, Ax21 was identified as a DSF in *Xoo* that interacts with two two-component regulatory systems: *raxRH* and *phoPQ*. Orthologs of *Ax21*, *raxRH*, and *phoPQ* were identified in the *Xf* genome and deletion knockout mutants were constructed to investigate the functional role of these orthologs in *Xf*. Using a biosensor assay, we determined *Xf* does not produce active Ax21, which is likely due to the lack of a sulfation system for Ax21 in *Xf*. Furthermore, a gene knockout in *Ax21* had no significant impact on biofilm formation but resulted in a 23% reduction in cell aggregation and a 29% reduction in population density after completion of the log phase growth. Knockout strains of *Xf* deficient in production of phoP and phoQ resulted in a 42% and 47% reduction in biofilm formation, respectively, and a 42% and 36% reduction in cell aggregation, respectively. Our preliminary results indicate that Ax21 and phoPQ play a role in density dependent gene expression during early colonization and infection.

Uptake and translocation of Penthiopyrad fungicide in wheat leaves and correlation to fungicidal control of key foliar diseases

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Penthiopyrad is a new succinate dehydrogenase inhibitor (SDHI) fungicide discovered and owned by Mitsui Chemicals, Inc. The active ingredient Penthiopyrad is being co-developed by E. I. du Pont de Nemours and Company for control of a wide range of fungal diseases of cereals and specialty crops. A combination of bioassays, LC/MS, and autoradiographic analyses was used to characterize the degree and rates of uptake and movement into wheat leaves of penthiopyrad in a commercial EC formulation. Penthiopyrad was applied at a field rate using in 10^{-1} uL droplets to an area near the base of the leaf. Immediately after treatment most penthiopyrad remained on the leaf surface. Within 28 hours sufficient compound had penetrated and translocated to give efficacious concentrations throughout the leaf. Concentrations sufficient for disease control were maintained throughout 21 days as demonstrated by bioassay results showing both curative and preventive control of wheat leaf rust (*Puccinia triticina*) and leaf blotch (*Septoria tritici*) in untreated zones.

Uptake, transport, and fungicidal efficacy of Penthiopyrad fungicide in wheat resulting in protection of treated and untreated foliage

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Phytopathology 101:S142

Penthiopyrad is a new succinate dehydrogenase inhibitor (SDHI) fungicide discovered and owned by Mitsui Chemicals, Inc. The active ingredient Penthiopyrad is being co-developed by E. I. du Pont de Nemours and Company for control of a wide range of fungal diseases of cereals and specialty crops. The objectives of this project were to analytically characterize the uptake and movement as well as the persistence of penthiopyrad in a commercial EC formulation applied to field-grown winter wheat at GS32 timing when the second leaf below the flag-leaf is fully expanded. Three hours after field application, approximately 50% of the active ingredient was located inside the fully exposed F-2 and the partially exposed F-1 leaves. Five days after application, significant decreases of penthiopyrad were observed in the surface residues of the fully exposed F-2 and in both surface and internal concentrations of the partially exposed F-2. By 14 days after application, concentrations of penthiopyrad in the F-2 and F-1 had not changed significantly since the last sample. Penthiopyrad was detected in the now fully emerged flag-leaf showing the highest concentrations. A subsequent bioassay on greenhouse-grown spring wheat demonstrated that when applied at the GS32 timing when the flag-leaf had not emerged, sufficient active ingredient was transported to the flag-leaf to protect it against leaf blotch (*Septoria tritici*).

Fungicidal efficacy and partitioning of Penthiopyrad in apple leaves in relation to application rate

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Phytopathology 101:S142

Penthiopyrad is a new succinate dehydrogenase inhibitor (SDHI) fungicide discovered and owned by Mitsui Chemicals, Inc. The active ingredient Penthiopyrad is being co-developed by E. I. du Pont de Nemours and Company for control of fungal diseases in various crops. Our objectives were to determine the partitioning of penthiopyrad applied in a commercial SC formulation between the surface, the cuticle, and inside the apple leaves as affected by application rate, as well as to determine the correlation of partitioning with preventive and curative fungicidal efficacy against apple scab (*Venturia inaequalis*). We used a 3-compartment partition model based on extraction solvent to evaluate penthiopyrad deposits i) on the leaf surface, ii) associated with the waxy cuticle on the exterior of the leaf, and iii) inside the leaf that was not extracted in previous steps. After application of formulated penthiopyrad to apples, the active ingredient was detected in all three leaf compartments. The relative partitioning of penthiopyrad was depended on the application rate as well as time of sampling after application. At rates similar to field use rates and independent of sampling time, most of the active ingredient was located in the cuticle with lower amounts on the leaf surface and inside the leaf. Both preventive and curative fungicidal activities were directly correlated with increasing application rates of penthiopyrad as well as increasing concentrations of penthiopyrad in the cuticle.

Identification of *Fusarium oxysporm* f. sp. *lycopersici* and *Fusarium oxysporum* f. sp. *radicis lycopersici* using specific primer

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Phytopathology 101:S142

Fusarium oxysporum f. sp. *lycopersici* (FOL) and *Fusarium oxysporum* f. sp. *radicis lycopersoci* (FORL) cause considerable damages in greenhouses. FOL is only pathogenic on tomato and FORL is pathogenic on other hosts from Cucurbitaceae family in addition to tomato. In this study, 35 fungal isolates from the border of healthy and infected tissues of tomato vessels with wilt symptoms were identified as *Fusarium oxysporum*. Pathogenicity of isolates was investigated on tomato and cucumber on seedling stage with conidial suspension inoculation (106 spores/ml). 13 isolates caused wilt in cucumber in addition to tomato. Study of growth rate of isolates on PDA in 18°C and 27°C temperatures showed that 13 isolates which were pathogenic on cucumber have more growth in 18°C. To confirm the above results, DNA of isolates were extracted and reproduced by using Uni and Sprl specific primers. All isolates with Uni primer made a 672 bp band. In addition, 13 isolates with Sprl primer made a 947 bp band. Therefore, the latter isolates were determined as FORL and the other 22 isolates were determined as FOL. In this way, PCR molecular method confirmed the accuracy of above results with more precision.

Identification of a novel fruiting structure produced by *Aspergillus niger* and *A. carbonarius* in grape berries affected by sour rot

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Aspergillus spp. infections of grapes can lead to sour rot. *Aspergillus* enters the berries through wounds caused by birds, insects, or other mechanical damage at veraison or later. Typical sour rot symptoms include soft rot of berry tissue followed by colonization by other fungi, acetobacter and yeasts. A novel sporulation structure caused by *Aspergillus* spp. has been observed in berries affected by sour rot. Sporulation occurs at the wounding site with the formation of a cavern-like structure of soft fungal tissue growing inside the berry. Isolates of *A. niger* and *A. carbonarius* were used to conduct field and laboratory experiments to monitor the formation and development of the fruiting structure over time. Identification of the fruiting structure was done by means of histological studies with GMS and H&E stains to better understand the developmental stages of both *Aspergillus* spp. on the table grape Red Globe. The effect of temperature was also studied. Individual berries were wound inoculated and incubated at different temperatures. It was observed that structures of two kinds were formed under dry conditions: a "cavern"-type structure that assumed a more tubular shape, and a thinner fungal tissue forming around the seeds of Red Globe grapes. Histological studies showed that conidia-bearing structures start forming around 16 days post inoculation on the fungal tissue closest to the seeds.

Tropical race 4: Current and future impact on export and subsistence banana production

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Phytopathology 101:S142

Norman Simmonds classified fusarium wilt of banana, caused by *F. oxysporum* f. sp. *cubense* (FOC), as one of the most destructive plant diseases. Susceptibility to race 1 of FOC doomed 'Gros Michel,' the banana cultivar on which the first export trades were based; it was replaced by race 1-resistant Cavendish clones by the 1960s. Currently, the tropical American trades depend on the Cavendish subgroup. A new variant of FOC, tropical race 4 (TR4), has recently decimated Cavendish monocultures in southeastern Asia. We summarize the threat that TR4 poses to global production. TR4 would affect 85% of total production were it disseminated to other important banana-growing regions. In the Americas, a hemispheric plan has been drafted to address courses of action for before and after TR4 would arrive in the region; it has been discussed with regional quarantine authorities, producers and scientists, and is used to focus activities that surround the issue and inform a developing contingency plan (OIRSA). Decades of research on race 1 resulted in no highly effective management options for infested soils other than the use of resistant genotypes. Thus, as expected, biological and cultural measures have proven to be ineffective long-term measures for managing the TR4 problem. We discuss the conventional and nonconventional improvement of banana with TR4 resistance, the recent development and application of a TR4 diagnostic, and future research objectives.

Genetic variability of Colorado Cherry rasp leaf virus

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Cherry rasp leaf virus (CRLV) is one of the most common viral pathogens of tree fruits in Colorado. Infected trees show different symptoms including mild to severe enations in orchards. To assess the incidence and genetic variability of CRLV, 267 samples were randomly collected from stone and pome fruit orchards in western Colorado from June-October 2010. The virus was detected from 80 cherry and apple samples by RT-PCR using virus-specific primers. A DNA fragment of 452-bp from a conserved RNA dependent RNA polymerase region was cloned and sequenced from 36 samples. Comparisons of the deduced amino acid (aa) sequence of 36 isolates with three isolates from the GenBank database show that the Colorado isolates share 92–100% (majority 96–100%) identities with each other and 87–100% with the three known isolates. Phylogenetic analysis of the deduced aa sequences indicates low genetic variation among the Colorado isolates, with a majority (35 of 36) grouped in a major cluster. There are three subclusters within the major cluster, with two of them containing isolates from different host species and orchards, indicating a lack of correlation among variants, hosts and orchards. A variant from sour cherry was the most distinct among the Colorado isolates.

Our results indicate that there is limited genetic variation among CRLV isolates, and no apparent relationship between different symptom types in cherry and apple trees and variants of the CRLV isolates.

Incidence of multiple viruses in western Colorado cherry orchards
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A limited survey for viruses in commercial cherry orchards in western Colorado in 2008 and 2009 suggested there may be a correlation between leaf symptoms and viral infection. Thus, further investigations were performed in 2010. Leaf and fruit samples from 116 trees of various sweet and sour cherry cultivars were collected from 25 orchards. Total nucleic acids were extracted and tested for viruses (RT-PCR) and viroids (dot blot hybridization) to correlate the severity of leaf enation symptoms, typically associated with Cherry rasp leaf virus, with the presence of other viruses or viroids. Eight viruses including CRLV, *Cherry virus A* (CVA), *Cherry green ring mottle virus*, *Cherry necrotic rusty mottle virus*, *Plum bark necrosis stem pitting associated virus*, *Prune dwarf virus*, *Prunus necrotic ringspot virus* and *Tomato ringspot virus* were found in various combinations in these trees. No viroids (*Peach latent mosaic viroid* and *Hop stunt viroid*) were detected. At least one virus was detected in 94% of the samples, with CRLV (62%) and CVA (53%) being the most common infections. Two or more viruses were present in 60% of the samples, and combinations of up to 7 viruses were detected in a given tree. The incidence of multiple infections did not correlate with symptom types, varieties or locations. Although trees with leaf enations were infected with CRLV, asymptomatic trees were also found to contain CRLV.

Efficacy of bio-fumigation and soil solarization on soil-borne onion pathogens
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Phytopathology 101:S143

Bio-fumigation can manage many soil-borne pathogens, but its efficacy is not known against Fusarium Basal rot and pink root diseases of onion. Studies were conducted to evaluate the efficacy of soil solarization, biofumigation, and their combination on these diseases within the context of existing growers cropping systems. The field studies used a replicated split-split plot completely randomized block design; these were followed by greenhouse experiments. Treatments included mustard vs no mustard as main plots; canola meal cake, chicken manure and control as sub plots; and plastic mulch vs no mulch as sub-sub-plot treatments. Mustard grown until flowering after sweet corn harvest in 2008 was incorporated into the soil along with chicken manure and canola meal cake in Sept. and temperature sensors were inserted at 15 cm depth to monitor soil temperature. The soil was covered from Sept. 19 to Oct. 30 with four mm thick transparent plastic sheets in designated plots that were made air tight from all sides. Onion was grown in the next season, and Fusarium basal rot and Pink Root incidence was measured toward the end of the growing season. Plastic mulch increased soil temperature, and soil amendments facilitated the process. Mustard, canola meal cake, chicken manure, and their combination did not affect Fusarium basal rot incidence; only chicken manure reduced the Pink Root incidence. A combination of mustard, chicken manure and soil solarization was more effective than each factor alone.

Impact and characterization of 'black shadow' on highbush blueberry
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Phytopathology 101:S143

There is an increasing occurrence of a disease that we call 'black shadow' on highbush blueberry (*Vaccinium corymbosum*) stems and buds. The disease is characterized by a blackening of the 1–2 year old shoots and the developing buds. Symptoms become apparent in the late summer and fall and are readily visible in the spring before the plants emerge from dormancy. The blackening is due to the growth of fungi in or on the plant cuticle. To characterize the problem, we isolated the causal agent(s). Small sections of affected bark were plated on PDA. At least three different types of fungi were isolated, some of which were similar to those seen in sooty blotch and fly speck of apple. However, the disease is distinct from a similar condition known as sooty mold which develops epiphytically on plant exudates and the sugary excretions of some insects. To quantify the impact, shoots were collected from affected bushes and evaluated for percent 'black shadow' coverage, length (current year growth), and number of flower buds. Preliminary results suggest that when black shadow covers greater than 90% of the stem surface, the inflorescence buds are reduced in size and number. Control of the disease is best accomplished with application of lime sulfur in the fall. Work is ongoing to confirm the causal agent(s).

Fruit rot resistance and heritability in cultivated cranberry
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Phytopathology 101:S143

Fruit rot is the primary threat to cranberry (*Vaccinium macrocarpon*) production in the northeast and an increasing problem in other growing regions. Cranberry fruit rot is incited by a complex of fungi from at least 12 genera. Despite the wide array of causal agents, we have identified a few accessions that exhibit field fruit rot resistance (FFRR). The FFRR selections were crossed and the progeny evaluated in the field. Thirty families and 1644 progeny were rated using a 1–5 scale, with 5 being 100% rotted. Some families were segregating, as a continuous distribution, for FFRR and targeted for further study. We now have a draft cranberry genome sequence and the opportunity to identify gene regions associated with resistance. Thus, we need a method to evaluate fruit for resistance to specific pathogens. Some fruit rot fungi do not readily sporulate and spray-inoculation of spores is unreliable. As an alternative, wooden toothpicks were colonized with *Coleophoma empetri*, *Colletotrichum acutatum*, *Colletotrichum gloeosporioides*, *Phyllosticta vaccinii*, or *Physalospora vaccinii*. The tips of the colonized toothpicks were inserted into fruit of susceptible and resistant selections. This method worked well, with susceptible selections having a larger diameter of rotten tissue. In some cases, production of anthocyanin was evident at the wound site. We are confident that this method will allow a more precise identification of resistance across a wide array of germplasm.

Potential of Furrier transform infra-red (FTIR) spectroscopy for differentiation of phytopathogens
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Phytopathology 101:S143

Phytopathogenic fungi are of the most common causes of plant disease. Distinguishing among different strains or isolates of a certain fungus species is difficult, and time consuming using common biological means. In this study we suggest a quick and low cost technique. IR spectra are well known for their sensitivity to composition and three dimensional structures of biomolecules. They allow measuring complex molecular vibrational modes of functional groups. FTIR spectroscopy has become a widely used analytical tool in the biomedical sciences for characterization of a variety of specimens, e.g., microorganisms, body fluids and tissues. In this study we used FTIR attached with attenuated total reflection (ATR) to examine fungi. Using this technique one can get information of the fungus chemical structure which is represented in its mid-infrared absorption spectrum. Comparing absorption spectra of various fungi genera shows significant differences among them. Using cluster analysis techniques, we differentiated between different genera with 100% success. Further examination of several isolates of the same species (*Fusarium oxysporum*) yielded more subtle differences among the absorption spectra. In order to distinguish among the isolates, we used advanced mathematical and statistical methods (PCA and LDA respectively) on the obtained spectra. That resulted in good distinction (90%) among the isolates, which is, to our knowledge, an unprecedented result in this field.

Molecular diversity of viruses in vegetable crops from farmers' fields in South and Southeast Asia
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Virus diseases are affecting the production of vegetables by farmers in South and Southeast Asia. The IPM-CRSP of the USAID has funded a project to implement ecologically-based IPM packages for key virus diseases and/or virus-vector complexes. For this purpose, we have collected samples suspected for virus infections, based on visual symptoms, from a variety of vegetable crops (tomato, chilli peppers, okra, bitter gourd, bottle gourd, cucumber, pumpkin and ridge gourd) in farmers' fields from India, Bangladesh, Nepal, Cambodia, and Indonesia. These samples were imprinted on FTA® cards or nitrocellulose (NC) membranes in the field, air dried and

shipped to Washington State University. Total nucleic acids recovered from FTA cards and NC membranes were tested by PCR and RT-PCR using group- and species-specific primers for the detection of a range of viruses. The DNA fragments amplified from these assays were cloned and nucleotide sequence determined. A comparison of these sequences with corresponding sequences available in GenBank revealed the presence of distinct virus species belonging to the genera Begomovirus, Potyvirus, Tospovirus and Cucumovirus. These results provided a foundation for a better understanding of the spectrum of viruses in vegetable crops across the regions and the development of field-based assays for their monitoring in multi-location varietal evaluations and IPM trials in host countries.

Rapid and real-time detection of grapevine leafroll associated viruses in grapevines and insect vectors

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Grapevine leafroll disease (GLRD) produces distinct symptoms in red- and white-fruited wine grape cultivars of *Vitis vinifera*. GLRD symptoms are sometimes mimicked by nutritional disorders, mechanical damage during viticultural operations and injury due to other abiotic factors, especially in red-fruited wine grape cultivars. Previous studies have shown that six grapevine leafroll-associated viruses (GLRaVs; GLRaV-1, -2, -3, -4, -5, and -9) are present in GLRD-affected grapevines in Washington vineyards. Due to low concentration and uneven distribution of viruses in grapevines and variation in disease symptoms, we developed reverse transcription-quantitative real time polymerase chain reaction (RT-qPCR) assay for the detection of GLRaV-2 and GLRaV-3 in grapevines infected with GLRD. For this purpose, we designed primers to amplify a 120 base pair fragments specific to the replicase gene module of GLRaV-2 and GLRaV-3 and optimized conditions for their amplification in grapevine samples and insect vectors. Using this method, we were able to estimate virus load in a given sample. The RT-qPCR provided higher sensitivity for the detection of the two GLRaVs in grapevines and vectors and allowed rapid discrimination of these viruses in mixed infections. This assay is being used in monitoring the spread of GLRD and discriminating GLRD from 'symptoms' due to nutritional and other abiotic factors.

Identifying quantitative trait loci (QTL) for resistance to Fusarium crown rot (*Fusarium pseudograminearum*) in two spring wheat populations

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Phytopathology 101:S144

Fusarium crown rot (FCR), caused by *F. pseudograminearum* and *F. culmorum*, reduces wheat yields in the Pacific Northwest (PNW) of the U.S. by as much as 35%. Currently there is no consistent durable resistance to FCR in PNW wheat cultivars. Significant QTL for crown rot resistance have been documented on chromosomes 1A, 1D, 2B, 3B, and 4B from resistant Australian cultivars. The objective was to identify major QTL for Fusarium crown rot resistance in the Australian spring wheat cultivar 'Sunco' to facilitate PNW breeding efforts. Two mapping populations consisting of 151 F5:F6 and 219 F6:F7 recombinant inbred lines (RIL), were derived from crosses between Sunco (partially resistant) by Otis (susceptible) and Sunco by Macon (susceptible), respectively. Plants were inoculated with *F. pseudograminearum* isolate (006-13) in growth room (seedling), outdoor nursery (adult) and field (adult) assays. Stem base crown tissues of seedling and adult plants were rated for disease severity on 0 to 10 scale during 2008, 2009 and 2010. Five significant QTL were identified on chromosomes 3B, 4B, 4A, and 7A with LOD scores ranging from 3.0 to 23. The most significant QTL was located on chromosome 3BL and inherited from Sunco with maximum LOD scores of 23 and 10 explaining 28% and 23% of the variation, respectively for each population. This is the first report of this unique 3BL QTL for resistance to Fusarium crown rot inherited from Sunco.

Spatial distribution of soybean cyst nematode in research plots

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Soybean cyst nematode (SCN), *Heterodera glycines* Ichinohe (Tylenchida: Heteroderidae), is the most important pathogen of soybean (*Glycine max* (L.) Merr.), in the United States. The spatial distribution of SCN in ten naturally infested sites in the Red River Valley of North Dakota was examined during 2006–2009. These sites, which ranged from 557 to 975 m^2, had been used as disease nurseries to screen soybean cultivars. SCN populations varied among plots from undetected to 25,000 eggs/100 cc soil, and in some sites the differences in egg densities observed between adjacent plots were as high as 6-fold. Lloyd's index of patchiness, which ranged from 1.09 to 3.34, suggested an aggregated distribution in nine of the ten sites evaluated. Egg densities were grouped in classes using two categorical scales based on the effect such populations have on soybean yield. Such data was used to estimate the effect of the spatial variability of egg populations on plot size and to estimate the minimum number of plots required to compare cultivars. In three of the sites, no minimal plot size could be determined due to the spatial distribution of SCN. For some sites, the use of categorical scales compared to using egg numbers, reduced the size and number of plots. The spatial distribution of SCN eggs in research plots can be a critical factor affecting outcomes of field experiments.

Survival and natural biological control of *Sclerotinia sclerotiorum* sclerotia in alfalfa seed production

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White mold caused by *Sclerotinia sclerotiorum* can be a major issue for alfalfa seed growers under cool wet weather conditions following row closure. After harvest in August, alfalfa seed growers in Touchet, WA manage stubble by burning it in February to March of the following year. Burning alfalfa stubble is practiced to help manage weeds, insects and white mold, however, pressure to limit or not permit burning is a current issue due to environmental concerns over air pollution. The present study determined that the total number of sclerotia in the soil of burned field plots was either less (41%) or significantly less (71%, $P = 0.015$) than non-burned control plots in 2009 and 2010, respectively. In addition, survival of sclerotia in burned verses non-burned plots was either less (9.4%) or significantly less (10.7%, $P = 0.021$) than non-burned field plots in 2009 and 2010, respectively. The percentage of sclerotia colonized by *Fusarium acuminatum* collected in plots that were burned (23.8% in 2009, 24.2% in 2010) was significantly greater than in plots where stubble was non-treated, mowed or tilled in 2009 ($P = 0.0586$) or mowed and tilled in 2010 ($P = 0.0533$). *F. acuminatum* and *Ulocladium atrum* were identified as the two dominant natural colonizers of sclerotia in field soil. These results support that field burning of alfalfa stubble is an important IPM practice for the management of white mold in seed production.

Biological control of Silvery threadmoss (*Bryum argenteum*) a weed problem of golf course putting greens and nursery crops

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Silvery threadmoss (Bryum argenteum) has become an increasingly problematic weed of golf courses, particularly since the loss of mercury and other heavy metal based pesticides, which controlled moss. A decrease in mowing height requiring increased passes of equipment over the green, decreased nutrient inputs, and an open turf canopy contributes to moss encroachment on putting greens. The only commercial herbicide labeled for moss control is carfentrazone which does not completely eradicate moss, so sequential applications are required once moss recovers. Aside from turf, silvery threadmoss can also be a weed problem of containerized nursery crops as well as nursery growth pads and stone hardscapes. There are no professional products labeled for moss control in these systems. A naturally occurring microorganism has been discovered that effectively controls silvery threadmoss. This organism was evaluated fulfilling Koch's postulates for disease in silvery threadmoss. Host specificity confirms the organism is active against silvery threadmoss, but does not cause disease in the most commonly managed turf species, creeping bentgrass or annual bluegrass. We are evaluating this organism for all three niche markets, looking at host specificity to be sure the pathogen is not a disease organism of the most commonly cultivated landscape ornamentals or common naturally occurring mosses found in the landscape.

Complete genome sequence of the stone fruit pathogen *Xanthomonas arboricola* pv. *pruni*

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Xanthomonas arboricola pv. *pruni* (Xap) causes bacterial spot on a wide range of *Prunus* spp. Outbreaks can result in severe economic losses to fruit quality and yield, branch/tree dieback, and orchard devastation. Xap is endemic in the U.S.A., NZ and is a quarantine regulated pathogen in Europe

and elsewhere. Almost nothing is known about the genetics of Xap compared to other xanthomonads. We have sequenced the complete genome of a genotypic-representative Xap strain from Europe (Italy, CFBP 5530). This is the first complete genome sequence for this species. Paired-end 454-pyrosequencing and primer walking on a fosmid library gave 3 contigs. The chromosome is 4.85 Mb with 65.6% GC ratio and 3912 predicted CDS. Xap has a unique 41.2 kb plasmid with a 62.3% GC ratio and 45 CDS. Automatic annotation using several sequenced *Xanthomonas* genomes as templates, and partial manual annotation, identified a suite of 21 type III secretion system effectors, iron acquisition, and other putative virulence/ecological fitness determinants, many apparently unique to *X. arboricola*. EDGAR comparisons against *X. axonopodis* pv. *citri* str. 306 and *X. campestris* pv. *campestris* LMG8004 indicated a pangenome of 2786 CDS and 848 singletons in Xap. Applied genomics has identified over 90 VNTR, several of which are currently being used for biodiversity analysis of Xap and related subspecies, and design of improved diagnostics.

MALDI-TOF mass spectrometry: Applications for rapid bacterial identification and phylogenetic analysis

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Rapid reliable identification of pathogenic bacteria is critical for effective implementation control measures, and is typically required at subspecies level. We have developed whole-cell matrix assisted laser desorption ionisation – time of flight mass spectrometry (MALDI-TOF MS) as a high-throughput, rapid and cheap alternative for routine diagnostics. MALDI-TOF MS is based on discriminatory peptide mass fingerprints cross-referenced in a comprehensive database. Commercial databases are largely limited to clinical bacteria, and we are filling this gap with super-spectra fingerprints for phytopathogenic bacterial groups (*Xanthomonas*, *Agrobacterium*, *Erwinia*, *Dickeya*, *Pantoea*). Sample preparation, analytical and statistical methods have been optimized to deliver robust taxa discrimination at the species and in many cases subspecies level. Further application for rapid phylogenetic analysis has been validated as a reliable alternative to sequence-based approaches delivering comparable phylogenetic resolution. MALDI-TOF MS methods, applications, comparison with standard identification and phylogenetic approaches, and potential as an emerging tool in phytobacteriology will be discussed.

Application of bioinformatics to study type III effector signals

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Type III effectors are secreted and translocated into the host cell via a specialized type III secretion system. The process of translocation of effectors is highly regulated. Effectors are believed to contain secretion and translocation signals in the N-terminal region that direct them through the secretion apparatus. Secretion and translocation signals have not been well characterized in xanthomonads. Although a few *Xanthomonas* effectors contain *Pseudomonas* effector-like features in the N-terminal region, the majority of the amino acid bias features do not strictly follow for *Xanthomonas* effectors, indicating the need to develop separate predictive rules and programs for identifying signal features in type III effectors. We have developed position-specific scoring matrices (PSSM) for the motifs identified based on amino acid biases in N-terminal region of *Xanthomonas*. These PSSMs can be used for screening for the candidate type III effectors from the sequenced genomes and upcoming draft genomes of xanthomonads. Calibration and validation of the PSSMs were carried using already sequenced *Xanthomonas euvesicatoria Xcv* str. 85-10 genome, from which type III effector repertoire has been well characterized. These matrices were searched for the candidate effectors within bacterial spot *Xanthomonas* genomes. Candidate effectors were tested for translocation using *in-planta* avrBs2 reporter gene assay. We have identified a few novel effectors from bacterial spot xanthomonads using this method.

Epidemiological studies on *Blackberry chlorotic ringspot virus*

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Blackberry yellow vein (BYV) has emerged as an important disease in all the blackberry growing areas of southeastern United States, with new viruses associated with the disease discovered continually. *Blackberry chlorotic ringspot virus* (BCRV), a recently identified ilarvirus, has frequently been detected in diseased samples. Other than blackberry, the virus also infects rose and raspberry making its survival more efficient. Apart from the apparent growing invasion of BCRV in blackberry and rose, there is no information on the epidemiology of this virus. The objective of this study is to acquire information on different aspects of the virus epidemiology including identification of initial sources of infection, alternate hosts and virus transmission. Several isolates of the virus infecting cultivated and wild-blackberry, raspberry and rose were collected from several states. The complete RNA 3 of the virus was amplified, cloned, and subjected to sequence analysis to determine isolate variability. Alternate host identification was performed by testing plant species present in areas with high BCRV incidence. BCRV was grafted on to herbaceous hosts and seed transmission evaluated an efficient transmission mode of ilarviruses. Rosa multiflora seeds, naturally infected with BCRV, were also collected and tested for seed transmission. The results of this study clarify factors contributing the epidemiology of BCRV by identifying the virus variability, alternate hosts and seed transmissibility.

Proteomic and biochemical analysis of heat shock responses in *Trichoderma* species

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Trichoderma strains are the best studied bioagents of plant pathogens and are successfully employed as biopesticides and biofertilizers. Biocontrol activity of these fungi is significantly affected by soil temperature. Identification of thermotolerant strains of *Trichoderma* would aid in their successful application in arid and semi-arid regions of agriculture. *Trichoderma* strains collected from varied agro climatic zones of India were screened for high temperature tolerance by exposing them to 48°C, 50°C and 52°C for 1 hr, 2 hr and 4 hr respectively. Four *Trichoderma* strains *viz*., Tv33, Tv7316, TvS3 and Tv79 with lethal temperature 50 (LT_{50}) of 44–50°C were identified as tolerant to high temperature stress and these strains have good biocontrol potential against root rot diseases caused by *Macrophomina phaseolina* and *Sclerotium rolfsii*. These isolates accumulated >25% of trehalose and mannose during heat stress compared to the susceptible strains. Raffinose concentration increased steadily with increased duration of heat stress in all isolates compared to controls and thus its role in thermotolerance is not clear. Cycling decrease and increase of protein concentration was observed in thermotolerant isolates during heat stress at 48°C, 50°C and 52°C for 1hr and 2D-PAGE analysis revealed accumulation of high molecular weight acidic proteins. Identification of these proteins by MALDI-TOF and their role in thermotolerance are discussed in this paper.

Characterization of bacteriophages PT21 and UASP infecting Ralstonia solanacearum: A potential bio-control agent

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Bacteriophage as biocontrol agent is at peak in the area of plant protection with great potential to replace the chemical control measures now prevalent. The present study aims at understanding the morphology, host specificity and phage preparation in order to effectively fight potentially adapting R. Solanacearum. The phages were found to be different on the basis of their plaque morphology; PT21 isolate from black clay soils of Bijapur produced bigger plaques, where as the UASP isolate from red sandy soils of Bangalore produced little smaller size plaques. The phage titre was found to be higher in both types indicating wide prevalence of phage in both soil types. Phages were thermo stable at 65°C for 30 min. The lower pH of 5 to 4 inactivated the phages and 6-10 was optimal for growth. The latent period ranged from 30 to 35 minutes while the burst size was found to be 35 pfu/bacterium. The electron microscopic studies revealed that phage belong to Siphoviridae with hexagonal head with non-contractale tail. Genomic DNA extracted from Bijapur phage (PT21) found to have molecular weight of 47,771 bp with restriction sites for Eco-RI, Eco-RV, Alu-I, Hinc-II, MLU-I. In vivo evaluation of phages in green house condition for its efficiency revealed that phages were effective in reducing the disease incidence upto 85 per cent and the pathogen population was reduced by 1000 times. Hence, bacteriophages are found to be potential candidates as biocontrol agents in the control of Ralstonia solanacearum.

Pseudomonas fluorescens SP007s reduces plant infection and increases γ-aminobutyric acid in seed infected by a complex pathogens of rice
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Phytopathology 101:S146

Field experiments using seed and foliar treatments to test efficacy of *Pseudomonas fluorescens* SP007s against brown spot (caused by *Helminthosporium oryzae*) and dirty panicle (caused by complex pathogens) were conducted. SP007s significantly reduced disease severity of both diseases, promoted plant growth and increased yield (P = 0.05). Expression of different defense-related enzymes including superoxide dismutase, guaiacol peroxidase, β-1,3-glucanase, peroxidase and phenylalanine ammonia-lyase was detected at 1 day post SP007s spray which inversely correlated with the reduction of AUDPC. Treatment of naturally infested seeds (dirty panicle caused by *H. oryzae*, *Fusarium semitectum*, *Cercospora oryzae*, *Curvularia lunata*, and *Alternaria padwickii*) with SP007s enhanced pathogen reduction, seed germination, shoot, and root length with 92, 22, 36, and 25% respectively. Populations of all causal pathogens were significantly and consistently suppressed in treated seeds over 3-month investigation. Increased γ-aminobutyric acid (GABA) accumulation in only treated seeds that positively correlated with pathogen reduction was also observed. The result supports GABA mechanism involving seed resistance induction against populations of infested seeds.

The effects of salinity on _Phytophthora ramorum_ viability and infectivity
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Phytophthora ramorum, a threat to Eastern U.S. forests, has been found in waterways outside the boundaries of infested ornamental nurseries. Very little is known about what factors are conducive to its survival and sporulation in water. Water collected from various sources with different salinity was used to better understand what effect salinity has on the life cycle of *P. ramorum* and infection of tissue. Water samples were collected from natural bodies of water with conductivities of 5.6, 30.5, 32.3, and 35.3 in May 2010. The water samples were added to cups containing *P. ramorum*-infested sand (1,000 chlamydospores/cc). Rhododendron leaf disks were placed on the water surface for 1 week at 20 C and then plated on *Phytophthora*-selective medium (PARPH+V8). Very few leaf disks (≤ 3%) were infected at the three highest conductivity levels while 100% infection occurred at 5.6 mS. Similarly, Rhododendron leaf disks were placed on the surface of different salt solutions added to *P. ramorum*–infested sand at two chlamydospore levels (100 and 1,000/cc) for 1 week and plated on PARPH+V8. The leaf disks were exposed to the conductivity levels of 10.3, 26.5, 36.0, 57.2, and 67.9 mS. The disk infection rates at 100 spores/cc were 61.1, 23.1, 3.3, 0, and 0%, respectively, while infection rates at 1,000 spores/cc were 100, 70.0, 55.6, 2.2, and 0%, respectively. This research helps to better understand the survival and factors affecting infectivity of *P. ramorum*.

Increased strawberry production in Florida over a generation is associated with adoption of favorable arthropod management practices
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Strawberry production in Florida, U.S.A. has increased from about 1,600 ha. valued about $65 million in 1978 to about 3,642 ha. valued at about $363 million in 2010. The entire production is for fresh-market winter sales along the U.S.A. east coast. The principal arthropod pests include the twospotted spider mite (Tetranychus urticae Koch), southern and fall armyworms (Spodoptera eridania (Cramer) and S. frugiperda (J.E. Smith)), Frankliniella bispinosa flower thrips, melon aphid (Aphis gossypii Glover), two Drosophila spp. fruit flies and three or more species of sap beetle (Nitidulidae), although several other arthropods occasionally reach economic pest status. During the past 34 years, management measures have incorporated new practices such as scouting and applying remediation as ecological conditions warrant, use of Phytoseiulus persimilis predator for control the main pest twospotted spider mite, elimination of all chlorinated hydrocarbon insecticides, elimination of 73% of the 11 previously used organophosphate insecticides, introduction of eight biologically derived insecticides where there were none before, and introduction of 18 modern insecticides largely environmentally and toxically more benign than the insecticides they replaced. These changes have been associated with increased production, reduced impact on the environment, and enhanced worker and consumer safety.

Disease progress of thousand cankers disease in Oregon
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Thousand cankers disease has spread throughout Oregon since first observed in the early '90's. Symptomatic, mature black walnut (*Juglans* sp.) trees are harvested for valuable lumber with the assumption they will rapidly decline and die. Disease progress was documented for 60 trees from 11 locations in the Willamette Valley from Sep 07 to Jul 10. The walnut twig beetle vector (*Pityophthorus juglandis*) and causal pathogen (*Geosmithia morbida*) were confirmed in each general location. The amount of canopy with dieback symptoms was recorded for each tree in Sep 07, Sep 08, Aug 09 and Jul 10. At the Jul 10 rating 15 trees had higher canopy dieback ratings, 36 had similar ratings and 8 trees had lower dieback ratings when compared to Aug 09. Trees with higher ratings had an average increase of 6.4%, with a range from 5 to 20%. Trees with lower ratings had an average decrease of 6.2% ranging from 5 to 10%. At the Jul 10 rating for trees on which data had been collected in Sep 07, 17 trees had higher canopy dieback ratings, 26 had similar ratings and 8 trees had lower ratings. Trees with higher ratings had an average increase of 17.2%, with a range from 5 to 70%. Trees with lower ratings had an average decrease of 6.3% ranging from 5 to 15%. Although some trees seem to die quickly, the vast majority die back very slowly, if at all. Based on these observations, disease progression in trees with thousand cankers disease is a slow process in Oregon.

Species profile and genetic variation of _Fusarium_ isolates sampled from koa trees in Hawaii
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The native Hawaiian koa (*Acacia koa*) trees suffer from wilts and diebacks which were considered to be caused by *Fusarium oxysporum*. To determine the causal agents of the koa wilt and their genetic variation, 92 isolates of *Fusarium* spp. were recovered from rhizosphere soil, roots and branches of wilted or die-backed koa plants, and characterized by sequence analyses of partial translation elongation factor (EF-1a) gene, the beta-tubulin gene and the nuclear ribosomal intergenic spacer region (IGS-rDNA). Based on sequence identity, 50 of the *Fusarium* isolates were identified as *F. oxysporum*. Other species identified includes *F. solani*, *F. pseudocircinatum*, *F. equiseti*, *F. boothi*, *Nectria rigidiuscula* and *Neonectria castaneicola*. The genetic variation of the *Fusarium* isolates was further evaluated using amplified fragment length polymorphism (AFLP) and simple sequence repeat (SSR) markers. The four AFLP primer pairs used (E-AC/M-CG, E-AT/M-CC, E-AA/M-CT, and E-AA/M-AT) generated polymorphism among the 92 fungal isolates. However, the nine SSR primer pairs derived from *F. oxysporum* only produced amplicons in the 50 *F. oxysporum* isolates and 18 of the other *Fusarium* isolates. Cluster analysis using the DNA markers indicated that isolates formed distinct clusters corresponding to the *Fusarium* species identified. The results indicate that *F. oxysporum* was the predominant species colonizing the roots of wilted koa plants and exhibited a certain level of genetic variation.

Antibiosis by _Pantoea agglomerans_ biocontrol strain E325 against _Erwinia amylovora_ on apple flower stigmas
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Pantoea agglomerans E325, the active ingredient in a commercial product for fire blight control, was previously shown in vitro to produce a unique alkaline- and phosphate-sensitive antibiotic specific to *Erwinia amylovora*. Antibiosis was evaluated as a mode of antagonism on flower stigmas using two antibiosis-deficient mutants. On King's medium B, mutants E325ad1 and E325ad2 have stable smooth-butyrous or hypermucoid colony morphologies, respectively, whereas the parental strain E325 exhibits phenotypic plasticity with predominantly hypermucoid colonies accompanied by slower-growing, smooth-butyrous colonies. Mutants were tested against *E. amylovora* on stigmas of detached flowers of crab apple (*Malus Mandshurica*) in growth chambers and apple (*Malus domestica*) in the orchard. Epiphytic fitness of the antibiosis-negative mutants was similar or greater than the parental strain as determined by relative area under the population curve (RAUPC). In laboratory and orchard trials, both mutants had significantly lower inhibitory activity against the pathogen (i.e., less reduction of *E. amylovora* RAUPC)

compared to the parental strain. E325 and the mutants caused similar decreases in pH in a broth medium, indicating that acidification, which was previously reported as a possible mechanism of pathogen inhibition on stigmas, is not directly related to antibiosis. In this study we provide the first evidence for E325 antibiosis involved in *E. amylovora* growth suppression on apple flower stigmas.

Genetic characterization and distribution of mating type genes in *Sclerotinia homoeocarpa* populations

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Dollar spot, the most economically important disease of turfgrass worldwide, is caused by the filamentous ascomycete *Sclerotinia homoeocarpa*. The objective of this research is to characterize the genetics and distribution of mating type (*MAT*) genes in *S. homoeocarpa* populations. In an early draft genome assembly of *S. homoeocarpa*, the *MAT* locus was found to contain regions with similarity to the *MAT1-1-1* genes, containing an alpha motif, and the *MAT1-1-5* genes in the available genomes of *S. sclerotiorum* and *Botrytis cinerea*. Primers anchored in the flanking DNA lyase and cytoskeleton assembly genes were used to amplify and sequence the *MAT1-2* idiomorph, which contained a high mobility group-box motif with similarity to *MAT1-2-1* genes. The *MAT* locus in *S. homoeocarpa* is similar to that of *B. cinerea* with respect to gene orientation and the presence of a truncated portion of *MAT1-2-1* flanking the *MAT1-1* idiomorph. However, unlike *B. cinerea*, elements of the *MAT1-2-3* gene and a deleted portion of *MAT1-1-1* were not detected in the *MAT1-2* idiomorph in *S. homoeocarpa*. In a limited survey, 55 of 121 isolates of *S. homoeocarpa* from North America and 3 of 49 isolates from Asia, Europe, and South America were determined to contain the *MAT1-1* idiomorph. A multiplex PCR assay is currently being developed to rapidly screen worldwide populations of the pathogen. Data developed from this study will be useful in population studies of *S. homoeocarpa*.

Development and characterization of microsatellite markers for *Sclerotinia homoeocarpa*

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Phytopathology 101:S147

Sclerotinia homoeocarpa causes dollar spot, the most economically important disease of turfgrass worldwide. The objective of this research is to develop and use microsatellite markers in population studies of *S. homoeocarpa*. Microsatellites were initially isolated using a bead capture enrichment protocol, and additional repeats were identified in silico from an early draft genome assembly of *S. homoeocarpa* using the Tandem Repeat Database. Microsatellites with sufficient flanking sequence to the end of the read or to adjacent repeats were deemed suitable for primer design. Next, candidate loci were examined for polymorphisms by Sanger sequencing. Candidates containing indels in the flanking region or compound polymorphisms were discarded as not usable. From the genome data, 6,075 microsatellites were identified based on minimum thresholds of repeat number, copy number, and perfection of repeat units. Two of 31 candidate loci from the enrichment protocol were selected as usable. Of the 791 candidate loci identified in silico, to date 5 usable loci have been selected and 42 have been discarded. Two to four alleles per locus have been found among a select group of cool- and warm-season isolates from four continents. Multiplex PCR protocols using fluorescently-tagged universal primers are being developed to enable rapid genotyping. These microsatellites will be useful in determining the diversity and structure among worldwide populations of *S. homoeocarpa*.

The effect of plant activators on salinity-induced predisposition in tomato to *Phytophthora* root rot and bacterial speck disease

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Episodes of abiotic stress such as water deficit and soil salinity are common in agricultural systems and can increase the incidence and severity of diseases. Abscisic acid (ABA) increases rapidly in roots and shoots under conditions of water deficit. ABA plays an important role in crosstalk between water stress and disease response signaling networks in plants, and modifying ABA levels in plants by osmotic stress can increase susceptibility to pathogens. Commercially available activators of systemic acquired resistance (SAR) have shown efficacy against plant diseases. To determine the effect of plant activators on salt-induced predisposition, we examined two SAR activators, Actigard (Syngenta) and Tiadinil (Nihon Nohyaku Co., Ltd), in hydroponically-grown tomato seedlings challenged with the root pathogen *Phytophthora capsici* or with the bacterial speck pathogen *Pseudomonas syringae pv. tomato* (*Pst*). Root treatment with the plant activators induced resistance in leaves to *Pst*, but not to *P. capsici*. Salicylic acid-deficient *NahG* transgenic plants displayed enhanced susceptibility and salt-induced predisposition to both pathogens. Pretreatment of roots with Tiadinil reduced salinity-induced predisposition to *Pst*, but not to *P. capsici*. Actigard and Tiadinil induced ABA in roots and shoots to levels observed with salt stress, suggesting that plant activators can override predisposition observed in some diseases associated with stress-induced ABA.

Temporal dispersal patterns of *Sclerotinia sclerotiorum* ascospores during canola flowering

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The temporal patterns of *Sclerotinia sclerotiorum* ascospores dispersal in canola field were studied at two North Dakota locations between 2005 and 2007. Seven-day volumetric spore samplers were used to monitor airborne ascospore population while electronic data loggers recorded hourly information on air temperature, relative humidity, soil temperature, and soil moisture under the conala canopy. Ascospore dispersal occurred during single period that lasted 4–6 hours. in 2005 and 2007 most ascospore were collected between 10 am and 1 pm; however, in 2006, a drier than normal year, most were collected between 2am and 7am. in 2005 and 2007 the first sharp increase in ascospore dispersal was preceded by a 10-unit drop in relative humidity from close to saturation, and an increase in air temperature of 5°C. in 2006, however, no significant changes in relative humidity, which remained around 90%, or air temperature, which hovered around 15°C, were recorded prior to the start of the discharges. these nighttime discharges lasted two hours longer than daytime discharges. daily discharges, daytime and nighttime, started when relative humidity was ≥90% and air temperature was around 10–16°C. multiple peak-days, days with mean ≥20 ascospores/m_3, were recorded in 2005 and 2007, but none were recorded in 2006. Peak-days were associated with preceding periods of seven consecutive days with mean relative humidity ≥85% in the canola canopy.

Physiological and genetic differentiation of Curvularia lunata and resistance evaluation on corn Curvularia leaf spot in northeast of China

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Curvularia lunata, 26 isolates of mainly isolated from corn Curvularia leaf spot in 15 different geographic regions in Jilin, Liaoning and Heilongjiang province, which were characterized by differential host and random amplified polymorphic DNA(RAPD). Two kinds of variation may have a certain correlation. No close correlation was found between genetic diversity and geographic orgins of isolates. The strains for test were identified as five pathogenic types by differential host which higher pathogenic types was distributed widely in Northeast, therefore that could be considered as the dominant pathogenic group. However highest pathogenic types was distributed in Baicheng in Jilin Province, weakly pathogenic types was mainly distributed in Baishan and Lishu in Jilin Province. Eight primers were employed for RAPD analyses of 26 isolates. Out of the 77 RAPD markers, 71 polymorphic markers (P = 92.2%) were obtain. Most strains with highest and higher pathogenicity was clustered together, however strain with weak pathogenicity was clustered at lower similar level. 200 maize cultivars were collected to assess their resistance to Curvularia lunata by artificial inoculation at the seedling and adult stages in the field. No immune genotypes were found. Highly resistant were 3%, resistant were 4% and intermediately resistant were 33%, and intermediately susceptible were 23.5 and susceptible were 36.5%.

Genetic analysis of gene conferring resistance to wheat stripe rust in Lankao5

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Stripe rust, caused by Puccinia striiformis f. sp. tritici, is one of the most serious diseases of wheat in the worldwide. China is the largest epidemic region in the world and severe losses in grain yield have been reported. The disease-rsistant variety is controls the wheat stripe rust to be most economical, the secrurity and effective method. To identify new resistance genes is significiant in wheat breeding. Lankao5 is a famous variety that has resistance to stripe rust, for identifing the genetically characterize, F1, F2, F3 and BC1F1

progenies from the cross of Lankao 5/Chinese Spring were tested with PST races CYR29, CYR30, CYR31, CYR32, SU11-4 and SU11-11 in the greenhouse, respectively. Genetic analysis showed that resistance of Lankao5 to SU11-4, CYR31 and CYR32 were conferred by two dominant genes, to SU11-11 conferred by one dominant gene, to CYR30 conferred by one dominant gene and one recessive gene and to CYR29 conferred by two recessive genes. Lankao 5 as resistant resource and its genetic information should be useful in breeding resistant cultivar to stripe rust.

Races of Exserohihun turcicum and evaluation of maize cultivars on the resistance to Northern corn leaf blight in Jilin Province of China

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Northern corn leaf blight (NCLB), caused by Exserohilum turcicum (Pass.) Leonard & Suggs, is an important foliar disease of corn in Northeast of China. The disease was observed and sporadic occurrence at first in Northeast of China in 1899. Gene-for-gene relationships have been demonstrate for E. turcicum on maize. 15 physiological race including 0, 1, 2, 3, 12, 13, 1N, 23, 2N, 3N, 12N, 13N, 123N, 123, 23N were have been identified according to virulence formula (Leonard,1989) in Northeast of China. It is quite complex race component in Jilin province of Northeast of China, and there were not dominant races as well as in mid-serotinous production areas of maize. Especially, new virulent races can overcome all multi gene of Ht resistance. 123N (0/Ht1, Ht2, Ht3, HtN) were prevalent in the middle and west of Jilin province. 100 lesions lamina were collected from 7 mazie-growing areas of Jilin province, of 11 were identified as race 123N with the frequency of 11%. 269 maize cultivars were collected from seeds market in Northeast of China, and evaluated for resistance to northern corn leaf blight under the level of greenhouse. The result showed that the susceptible varieties with the frequency of 64.31%, mid-susceptible varieties occupy 21.56%, mid-resistance varieties occupy 10.40% and resistance varieties only 3.72%.

Nematicidal activity of two components from the broth filtrate of *Aspergillis niger* Y-61

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Phytopathology 101:S148

Application biological pesticides are more concern in China. A fungus strain of *Aspergillus nigerY-61* was isolated from farming soil of Beijing and against *Meloidogyne incognita*. Using the method of immersion, The hatching rate of individual egg and egg masses were suppressed average 82.11% and 98.47%, respectively. The corrected mortality of J_2 was 97.25% treated by the broth over 12 h. and the J_2 mortality could be remained upon 95% treated with UV for 10 h. Two activated components were obtained by means of Sephadex LH-20 column chromatography with ethanol as eluant. Molecular weight was 90 Da and 192 Da, respectively assayed by the MS. The formula of activated component were $C_2H_2O_4$ and $C_6H_8NO_7$ determined by MS, IR, ^1H and ^{13}C NMR. $C_2H_2O_4$ is oxalic acid and $C_6H_8NO_7$ is citric acid by repeated analysis of variance and contour diagram. A synergy action appeared for mixing with oxalic acid and citric acid. Suggested that citric acid and oxalic acid played a synergistic effect when LC_{50}, add > LC_{50}, mix. Statistical analysis of experimental LC_{50}, mix and theoretical LC_{50}, add with different proportions of two components were treated that the lethal rates of J_2 were significantly different between along or mixed of two acid. Therefore, oxalic acid and citric acid displayed a synergy action of killing root-knot nematodes preliminarily.

Novel heat-stable protein elicitor from *Alternaria tenuissima* activates plants resistance and growth

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This paper describes the discovery of a new microbial protein elicitor named PeaT1 that significantly enhances plant disease resistance and crop growth. PeaT1 obviously suppresses TMV on tobacco leaf, grey mould on tomato and greatly increases plant growth in rice, tomato, tobacco and cucumber. We have identified the gene sequence of PeaT1 and described the processes of purification and identification through electrophoresis, anion exchange chromatography and mass spectrometry. Action research results suggest that PeaT1 could induce plant chlorophyll formation and nutrition uptake by up-regulating a series of related genes using the rice oligo microarray. The molecular mechanism of PeaT1 inducing disease resistance in tobacco was likely through the systemic acquired resistance pathway mediated by salicylic acid and the NPR1 gene.

Comparative analyses of endogenous small RNAs in *Sclerotinia sclerotiorum* and *S. trifoliorum*

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Endogenous small RNAs (sRNAs) of *Sclerotinia sclerotiorum* and *S. trifoliorum* were compared to gain insight into the biology of the two closely related plant pathogens. Random samples of 53 unique sRNAs of *S. sclerotiorum* and 55 unique sRNAs of *S. trifoliorum* had, respectively, 221 and 229 target loci in the *S. sclerotiorum* genome database. More than half of the sRNAs targeted at exons in both species. The sRNA target loci were not evenly distributed among the 37 supercontigs of the *S. sclerotiorum* genome. The sRNA target loci from *S. trifoliorum* had the highest frequency per megabase sequence in Supercontig 35, whereas Supercontig 36 had the highest frequency of sRNA target loci of *S. sclerotiorum*, suggesting that supercontigs 35 and 36 are hot-spots for sRNA biogenesis. Four sRNAs were found in both species (four pairs). Two pairs targeted five orthologs of the same Tf2 retrotransposon. The other two pairs targeted exons, one of which is a microRNA-like sRNA. However, BLAST searches found no similar sequences in the microRNA database (MIR-BASE), suggesting fungi have different mechanisms of sRNA biogenesis than other higher eukaryotes. Two putative dicer-like (DCL) genes were identified, and DCL-2 was expressed at higher levels than DCL-1 in both species. Similarly, among three putative Argonaut protein gene transcripts AGO1 is the highest expressed Argonaut protein gene in both species, suggesting prominent roles of DCL-2 and AGO1 in sRNA biogenesis of *Sclerotinia* species.

Comparative transcriptome analysis in *Sclerotinia sclerotiorum* and *S. trifoliorum* by 454 Titanium RNA sequencing

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Sclerotinia sclerotiorum and *S. trifoliorum* are two closely related devastating plant pathogens. Extensive research has been conducted on *S. sclerotiorum* and its genome sequences are available. To take advantages of the genomic information of *S. sclerotiorum*, we compared the transcriptome of *S. trifoliorum* with that of *S. sclerotiorum* in order to gain a better understanding of the biology of both species. Total transcripts of both species during vegetative growth were extracted and sequenced using the latest 454 Titanium RNA sequencing technology. A total of 23325 unique transcripts with average length of 534 nt (12.5 mb genome coverage) were obtained from *S. sclerotiorum*, whereas 21214 unique transcripts with average length of 509 nt (10.8 mb genome coverage) were obtained from *S. trifoliorum.* About 80% of the unique transcripts of both species were found in the *S. sclerotiorum* genome database, and about 60% of the transcripts were found between the two species. About 150 transcripts from each species were found in DNA regions that are not considered as coding regions, and 15 of those transcripts were the same in both species, suggesting they are functional. Twenty-eight contigs (transcripts with more than one read) of *S. trifoliorum* were not found in the *S. sclerotiorum* genome database. Additionally, differences in expressed genes involved in pathogenesis like oxalate biosynthesis and endopolygalacturonases were detected between the two species.

Rice mutated lines showing improved resistance to *Magnaporthe oryzae* induced by space mutation

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The space mutated offspring from Zhonger Ruanzhan were evaluated on resistance to rice blast and analyzed on their resistant inheritance and R genes in system. The mutated lines H4, H11 and D69 conferring improved resistance, their leaf blast scale was 0 tested by isolate GD0193. They conferred broad-spectrum resistance, reaching 100%, the wild-type conferred moderate-spectrum resistance with about 60%, tested by 150 different isolates. They were demonstrated to show high level of field resistance tested in a natural nursery for 5 successive cropping seasons, while the wild-type was highly susceptible. H4 to isolate GD0193 was controlled by a single dominant gene and two independent dominant genes controlling its resistance to isolate GD08T4. The R gene to isolate GD0193 in H4 has been finely located in the long arm of rice chromosome 11, linked to markers RM224 and

RM27360 with ≈ 1.04 cM and 1.2 cM respectively. One of the R gene to GD08T4 in H4 was located in a region on the short arm of chromosome 1, where no other blast R genes has been reported. This could be attributed to mutagenesis of the seeds, which gave rise to new resistance.

Investigating the genetic structure of *Phytophthora capsici* populations
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Phytopathology 101:S149

Phytophthora capsici is a destructive soilborne pathogen that infects economically important vegetable crops. The objective of this study was to investigate the genetic structure of 255 *P. capsici* isolates assigned to predefined host, geographical, mefenoxam sensitivity and mating type categories. Isolates from six continents, 21 countries, 19 U.S. states, and 26 host species were genotyped for four mitochondrial and six nuclear loci. Bayesian clustering revealed some population structure by host, geographic origin and mefenoxam sensitivity with some clusters occurring more or less frequently in particular categories. Bayesian clustering, split networks, and statistical parsimony genealogies also detected the presence of non-*P. capsici* individuals in our sample corresponding to *P. tropicalis* and isolates of a distinct cluster closely related to *P. capsici* and *P. tropicalis*. Our findings of genetic structuring in *P. capsici* populations highlight the importance of including isolates from all detected clusters that represent the genetic variation in *P. capsici* for development of diagnostic tools, fungicides, and host resistance. The population structure detected will also impact the design and interpretation of association studies in *P. capsici*. This study provides an initial map of global population structure of *P. capsici* but continued genotyping of isolates will be necessary to expand our knowledge of genetic variation in this important plant pathogen.

The genetic structure of *Pseudoperonospora cubensis* global populations
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Phytopathology 101:S149

Pseudoperonospora cubensis is a destructive foliar pathogen that infects economically important Cucurbitaceous crops in the United States and worldwide. In this study, we investigated the genetic structure of 465 *P. cubensis* isolates from three continents, 13 countries, 19 U.S. states, five host species and spanning 28 years. Isolates were assigned to predefined host, geographic and year categories and genotyped for two mitochondrial and five nuclear loci. Bayesian clustering resolved six genetic clusters and suggested some population structure by geographic origin and host, as some clusters occurred more or less frequently in particular categories. Since genetic structuring was detected in P. cubensis populations, it is important to include isolates that represent the genetic variation in P. cubensis when developing diagnostic tools, fungicides, and resistant host varieties. The population structure detected should also be taken into account when designing and interpreting association studies in this pathogen. While this study provides an initial map of global population structure of *P. cubensis*, it highlights the need of an assembled genome to generate single-copy markers for evolutionary and populations studies. Future genotyping of additional isolates would be useful to determine population structure within specific geographic regions or across a wider range of hosts.

The phenomics of rice blast: Using extensive nutritional profiling to understand how the devastating plant pathogen *Magnaporthe oryzae* causes disease
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Phytopathology 101:S149

The filamentous fungus, *Magnaporthe oryzae*, responsible for rice blast disease, destroys 10–30% of the world's rice crop annually. Although its pathobiology has been studied for many years, hardly anything is known about nutrient acquisition from the host and the underlying physiology of the fungal cells. *M. oryzae* is ideally suited for *in vitro* study because it can be cultured away from its host plant, it is amenable to rapid gene functional analysis, and both *M. oryzae* and rice have sequenced genomes. Nitrogen and carbon utilization play an important role in many aspects of fungal biology especially in pathogenesis. The aim of this study is to identify key regulators of nutrient acquisition that influence pathogenesis. To facilitate our understanding of these processes, we have extensively tested our mutant strain collection on different carbon and nitrogen sources. Our results are presented in heat map form, which uses color to represent colony diameter and growth for accurate comparisons of nutrient utilization among and between different mutant strains. The data demonstrate that non-pathogenic mutant strains have a widely different nutritional profile compared to the wild type. This supports the hypothesis that some nitrogen and carbon utilizing capabilities can be important indicators of pathogenesis in the rice blast pathogen. This study is the first step in a high-throughput gene functional analysis using nutritional profiling for pathogenic gene discovery.

***Raspberry latent virus* a plant reovirus that is aphid transmitted in a replicative persistent manner**
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Phytopathology 101:S149

Raspberry latent virus (RpLV), is a newly characterized reovirus found in commercial raspberry fields in the Pacific Northwest (PNW). Phylogenetic analyses showed that RpLV is related most closely to *Rice ragged stunt virus* (RRSV), the type member of the genus *Oryzavirus*. RRSV and all members of plant reoviridae are transmitted by species of leafhoppers in a replicative persistent manner. After several failed attempts to transmit RpLV using leafhoppers, *Amphorophora agathonica*, the common raspberry aphid in the PNW, was tested as a vector of RpLV. RpLV was detected in aphids after a 12h-acquisition period using quantitative RT-PCR. Using a standard curve generated for quantifying RpLV, it was shown that the virus titer in aphids continued to increase after the acquisition period even when aphids were maintained on healthy plants with successive transfers onto fresh healthy plants every two days. This suggests that the virus replicates in the vector. Serial transfers of virus-carrying aphids to healthy plants demonstrated that the virus has a 7-day latent period in the aphid before it can be transmitted. A low percentage of plants tested positive for RpLV, 60 days post-inoculation, using aphids that tested positive for the virus, suggesting that aphids are inefficient vectors of this virus. Further experiments showed that RpLV is not transmitted transovarially to the next generation. To our knowledge this is the first report of an aphid transmitted plant reovirus.

Significant increase in titer of *Raspberry bushy dwarf virus* when present with *Raspberry leaf mottle virus* and its effect on raspberry plants
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Phytopathology 101:S149

Raspberry crumbly fruit is a virus-induced disease widespread in the Pacific Northwest (PNW). *Raspberry bushy dwarf virus* (RBDV) has been attributed as the causal agent of the disease. Recently, the identification of two new viruses: *Raspberry leaf mottle virus* (RLMV) and *Raspberry latent virus* (RpLV) in northern Washington (WA) and British Columbia, where crumbly fruit is more prevalent, suggested the existence of a new virus complex responsible for the increased severity of the disease. In efforts to determine the role of the new viruses in crumbly fruit, 'Meeker' plants containing single and mix infections of RBDV, RLMV, or RpLV were developed and used to establish field trials. Plant growth during the first year was significantly reduced in plants infected with all three viruses and the combination RBDV/RLMV when compared to control and singly-infected plants. Quantitative RT-PCR tests revealed that the titer of RBDV was increased 800-fold when it occurred in mixed infections with RLMV compared to RBDV in single infections. In addition, a survey of RpLV and RLMV in WA and Oregon revealed that RLMV is present at very high incidence (up to 100% in 5-year old fields) in northern WA; whereas the incidence in southern WA and Oregon, where crumbly fruit is not a problem, was considerably lower (40% in 8-year old plantings). These findings open the possibility that crumbly fruit disease could be managed by targeting RLMV's vector, the aphid *Amphorophora agathonica.*

Soybean susceptible leaves response to *Fusarium virguliforme* toxin in a manner resembling an incompatible interaction
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Sudden death syndrome is an important disease, caused by *Fusarium virguliforme*. This fungus colonizes soybean roots causing rot, and releases a phytotoxin that is translocated to leaves causing interveinal chlorosis. In this study, we report on an Affymetrix analysis measuring transcript abundances in resistant (PI567.374) and susceptible (Essex) leaves from the same plants used to study gene expression in roots (Radwan et al., 2011, MPMI) upon infection by *F. virguliforme*. Analysis of the leaf response to *F. virguliforme* infection versus mock inoculated plant identified 2671 transcripts as being differentially expressed. Gene expression analysis has led to a working

hypothesis that the fungal toxin activates HR-defense pathways of susceptible leaves in a manner that resembles an incompatible interaction. The response was slow and led to disease induction instead of defense. Molecular markers related to senescence and cell death were induced in susceptible leaves reflecting in part the role of HR in disease symptom development. On the other hand, soybean resistant leaves may employ a lipid biosynthesis pathway to reduce the damage fallout from fungal toxin. Cross comparison of gene expression between leaves and roots indicated that while root employ mechanisms to restrict the fungal infection, leaves employ different mechanisms to reduce the toxicity of the fungal toxin. Changes in small RNA levels between inoculated and mock treated samples were also studied and will be presented.

A 14-3-3 protein appears to be required for establishing normal nodulation in soybean

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The soybean genome contains 18 members of 14-3-3 proteins that play key functional roles in many critical physiological pathways that are regulated by phosphorylation. Transcriptomic and proteomic analyses of soybean inoculated by *Bradyrhizobium japonicum* revealed that a 14-3-3 transcript and protein was induced in inoculated roots versus control. To investigate the role of the 14-3-3 during the establishment of the symbiotic relationship between the root and *B. japonicum*, we used RNAi to silence the 14-3-3 transcript using *Agrobacterium rhizogenes*-mediated root transformation. The transformed roots exhibited reduced numbers of mature nodules. Inoculated 14-3-3 silenced roots contained large numbers of arrested nodule primordia and empty nodules instead of mature nodules. In addition, electron microscopy images showed that in the empty nodules the host cytoplasm was absent; in addition all membranes, but the symbiosome membrane, were gone. There are two highly similar paralogs of the 14-3-3 gene in soybean that was targeted for silencing. Specific differentiating primer pairs were designed to establish the expression patterns of each of these paralogs. Although these two paralogs most likely descended from a common ancestral DNA sequence, q-PCR suggested that the Glyma05g29080 transcript was induced preferentially by *B. japonicum* inoculation. Investigating of the 14-3-3 gene expression using promoter-reporter gene fusions and protein-protein interaction using co-immunoprecipitation are being conducted.

RT-PCR detection and partial characterization of *Prunus necrotic ringspot virus* isolates occurring in Iran

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Phytopathology 101:S150

The main areas for stone fruits production in Iran were surveyed for the occurrence of *Prunus necrotic ringspot virus* (PNRSV) during the April to October of 2008 to 2010. Leaf samples from 544 *Prunus* spp., Peach (*P. persica*), apricot (*P. armeniaca*) and plum (*P. domestica*) trees showing symptoms of virus infection were collected from commercial stone fruits and tested for the occurrence of PNRSV using DAS-ELISA and RT-PCR. Our study revealed a higher incidence and diversity of virus in the Tehran (19.6%) compared with the Fars (14.9%) and Golestan (11.4%) provinces. The infection levels for single species in each province were: Fars (peach, 10.6%; apricot, 17.1% and plum, 16.6%); Tehran (peach, 26.08%; apricot, 18.6% and plum, 10.9%) and Golestan (peach, 15%; apricot, 2% and plum, 7.5%) respectively. The peach isolate of PNRSV was differentiated from the apricot and plum isolates by nine differential host species. Electron microscopy examination showed spherical virions with ca 29-32 nm in diameter. All isolates had molecular weight of coat protein subunits of 29 kDa, determined by western blotting method. Three primers (VP90,91 and VP96) were used to amplify movement protein (MP) gene of three Iranian isolates of PNRSV isolated from Peach, apricot, and plum trees. Amplicons of the correct size (~ 283 bp) for the MP gene obtained from the all examined isolates of PNRSV. Most of the PNRSV isolates were identified as members of group PV32, none of the isolates belonged to group PV96 and PE5.

Analysis of Citrus Huanglongbing-associated *Candidatus* Liberibacter strains from Pakistan

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Citrus Huanglongbing is a serious disease known to cause significant damage to citrus industries world-wide. HLB has been known to be present in Asia for at least a century and one of the earliest records of the disease dates to early 1900s from the Punjab region of Pakistan. Molecular analysis of the isolates of HLB associated *Candidatus* Liberibacter asiaticus (LAS) is of importance for understanding the disease. Isolates of LAS from several regions of Punjab region from the main commercial cultivar, Kinnow mandarin, were selected. Seven different genomic regions of LAS were analyzed from the selected isolates. We included regions from different locations of the bacterial genome to better understand the extent of variability that may exist in the *Candidatus* Liberibacter strains from Pakistan. Preliminary results indicated the presence of at least two populations of LAS in the symptomatic plant samples. In the variable regions, the sequence of Pakistan isolates showed a significant number of differences when compared to the completely sequenced psy62 LAS strain from Florida.

Small RNAs of *Magnaporthe oryzae*, and the role of different sRNA biosynthetic genes on pathogenicity

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Phytopathology 101:S150

The Rice Blast fungus, *Magnaporthe oryzae*, causes serious disease to rice and other cereal crops worldwide. The deep sequencing analyses of small RNAs (sRNAs) from various *M. oryzae* mycelia exposed to different physiological stress conditions revealed the presence of more than 37 million total genome matched reads mapping to intergenic regions, coding sequences, retrotransposons, inverted repeats, tandem repeats and other repeats of the genome with more than half of the reads are from intergenic regions. The 24-nt class of sRNAs was predominant, likely reflecting a high degree of heterochromatic siRNAs. Based on the matching genomic region, sRNAs are divided into several classes, and characteristics of these classes are analyzed in detail. We also made targeted deletions of sRNA biosynthetic genes resulting in mutants having phenotypes different from wild type. The phenotypic analyses of several mutants are discussed.

Characterizing whitefly species and/or biotypes vectoring geminiviruses on peppers in Indonesia

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Phytopathology 101:S150

Whitefly transmitted Geminivirus is one of the key diseases on peppers in Java, Indonesia. Pesticides are applied as frequently as 40 times over the course of the crop without significant benefit. One of the key steps in developing integrated management strategies for the vectors is to accurately identify the vector species and/or biotypes transmitting geminiviruses. Whitefly samples were collected from pepper, other crop and weed plants in pepper production systems of Java. The collected whitefly samples were identified as *Bemisia tabaci*, *Trialeurodes vaporariorum* and *Aleurodicus dispersus* based on the morphological characters. Since *B. tabaci* has several biotypes, the biotypes were confirmed using the partial mitochondrial COI gene sequences. The COI specific primer pairs (C1-J-2195 and L2-N-3014) amplified a PCR product of approximately 900 bp size. The sequence alignment and editing resulted in a consensus sequence of 750–800 bp across all samples. The phylogenetic analysis showed two biotypes of *B. tabaci*: *Asia I* and *Asia II*. More than 90% of the samples clearly grouped with the biotype *Asia I*. This was already documented in few other studies in Indonesia and our study also confirmed the results. However, two samples from *Ipomoea aquatica* and *Trianthema* sp. grouped with *Asia II* biotype. Thus, it could be concluded that the predominant biotype in hot pepper production systems of Java is *Asia I*, and the development of management strategies should specifically focus on this biotype.

Incidence of criniviruses in multiple crops in Costa Rica

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Members of the whitefly-transmitted genus *Crinivirus* within the family *Closteroviridae* are emerging threats to both vegetable and fruit production worldwide. Recent surveys for criniviruses using symptomatology, RT-PCR, and real-time quantitative RT-PCR were performed in vegetable crops in the Cartago province of Costa Rica, one of the most important agricultural areas in the country. We identified *Beet pseudo yellows virus* in field-grown cucurbits and *Tomato chlorosis virus* (ToCV) in field- and greenhouse-grown tomatoes, and greenhouse-grown sweet peppers using virus-specific primers. In addition, newly discovered natural hosts of ToCV, including multiple species of common weeds growing adjacent to tomato nurseries and in production greenhouses in Cartago, were identified and may serve as virus reservoirs for agricultural crops. The most prevalent whitefly species found were the greenhouse whitefly (*Trialeurodes vaporariorum*) and biotype B of *Bemisia tabaci.* Using molecular tools we identified, for the first time in the agronomical region of Alfaro Ruiz, Costa Rica, the presence of the insecticide-resistant exotic Q biotype of *Bemisia tabaci* in greenhouses where tomatoes and peppers are grown. The results of our studies provide a better understanding of the epidemiology of criniviruses and their insect vectors in Costa Rica and will be used to develop improved disease management strategies.

Multiplication and movement of *Xylella fastidiosa* in Australian native plant species

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Xylella fastidiosa Wells is a xylem-limited plant pathogenic bacterium that causes diseases in numerous host species including food and fodder crops, ornamentals and weeds. The pathogen is vectored by insects, predominantly *Homalodisca vitripennis* Germar (Hemiptera: Cicadellidae). *Xylella fastidiosa*, not yet detected in Australia, is native to the Americas and is considered to be highly invasive. Australian climatic conditions are favourable for pathogen establishment and there is a need to develop the capacity for rapid detection and containment of an incursion, including knowledge of host plant species to target monitoring. In Riverside, California, twelve Australian native plant species were inoculated with *X. fastidiosa* and assayed for the pathogen after ten months using culturing and PCR to determine host status, symptom development, systemic spread and persistence of the pathogen over winter. The host status of several Australian native plant species will be presented and these host species may act as reservoirs from which further spread of the pathogen can take place should it reach Australia. The implications of these findings will be discussed and placed in an Australian invasion context.

Detection of '*Candidatus* Liberibacter asiaticus' in psyllid and citrus hosts in Pakistan and analysis of psyllid populations

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The occurrence of *Diaphorina citri* and a description of huanglongbing (HLB) or greening disease were reported by Husain and Nath from the Punjab area of Pakistan in 1927. The disease has been "lived with" for several decades. Recent research efforts focus on improvement of the nursery production system and systematic study of the psyllid vector and the bacterium associated with HLB, '*Candidatus* Liberibacter asiaticus' (LAS). Psyllid populations were monitored by use of yellow sticky traps from twelve orchards in Sargodha region. Population peaks of psyllids were observed in April-May and Oct-Nov, with the highest populations occurring in Oct-Nov. Upon testing the psyllids for presence of LAS using qPCR, the bacterium was detected in psyllids from all 11 locations indicating that HLB is widespread in the region. However, psyllid samples collected during the months of June/July tested negative by qPCR for the bacterium. Presence of the bacterium associated with HLB in plants was demonstrated by testing the DNA extracted from plant samples using qPCR and conventional PCR, and further confirmed by sequencing. Plant samples collected in June/July showed very low titers of LAS.

Postharvest control of gray mold of blackberry caused by *Botrytis cinerea* with preharvest applications of fungicides in Michoacan México

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Phytopathology 101:S151

Grey mold of blackberry caused by *Botrytis cinerea* is one of the most important diseases in Michoacan México. After harvest infected fruits are covered with gray mycelium and spores that make the fruit unsuitable for marketing. The objective of this research was to evaluate the preharvest effect of chemical, biological and biorational fungicides against gray mold of blackberry in Michoacán México. The experiment was conducted in the growing seasons of 2009 and 2010 in a commercial plot located at Ziracuaretiro, Michoacan. Commercial formulations of Copper sulfate, captan *Bacillus subtilis*, Hydrogen Dioxide, Grapefruit Seed extract, Harpin protein, Fenhexamid, Iprodione, Cyprodinil+Fludioxonil and Boscalid+Pyraclostrobin were sprayed in a full season program, during bloom or preharvest. In order to determine disease incidence harvested fruits were incubated at room temperature for 7 days. In both years of testing there were significant differences among treatments ($P < 0.001$). Two sprayings of Fenhexamid, Iprodione, Boscalid+Pyraclostrobin and Cyprodinil+Fludioxonil either during bloom or before harvest (green to red berry) complemented with 2 sprayings of Captan gave the lower disease incidence in postharvest. Full season programs based on Copper Sulfate, Captan, harpin protein and Grapefruit seed extracts provided good to moderate control of gray mold, but Hydrogen Dioxide did not provide acceptable disease control in the 2 years of testing.

Responses of maize (*Zea mays* L.) near isogenic lines carrying *Wsm1*, *Wsm2* and *Wsm3* to three viruses in the *Potyviridae*

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Genes on chromosomes six (*Wsm1*), three (*Wsm2*) and ten (*Wsm3*) in the maize inbred line Pa405 control resistance to *Wheat streak mosaic virus* (WSMV), *Maize dwarf mosaic virus* (MDMV) and *Sugarcane mosaic virus* (SCMV). Near isogenic lines (NIL) carrying one or two of these genes were developed by introgressing regions of the respective chromosomes into the susceptible line Oh28, and tested for their responses to WSMV, MDMV and SCMV in the field and greenhouse. F_1 progeny from NIL x Oh28 were also tested. *Wsm1*, or closely linked genes, provided resistance to all three viruses, as determined by symptom incidence and severity. *Wsm2* and *Wsm3* provided resistance to WSMV. *Wsm2* and/or *Wsm3* provided no resistance to MDMV, but significantly increased resistance in plants with one *Wsm1* allele. NIL carrying *Wsm1*, *Wsm2* and *Wsm3* had similar SCMV resistance in the field, but NIL with *Wsm2* and *Wsm3* were not resistant in the greenhouse. Addition of *Wsm2* to *Wsm1* increased SCMV resistance in the field. For all viruses, symptom incidence was higher in the greenhouse than in the field, and relative disease severity was higher in the greenhouse for WSMV and MDMV. An Italian MDMV isolate and the Ohio SCMV infected the *Wsm1* NIL, while the Ohio MDMV and Seehausen SCMV isolates did not. Our results indicate that the three genes, or closely linked loci, provide virus resistance. Resistance is influenced by interactions among the genes, the virus species, the virus isolate and the environment.

Are plant communities shaped by fungal root endophytes?

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Plant communities are the result of a complex interplay among plants, symbionts and the environment. Dark septate endophytes (DSE) of the *Phialocephala fortinii* s.l. – *Acephala applanata* species complex (PAC) are ubiquitous fungal root colonizers of a wide variety of woody plant species but their ecological role is largely unknown. Sterile seedlings of *Betula pendula* (birch) and *Picea abies* (spruce) in monoculture and in mixed culture were exposed to four PAC strains, either singularly or paired in all possible

combinations at 18°C and 23°C. Plant and fungal biomass was determined after four months. Colonization by PAC reduced biomass gain of either host. One of the strains was more virulent than any other strain to spruce but not to the birch. Biomass gain of spruce was slightly reduced and that of birch enhanced at higher temperature. Virulence of pathogenic strains was reduced in some strain mixtures, highlighting the importance of high genotype diversity on small spatial scale. The effect of PAC on plant biomass gain depended mainly on the mixture of PAC genotypes and the host plant species. Fungal biomass was higher in spruce than in birch and at lower temperature. Our results indicate that the presence of particular PAC genotypes can have an impact on the result of competition among plant species and thereby contribute to plant community formation.

Identification of solanaceous and non-solanaceous species as hosts of *Stemphylium solani* isolates in Brazil

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Stemphylium solani is one of the causal agents of the gray leaf spot of tomatoes in Brazil and it has been reported on distinct hosts in the Solanaceae and in other botanical families. In this work, we tested the Koch's postulates for the new *S. solani* isolates and evaluate a collection of solanaceous and non-solanaceous accessions for their reaction to *S. solani* isolates. Identification as *S. solani* was based upon morphology and the ITS sequence. All the isolates were virulent when inoculated on their original hosts and on tomato cultivar 'Ponderosa'. In addition the pathogen was reisolated from the symptomatic plants, fulfilling the Koch's postulates. In the host range assay, 72 accessions (13 families, 30 genera, and 58 species) were inoculated with four (tomato, gilo, eggplant, and *Capsicum*) isolates. The following species were confirmed as hosts: potato, peppers (*Capsicum* spp.), gilo (*Solanum aethiopicum* var. *gilo*), and eggplant. This updated host list contains new reports for Brazil: *C. chinense*, *C. frutescens*, *Physalis* sp., *Nicandra physaliodes*, *S. paniculatum*, *S. palinacanthum*, *S. betacea*, and *Datura stramonium.* Non-solanaceous hosts were confirmed: cotton, *Ocimum basilicum* (Lamiaceae), *Zinnia elegans* (Compositae), *Tabebuia impetiginosa* and *T. serratifolia* (Bignoniaceae). Differential interaction of host accessions and fungal isolates were observed for eggplant, *N. physaliodes*, and *S. paniculatum*, suggesting the presence of physiological races in *S. solani.*

Silicon and its interaction with fungicide on the control of anthracnose in susceptible and resistant sorghum lines

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This study aimed to evaluate the effect of silicon (Si), and its interaction with fungicide, to decrease the severity of sorghum anthracnose in a field experiment carried out in a silicon-deficient soil area. The experimental design was a split, split, split-plot design with four replications. The whole plot corresponded to calcium silicate (AgroSilício) (+Si) or lime (-Si) applications at the rates of 6 and 5 ton/ha, respectively. The slip plots where the fungicide Epoxiconazole + Pyraclostrobin (spray and non-spray) and lines (BR-008 (resistant) and BR-009 (susceptible)). The area under anthracnose progress curve (AUAPC) was reduced by 42 and 39%, respectively, for lines BR-009 and BR-008 with the application of calcium silicate. The AUAPC was reduced by 42 and 35%, respectively, for lime and calcium silicate treatments with fungicide spray. The application of calcium silicate contributed to decrease AUAPC by 44 and 37%, respectively, for non-spray and spray of fungicide. Fungicide spray decreased AUAPC by 39 and 50%, respectively, for lines BR-009 and BR-008. For non-spray and spray of fungicide, AUAPC was reduced, respectively, by 88 and 90% for line BR-008 in comparison to line BR-009. The Si concentration on leaf tissue significantly increased with calcium silicate application (5.9 g/kg) as compared to lime application (0.3 g/kg) regardless of the sorghum lines used. Financial Support: Excell Minerais e Fertilizantes Ltda.

RpfG interaction in *Xanthomonas axonopodis* pv. *manihotis*

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Xanthomonas axonopodis pv. *manihotis* (Xam) is the causal agent of the cassava bacterial blight (CBB), an important disease that can generates losses up to 100% in cassava crop fields. The cell communication system known as quorum sensing appears to be an important factor for the coordinated expression of virulence genes of this pathogen. In other Xanthomonads, like *Xanthomonas campestris* pv. *campestris* (Xcc), this communication system is encoded in an uncharacterized gene cluster called rpf. This cluster encodes RpfG, a two-component regulator with a CheY-like receiver domain attached to a HD-GYP, a protein of which little is known but seems to have an important role inside the signal network. The aim of this study was to manually annotate the rpf cluster in Xam genome and to assess the interaction of rpfG with two proteins, XC_0420 and XC_0249 corresponding homologues in Xam using the yeast two-hybrid system. According to a Bayesian analysis performed, Xac did not form a monophyletic group with the other *X. axonopodis* pathovars, in contrast with the pathovars of the species *Xanthomonas campestris*, *Xanthomonas oryzae* and *Xanthomonas vasicola* that were grouped together. Additionally, we found a direct interaction of proteins XC_0420 and XC_0249 with RpfG, showing that these proteins are relevant for the functioning of the system network in Xam. These results are critical in the elucidation of the function of the quorum sensing system in Xam, which has not been studied in detail yet.

Cassava's immunity suppression mediated by Type III effectors of *Xanthomonas axonopodis* pv. *manihotis*

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Xanthomonas axonopodis pv. *manihotis* (*Xam*) relies on the Type III Secretion System to translocate effector proteins, involved in the suppression of plant defense, to cause Cassava Bacterial Blight. Plant defenses can be induced by recognition of Microbial Associated Molecular Patterns, triggering a response known as PTI or basal defense. This line of defense involves responses such as strengthen of cell wall through callose deposition. Nonetheless this basal defense can be overcome by effectors of pathogens. A second line of plant defense known as ETI, can be triggered by effectors recognition and is associated with the development of a Hypersensitive Response, a type of programmed cell death. The objective of this study was to identify effectors of *Xam*'s repertoire with the capability of suppress ETI, PTI or both. For this purpose we co-expressed *Xam*'s effector proteins in *P. fluorescens* (pHIR11) or *P. fluorescens* (pLNA1965) to evaluate the suppression of markers for ETI (HR development) and PTI (decrease of callose deposition) respectively, according to a previously reported plant suppression methodology. Our results suggest that the majority of *Xam*'s effectors tested in our study can suppress plant immunity. As this is an artificial system, it will be interesting to demonstrate the impact of the suppression of these effectors in cassava plants.

Detection and localization of Undifilum oxytropis fungi in locoweed tissues

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Locoweed hosts an endophyte Undifilum oxytropis that produces the alkaloid swainsonine, which is responsible for locoism in grazing animals. The livestock industry in western United States is strongly affected by locoweed poisoning. It is imperative to develop a method using electron microscopy to confirm the presence of vegetative hyphae of the endophyting fungi. We are interested in determining the endophyte Undifilum location and plant cell attachment characteristics within different plant tissues. Two types of plants were used to compare and confirm the presence or absence of the endophyte. Endophyte free plants (from seed coat removed seeds), and endophyte infected plants (from intact seeds) were grown in agar. An analysis of different plant tissues between the two treatments was made using scanning electron microscopy (SEM), transmission electron microscopy (TEM) and confocal microscopy (CM).

Systemic resistance phenomena from an evolutionary perspective

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Systemic acquired or induced resistance has been studied for several decades. Aside from theoretical interest the phenomena may have practical application in disease management. The practical use of SAR/SIR for disease management raises the issue of the potential fitness cost of resistance priming. We contend that the on-going debate over this issue is a red herring which arises from two sources. First, physiological studies which focus on fitness, but not on fitness in the complete sense, may present a misleading impression of the relative importance of measured differences between induced and non-induced plants; fitness may be constrained by covariance between components. We highlight well known results from population ecology which define the appropriate experimental framework for measuring the impact of resistance priming on host fitness. Secondly, we contend that the physiological and biochemical resistance mechanisms lined with systemic resistance phenomena are a distraction from the truly significant aspect, which is the ability of plants to detect environmental cues linked to an increased

probability of disease and to respond to those cues. We describe results from information theory and theoretical ecology which support this view. Empirical studies using *Pinus*/*Fusarium* and *Arabidopsis Alternaria* as experimental long- and short-term systems, respectively, are being combined with modeling to investigate our hypotheses.

Remote sensing for detection of Rhizoctonia crown and root rot in sugar beet fields

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Rhizoctonia crown and root rot (RCRR) of sugar beet is caused by *Rhizoctonia solani* AG-2-2. Disease ratings are based on subjective, visual estimates of root rot severity (0–7 scale). Remote sensing was evaluated as an alternative method to assess RCRR. Field plots of sugar beet were inoculated at the 10-leaf stage with *R. solani* AG 2-2 IIIB at a range of inoculum densities in 2008 and 2009. Data were collected for 1) hyperspectral reflectance from the sugar beet canopy and 2) visual ratings of RCRR in 2008 at 2, 4, 6, and 8 weeks after inoculation (WAI) and in 2009 at 2, 3, 5 and 9 WAI. Seven narrowband and five wideband vegetation indices (VIs) were assessed; the wideband optimized soil adjusted vegetation index (OSAVI) provided the best overall fit with disease severity ratings. Values of VIs were constant until 25–50% of the root surface rotted and then decreased significantly as disease severity increased. RCRR also was detected using airborne, color-infrared imagery at 0.25 m. Remote sensing detected RCRR, but not before initial appearance of foliar symptoms. In 2010, OSAVI image analysis of a series of aerial images obtained with a multispectral camera were used to identify areas within commercial fields that were symptomatic of RCRR. Fields were then ground-truthed for RCRR, potential insect populations and soil nutrient problems. Analysis of aerial-obtained multispectral imagery has promise in identifying areas of RCRR in the field.

Defining the interactome underlying Sudden Death Syndrome of soybean

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The soil-borne ascomycete Fusarium virguliforme is the causal agent of Sudden Death Syndrome (SDS) of soybean, a devastating disease that has recently emerged as one of the most important diseases of soybean in the U.S. F. virguliforme colonizes the roots of soybean causing severe root rot, and also produces a potent phytotoxin that is translocated to leaf tissue leading to foliar necrosis. Despite the widespread importance of this disease, surprisingly little is known about the molecular mechanisms underlying pathogenesis. The goal of this project was to identify plant and fungal genes involved in pathogenesis based on expression profiles. To this end, total RNA from infected roots was extracted, and digital gene expression data were acquired with next-generation sequencing techniques. Highly expressed fungal genes were predicted to regulate signal transduction, secondary metabolism, and carbohydrate hydrolysis. Selected candidate genes involved in pathogenesis and host defense were further evaluated with quantitative PCR. This study provides a molecular perspective on SDS and identifies candidate fungal genes for future functional studies.

Where does it come from?: Determining initial inoculum for dollar spot

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Dollar spot, caused by *Sclerotinia homoeocarpa* (F.T. Bennett), is one of the most devastating diseases of turfgrasses worldwide. Understanding the basic biology of this pathosystem is critical because management has been complicated by the loss of broad spectrum fungicides and advent of fungicide resistance. Experiments were designed to determine the source of initial inoculum, seedborne or as mycelium within host tissue. Plant material, obtained by taking 40 soil cores adjacent to and three inches from dollar spot infection centers on creeping bentgrass, was collected in the late fall and early spring. Roots and shoots were separately removed from each core and plated on semi-selective media. Colonies resembling *S. homoeocarpa* were subcultured and identified using morphological and molecular techniques. Results indicate that *S. homoeocarpa* survives predominantly on shoots at the margins of infection centers. Seed assays consisted of four treatments: Non-sterilized (NS)/non-infected (NI), Sterilized/infected (I), Bleached/NI, and NS/I. Seeds from each treatment were plated on semi-selective media individually and as a slurry. Colonies resembling *S. homooecarpa* were identified as above. The highest rates of isolation were obtained from Bleached/NI and NS/I seed, suggesting that *S. homoeocarpa* can colonize seed and likely infiltrates beyond the surface. This research indicates two possible sources of initial inoculum for dollar spot that may be targeted as a means of delaying disease onset.

Deployment of rapid diagnostic tools for *Phytophthora* on horticultural crops in Central America

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Plant disease is a limiting factor in agricultural production in Latin America due to high rainfall conditions and the presence of a diversity of plant pathogenic microorganisms. Losses estimated to be as high as $30 billion per year. The accurate identification of *Phytophthora* has important implications for growers in Latin America and the U.S. and can improve our knowledge of pathogen biology and ultimately treatment and control of tropical plant diseases. Our overall objective is to produce a platform of tools needed to detect, identify, and ultimately prevent entry of novel species of *Phytophthora* into the U.S. with a major focus on development of surveillance tools for common and high threat species of *Phytophthora* on horticultural crops including potato, cacao and floriculture crops from Central America. We deployed a series of "shovel ready" technologies including: a *Phytophthora* diagnostics workshop held in San Jose Costa Rica in 2010, a Lucid key for species identification, PCR-RFLP and Padlock probes and digital diagnostic identification systems to identify *Phytophthora* species and improve the diagnostic capabilities for important plant disease clinics in the region. We are working with collaborators including, FHIA in Honduras, Universidad de Costa Rica, CATIE, The World Cacao Foundation, DOLE Foods, and the Organization of Tropical Studies to conduct surveys of *Phytophthora* species on horticultural crops in the region.

A Lucid key to the common *Phytophthora* species

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The Key to the Common *Phytophthora* species (Lucid v 3.4) is a matrix-based computerized identification key and includes important morphological and molecular characters that are useful for identification of 55 common *Phytophthora* species. A set of 20 features are used to make a correct species identification. The user enters responses to known character state options into Lucid Player and the correct species is identified. Illustrations of each feature state are included in the key. The main features included in the key are: asexual structures, sexual structures, and chlamydospore, hyphae and cultural characteristics. The user can read an illustrated "Fact Sheet" on each species. A cross-linked glossary of terminology is included in the "Fact sheets". In addition, a DNA Search function of ITS and Barcode of Life (5′ end of the cox1 gene) sequences for each species can be queried. The key was created to provide diagnosticians, regulatory personnel and teachers with easily accessible tools to distinguish common species based on a number of important morphological and molecular characteristics and is now available from APS Press.

Development of a PCR-RFLP method to rapidly identify common entomopathogenic fungi infecting soybean aphid

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Soybean producers face pest pressures from soybean aphid (*Aphis glycines* Matsumura) and foliar fungal diseases (e.g. brown spot, frogeye leaf spot, Cercospora leaf blight). Soybean aphid is an extremely economically important insect pest in many parts of the United States and Canada, causing yield reduction of 14–50% if left untreated. Foliar disease impacts on yield are not well established, but fungicides are frequently applied prophylactically. *Conidiobolus thromboides* and *Pandora neoaphidis* are two common fungal

pathogens of soybean aphid in North America. *In vitro* testing of the impact of several fungicides on these aphid pathogens has shown reduced and, in some cases, complete inhibition of germination and infectivity. Aphid-pathogenic fungi are a source of natural soybean aphid population control and are potentially valuable biocontrol agents. A cultivation independent PCR-based diagnostic tool has been developed for detection of *P. neoaphidis* in the environment, but there is no similar tool for monitoring *C. thromboides*. Universal primers were used to amplify the ITS rDNA regions of both species and ITS-RFLP analysis was used to identify fingerprints to distinguish between the two species. ITS-RFLP analysis was successfully performed on fungal isolates and artificially infected soybean aphids. The analysis was then used to determine the incidence of *P. neoaphidis* and *C. thromboides* in naturally occurring populations of soybean aphid during the 2009 and 2010 growing seasons.

Open access online database of powdery mildews (Order Erysiphales) in Puerto Rico

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An open access online database was developed containing information of powdery mildews (Ascomycetes: Order Erysiphales) of Puerto Rico. In the tropics, there is very limited knowledge of these species that affect our crops. From 2010 to 2011 we conducted a series of surveys to examine powdery mildew diversity in the island. Tissue samples were collected from more than 15 plant hosts and have been analyzed revealing the presence of at least 6 different powdery mildew genera. The database is focused to offer a friendly guidance to the diagnostic of powdery mildew pathogens occurring in the island. It includes morphology using light and scanning electron microscopy and phylogenic analysis of data-sequence profiles and alignments- in order to standardize genetic identification of Erysiphales occurring in the Caribbean. The specific identification of these pathogens will help enforced quarantines regulations to stop the introduction of new species of pathogens in an already fragile island ecosystem.

A novel vitivirus isolated from *Ribes* species in Alaska

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A new virus of domesticated black and red currants *(Ribes nigrum L. and R. rubrum L.)* was isolated in 2008 from a home garden on the Kenai Peninsula near Homer, Alaska. Leaf symptoms consisted of mosaic with vein-clearing and chlorotic spots. *Nicotiana benthamiana*, *C. quinoa* and *C. amaranticolor* Coste. and Reyn. developed mosaic or local lesions (latter two) when inoculated with either leaf sap or partially purified particle preparations. Protein and ds-RNA extracts from inoculated *N. benthamiana* that exhibited mosaic symptoms contained a tentative coat protein ~22 kDa and a prominent dsRNA ~7.5 kb, respectively. The dsRNA was sequenced using conventional and Illumina platform sequencing. A complete genomic sequence of 7,729 nt determined that the virus belonged to the genus *Vitivirus* and was most similar to *Grapevine virus E*. The Ribes vitivirus had amino acid sequence identities of 49% with the RNA dependent RNA polymerase, 44% with the movement protein and 43% with the coat protein of GVE. The Ribes vitivirus has a 382 bp long intergenic region that is absent in all GVE isolates reported thus far. The Ribes vitivirus also lacks a small ORF closest to the 3′ end that encodes for a putative nucleic acid binding protein. This ORF has been found in all the three GVE isolates sequenced so far. These data taken together suggest that this virus represents a new member of the vitivirus genus. Also, there was no evidence of additional viruses in the *Ribes* plants.

Molecular characterization of *Tobacco rattle virus* RNA1 from *Dicentra spectabilis* (L.) Lem (bleeding-heart)

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Dicentra spectabilis (L.) Lem. (*Papaveraceae*) is a popular cold-hardy ornamental grown in Alaskan home gardens as a perennial for its heart-shaped flowers. In 2010, virus-like symptoms with ringspots occurred on leaves of a *D. spectabilis* plant in a flower garden in Wasilla, AK. Total RNA was extracted from symptomatic leaves with a RNeasy Kit (Qiagen Inc., Valencia CA), and used as a template for randomly primed cDNA synthesis, followed by reverse transcription (RT)-PCR polymerase chain reaction using five sets of *Tobacco rattle virus* (TRV) primers. The PCR results gave the expected nucleotide sizes of 1771, 1,252, 818, 515, and 290 bp spanning RNA1. Virion extracts from infected leaves produced symptoms on inoculated *Nicotiana benthamiana* and the plants tested positive for TRV by RT-PCR. The nucleotide sequences contained identities as high as 97% with other TRV isolates in GenBank. *Tobacco rattle virus* (TRV) infected *D. spectabilis* was reported for the first time in North America in 2000 from nurseries in Minnesota, Michigan, and Massachusetts as determined by serology and electron microscopy. The first report of TRV in Alaska occurred in 2009 from peonies (*Paeonia lactiflora*). This is the first detection of TRV from *D. spectabilis* in Alaska, and the first TRV molecular characterization from bleeding-hearts. The importation of vegetatively propagated ornamentals such as *D. spectabilis* that harbors viruses presents a method of introducing plant pathogens to a relatively isolated geographical region.

Survey of *Potato virus Y* isolates in potato in Chihuahua, Mexico

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More than 1,200 random potato leaf samples were collected in August 2009 and in June 2010, from cultivars Snowden, Atlantic, FL1867, Felsina, Fianna, Gigant, Adora, Vivaldi, and Alpha, in two field surveys in the State of Chihuahua. Nine were found *Potato virus Y* (PVY)-positive, collected from cultivars Fianna, Snowden, Adora, and FL1867. The PVY status of the collected samples was initially determined with the PVY-specific Immunostrips, and by DAS-ELISA using the polyclonal PVY detection. In order to determine the strain specificity of these PVY isolates, original potato leaf samples were inoculated onto tobacco plants (*Nicotiana tabacum* cv Burley), and symptom appearance and development were observed for 8 weeks, followed by the standard typing by RT-PCR. Of the original nine PVY-positive samples, three induced systemic PVY infection in tobacco producing stunting, mosaic, and vein clearing; two of the three induced also systemic vein necrosis. One isolate, PVY-M3, was typed as recombinant PVY^{NTN} isolate by RT-PCR. It was further analyzed by TAS-ELISA using four PVY^{O} and PVY^{N} strain-specific monoclonal antibodies, and confirmed to have N-specific serology, characteristic of other PVY^{NTN} recombinants. Based on the combination of biological, serological, and molecular characteristics, this recombinant strain from Mexico may belong to the PVY^{Z} strain group represented by the isolate PVY-L26.

Identification of curly top virus infection in Jalapeño pepper in Chihuahua, Mexico

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Curly top is a serious problem in many irrigated crops in the dry semi-arid climates in North America. The disease is caused by a complex of leafhopper-transmitted curtoviruses, one of which, *Beet mild curly top virus*, was previously found in pepper in Zacatecas, Mexico. In the past few years, sporadic symptoms similar to curly top disease were observed in Jalapeño pepper in the South Central area of Chihuahua State. The symptoms featured stunted, yellowing plants scattered in the otherwise healthy looking pepper stand. Affected leaves were brittle, showed upward curling, and distinct green vein pattern with interveinal yellowing. In June and August of 2010, two field surveys were conducted in Cordillera-Escuadra, Meoqui-Estacion Consuelo, Meoqui-Lomas del Consuelo and Delicias-Presa Francisco I Madero locations. Ninety-four pepper leaf samples were collected from symptomatic Jalapeño pepper plants. The curly top virus status of the collected samples was determined by TAS-ELISA using the recently developed polyclonal curly top detection assay. Of the collected 94 samples from pepper plants showing stunting and yellowing 11 were found curly top-positive by TAS-ELISA. This is the first report of the curly top virus in the State of Chihuahua, it demonstrates that curly top is established in the state, and will need attention from other vegetable crops under irrigation, notably beans.

Ganoderma lucidum* and *Streptomyces lydicus* as biological control agents of *Xanthomonas campestris* pv. *vesicatoria

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Bacterial spot of pepper is one of the most destructive diseases of pepper in Chihuahua, Mexico. No biocontrol of this disease has been reported previously. The objective of this study was to determine the efficiency of *Ganoderma lucidum* and *Streptomyces lydicus* 5US-PDA8 to control this disease under *in vitro* and greenhouse conditions. *In vitro* bioassays were conducted using 20 bacterial isolates and two antagonists. In greenhouse a CRB design was carried out with 16 treatments and 5 replications.

Streptomyces was applied in rhizosphere, *Ganoderma* in phyllosphere and pathogens in selected leaves by syringe infiltration. *Ganoderma* showed total inhibition *in vitro* of all isolates after 24 hours and 88.9% of them after 48 hours. *Streptomyces* inhibited all isolates within a range of 6.59–100% after 24 hours. Plants inoculated with the pathogen in greenhouse showed the disease symptoms but when they were treated with the antagonists, a significant infection reduction was observed, being *Ganoderma* the most effective. *Streptomyces* reduced pathogen population but infection was not diminished in the same proportion. Treatments with the antagonists combined with the pathogens showed the highest plant height, and chlorophyll and biomass content. This is the first study that deals with biocontrol of the bacterial spot of pepper in Chihuahua, showing its potential effectiveness under field conditions.

Spatial characterization of favorable climate conditions for soybean rust progress on current and future scenarios in Brazil

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Phytopathology 101:S155

Current maps of spatial distribution of areas with favorable climate conditions for soybean rust progress have been developed for Brazil using data from the average monthly temperature and relative humidity obtained from the Climate Research Unit. Data refer to historical averages of these variables from 1961 to 1990. The A2 and B2 future scenarios were designed for the decades of 2020, 2050, and 2080 from six climate models of the Intergovernmental Panel on Climate Change. Based on them, maps of future scenarios for favorability of rust occurrence on different regions in Brazil were developed. The maps of future scenarios were prepared according to estimates of severity and rate of rust progress based on multiple regression models. Regarding the current condition, it was observed that in the rainy season (Dec., Jan., and Feb.), rust progress was favorable mainly due to better temperature and rainfall conditions in the main growing soybean regions. By contrast, during the dry season (Jul. and Aug.), rust progress was not favorable due to the low temperatures. Analyzing the future projections, there will be an increase in areas highly favorable for rust in the next decade. In the years 2050 and 2080, there will be a decrease in rust favorability in some growing regions with decline for A2 scenario with the highest temperature increase. Regarding to the climate, there will be reduction in areas favorable for soybean rust progress as compared to the current period. Financial support: CNPq.

The burden of truth: Visual representations of genetic engineering and genetically modified organisms in the online media

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Groups for or against genetic engineering have used visuals to capture public attention, stir emotions, and mobilize support—tasks that are now easier to accomplish through the web. Although images of biotechnology saturate the media, do they accurately portray the science and the process? What is their tone toward GE and GMOs? A content analysis of web images—photographs, illustrations, photoillustrations, cartoons, diagrams, and logos—collected over a seven-day period was conducted. The results show an abundance of visuals in personal and special interest group sites, stock photo and cartoon banks. Images with a negative valence trounced those with a positive tone in frequency and intensity. The visuals presented a range of perspectives on GE, but many failed the accuracy test. Recurring inaccuracies fall along the following lines: incorrect representations of the process; almost non-existent depictions of biosafety protocols; overly artistic images that obscure the rationale for GE; the stigmatization of Monsanto; monsters result from mixing plant and animal genes; (6) scare tactics exaggerate risks to human health; and limited comparative analyses. The findings indicate that people's ability to produce attractive graphics has outstripped knowledge of how to use them well. The result is a glut of captivating visuals clogging the information stream and impairing audiences' understanding of an important innovation.

Evaluation of *Arabidopsis thaliana* as a model host for *Xylella fastidiosa*

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Phytopathology 101:S155

Pierce's disease of grapes and almond leaf scorch are agronomic diseases caused by the bacterium *Xylella fastidiosa*. To date, progress determining mechanisms of host plant susceptibility, tolerance or resistance has been slow, due in large part to the long generation time and limited available genetic resources for grape, almond and other known hosts of *X. fastidiosa*. To overcome many of these limitations, *Arabidopsis thaliana* has been evaluated as a host for *X. fastidiosa*. A pin-prick inoculation method has been developed to infect Arabidopsis with *X. fastidiosa*. Following infection, *X. fastidiosa* multiplies robustly and can be detected by microscopy, PCR and isolation. The ecotypes Van-0, LL-0 and Tsu-1 all allow more growth of *X. fastidiosa* strain Temecula than the reference ecotype Col-0. Various *X. fastidiosa* strains also show differential growth in Arabidopsis. Affymetrix ATH1 microarray analysis of inoculated vs. non-inoculated Tsu-1 reveals gene expression changes that differ greatly from changes seen after infection with apoplast colonizing bacteria. Many genes responsive to oxidative stress are differentially regulated while classic pathogenesis-related (PR) genes are not induced by *X. fastidiosa* infection.

Validation of a single nucleotide polymorphism genotyping method for Wheat streak mosaic virus

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Wheat, one of the most economically valuable crops in the United States, ranks first in crop exports. Wheat pathogens threaten both national and international commerce, and could be deployed intentionally to impact trade streams. Attribution of such agricultural crimes will require new forensic tools that are more stringent and targeted. An assay using primer elongation with fluorescent dideoxynucleotides at potential single nucleotide polymorphic (SNP) sites was developed and tested for its ability to discriminate reliably among plant pathogen strains using Wheat streak mosaic virus (WSMV) as a model. Fifteen SNPs were identified in the coat protein (CP) and helper-component protease (HCPro) regions of the genome and a unique primer was designed for each. Consistent, distinguishable SNP fingerprints, consisting of patterns of chromatographic peaks, were obtained using six strains and eighteen field isolates of WSMV. The sensitivity of the assay was determined using a synthetic plasmid of the WSMV CP and HCPro genes. The specificity to WSMV was demonstrated by testing an exclusivity panel consisting of near-neighbors to WSMV. The SNP genotyping method appears to be an appropriate method for forensic discrimination among WSMV strains.

Effects of hot water treatment for seed disinfection and seed germination in rice

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Bakanae disease caused by the *Fusarium fujikuroi* is a significant disease in rice growing region of the world. This disease is spread by seeds infection so seed disinfection need for prevent disease spread primarily. But environment friendly farming system doesn't permit using any chemicals for seed disinfection. Thus cold-hot water treatment method has been widely using as a seed disinfection for controlling seed infectious diseases in environment friendly farming. Hot water treatment method was developed by omitting of cold water treatment and it's control effects for bakanae disease was equal to compared with cold-hot water treatment. Appropriate proportion of seeds and water in hot water treatment method was 250 g in 2,000 ml for stable seed disinfection. In germination test with 60 rice cultivars by hot water treatment, most of rice cultivars were showed safe germination in 60°C for 20 minutes. But eight rice cultivars Goonbyeo, Dongjin1, Seoan1, Pungmibyeo, Ilmibyeo, Samgwangbyeo, Sinunbong1, Ungwangbyeo, were showed over than 20% reduction of germination ratio in 60°C for 15 minutes and in 60°C for 20 minutes. Seed germination ratio was decreased 40 to 50% when pre-soaked rice seeds in plane water over 3 hours before hot water treatment in seed disinfection but represented normal seed germination when pre-soaked in the brine.

First report of Alternaria mali on apples in Brazil

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Phytopathology 101:S155

A species of Alternaria was isolated from leaf spots on Gala apples (*Malus domestica* Borkh.) collected from four orchards in Paraná State, southern Brazil, in 2007 and in 2010. The leaf spots were circular, tan to brown, 2 to 5 mm in diameter and were often initially bordered by a purple halo. The fungus was identified as A. mali Roberts based on conidial morphology and completion of Koch's postulates. Isolates produced abundant conidia in a dark, carpet-like mycelial mat on potato dextrose agar. Conidia had one to eight septations. The average length and width of conidia of a representative isolate (UFPR A-017) were 20.7 and 9.1 µm, respectively, which are similar to reported measurements for *A. mali*. Symptoms similar to those observed in orchards, and characteristic of Alternaria blotch, were reproduced on leaves of

Gala following inoculations in the laboratory with a conidial suspension at 104 conidia.ml^{-1} and the fungus was reisolated from the lesions. This is the first report of A. mali from Brazil. The importance of this disease is not known because it is often found on leaves severely affected with Glomerella leaf spot, caused by *Colletotrichum gloeosporioides*, *C. acutatum* and *G. cingulata.*

Species identification of the causal agent of Eutypa dieback of grapevine in northeastern U.S. and southeastern Canadian vineyards

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Eutypa dieback of *Vitis* grape is caused by the Ascomycete fungus *Eutypa lata*. The pathogen infects grapevine through wounds, and cause wood canker and dieback symptoms. *E. lata* has been identified in all major grape production areas in the world. The first report of Eutypa dieback from the northeastern United States identified the causal agent as *E. lata*. However, our recent studies questioned the species identity of the causal agent of Eutypa dieback in these regions. Our objectives were to; 1) survey Eutypa-affected vineyards in northeastern U.S. (CT, MA, MI, NY, OH, RI) and Ontario, Canada and measure disease incidence; 2) identify the *Eutypa* species using multi-gene phylogeny, microsatellite analysis and secondary metabolite profile; 3) determine the pathogenicity of the *Eutypa* species recovered. Our results indicated that the incidence of Eutypa dieback increased with the density of vineyards. Based on phylogenetic analyses of three nuclear loci, we identified *E. lata*, *E. laevata*, and two new undescribed species that are closely related to *E. lata*. *E. lata* was only found in two vineyards (Rhode Island and Ontario) suggesting that these collections may represent introductions from outside the eastern U.S. and Canada. All *Eutypa* species produced phytotoxins and were pathogenic on *Vitis labruscana* 'Concord' and *V. vinifera* 'Chardonnay'.

A new approach to manage phytoplasma diseases: Field treatments with resistance inducers to contain grapevine Bois noir

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Bois noir is one of the main phytoplasma diseases of grapevine in the Mediterranean basin. It induces severe loss of production due to the early drying of most of the bunches of grapes on the plant, and to stunted plant growth and general decline. At present, there are no known methods of containing this disease. The aims of this study are to investigate the spread of Bois noir in vineyards, to record the physiological changes of the infected plants, and to promote symptom remission or recovery through spraying of the canopy of symptomatic vines with resistance inducers. Some vineyards with sensitive cultivars, e.g. Chardonnay, show up to 60% symptomatic plants. Gene expression analysis carried out on symptomatic and recovered plants showed profound physiological changes, which included enzymes involved in plant defence mechanisms and secondary metabolism. Weekly applications of the resistance inducers Bion, Olivis and Kendal on the plant canopy from the beginning of May to the end of July decreased the number of symptomatic plants by about 50%. This increase in plant resistance provides an innovative approach to the management of grapevine Bois noir, and contribute to the opening of new possibilities for the containment of phytoplasma disease.

Characterizing microbial communities of potato common scab suppressive soil using pyrosequencing

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Potato common scab (PCS), caused by *Streptomyces* spp. is an annual production concern for commercial potato growers. A naturally occurring PCS suppressive soil has been identified in Michigan. To characterize the microbial community structure of the disease suppressive soil, pyrosequencing was employed. Soil was sampled from disease conducive and suppressive fields in the same location, and total genomic DNA was extracted. The pyrosequencing-based approach was used to analyze amplicon libraries from polymerase chain reaction amplification of a phylogenetically informative region (variable across taxa) within the 16S rRNA gene. Data was analyzed using operational taxonomic unit (OTU)-based, taxon-based and phylogenetic-based methods. The number of OTUs (10% dissimilarity) identified from disease conducive soil, disease suppressive soil and shared between sites was 565, 859, and 300 respectively. 26.69% of OTUs were shared between conducive and suppressive soil and the total number of OTUs was 1,124. Additionally phylogenetic analysis of pyrosequencing tag data of samples from conducive and suppressive soil samples found that the total number of phyla, classes, orders, families and genera set was 20, 49, 87, 173 and 335 respectively. The results of this study will provide information for potato crop production on how to enhance beneficial soil microbial communities and will have significant effects on plant health and in soil disease suppressiveness, particularly in the case of PCS.

ASI-261: A potential non-fumigant alternative to methy bromide

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A novel compound, ASI-261, is in development as a pre-plant soil treatment for broad-spectrum pest control. The material had activity against fungi, oomycetes, and nematodes when tested in vitro. In greenhouse assays, no phytotoxicity was observed on tomato or bell pepper when transplanted 5 days after soil treatment. Two small-scale field trials on bell pepper and tomato were conducted and a strawberry trial is underway. Bell pepper yields in small plots were similar to the methyl bromide control. Weed biomass emerging through plant holes in plots receiving the highest ASI-261 rate were slightly greater than the methyl bromide treatment, but were lower than the untreated control (UTC). In the strawberry trial, mortality of introduced inoculum of *Macrophomina phaseolina* was significantly increased with the high rate of ASI-261 compared to the UTC. *Trichoderma* colony forming units were higher in both ASI-261 treatments than in the untreated and 1,3-dichloropropene treated plots. Sting nematode numbers, while relatively low, were significantly reduced immediately after treatment and were equivalent to numbers extracted from plots treated with 1,3-D. Advantages of using this experimental material include the ability to make applications via drip irrigation with no volatile organic compounds generated, which should result in minimal worker exposure and few regulatory constraints and the broad-spectrum of activity against soilborne pests.

Genome sequence of an unassigned *Citrus tristeza virus* genotypic isolate from Puerto Rico reveals a trifoliate resistance breaking genotype

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The use of our recently developed genotype specific multiplex (GSM) reverse transcription polymerase chain reaction (RT-PCR) analysis left the *Citrus tristeza virus* (CTV) isolate B301 as an unassigned CTV genotype. The GSM RT-PCR method can detect CTV in any infected plants but failed to distinguish the resistance breaking (RB) genotype from other known CTV genotypes (T3, T30, T36, VT and B165). Puerto Rican CTV isolate B301 was obtained from the Exotic Pathogens of Citrus collection (EPCC) at the USDA-ARS, Beltsville, MD, U.S.A. but was originally collected from Aguas Buenas, Puerto Rico in 1992. Biologically, B301 induces symptoms similar to mild CTV-T30 like isolates and does not induce seedling yellows or stem pitting reactions in indicator Citrus spp. A primer pair was designed from a selected region of open reading frame (ORF) 4 for the identification of the CTV-RB genotype. The New Zealand (NZ) RB isolates also from the EPCC were used as positive controls. The CTV-RB genotype specific primer successfully amplified the B301 amplicon. In addition, complete sequence analyses showed a 97–99% nucleotide sequence identity with NZ-RB genotypic isolates. Phylogenetic analysis of ORF1a and 1b from the 5′ terminal half region and ORFs 2-11 from 3′ terminal half region revealed that B301 shared the RB clade with five CTV isolates from NZ. Although this is the first report of a CTV-RB genotype from Puerto Rico, this RB genotype has been present there prior to 1992.

Characterization of the *Pi-b* rice blast resistance gene in the National Small Grains Collection (NSGC)

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The *Pi-b* gene in rice confers resistance to a wide range of races of the rice blast fungus, *Magnaporthe oryzae*, including race IE1k that overcomes *Pi-ta*. In the present study, *Pi-b* was identified in 164 rice germplasm accessions from the National Small Grains Collection using DNA markers and pathogenicity assays. The existence of *Pi-b* in rice germplasm was detected by

using two simple sequence repeat (SSR) markers, RM 208 and RM 166, and a dominant marker Pibdom derived from *Pi-b*. Pathogenicity assays using an avirulent race (IE1k) and a virulent race (IB54) were performed to verify resistance specificity of Pi-b. Among the 164 germplasm accessions evaluated, 130 were found to contain the *Pi-b* gene using both SSR markers and pathogenicity assays, although with different haplotypes. The remaining 34 germplasm accessions were found to be different in their responses to the blast races IB54 and IE1k, suggesting the presence of *Pi-b* independent *R* gene(s). These characterized germplasm accessions can be used for genetic studies and marker-assisted breeding for improving blast resistance in rice.

IPM programs for winter wheat in Oklahoma: A team approach to manage insects, diseases and weeds

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Wheat is a multi-million dollar crop in Oklahoma, grown on more than 2 million hectares. Insects, plant diseases and weeds all provide major challenges for producers to profitably grow winter wheat. Scientists and Extension professionals and resources from Oklahoma State University, the USDA's Agricultural Research Service, and the National Institute of Food and Agriculture join in partnership with Oklahoma's wheat producers to successfully address many of these pest problems in a collaborative "team approach". This presentation describes how the teams are organized and outlines current successes and future challenges that are being addressed. These challenges include development of winter wheat varieties that are resistant to insects and diseases, development of weed management programs that integrate rotational cropping systems with herbicide resistant varieties, development of insect management programs that incorporate assessment of natural enemy activity in conjunction with pest activity and methods for effectively delivering information to Oklahoma's producers.

Previous reports of bacterial diseases on crucifers attributed to *Pseuomonas syringae* pv. *maculicola* were caused by *P. cannabina* pv. *alisalensis*

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Pseudomonas cannabina pv. *alisalensis* (*Pca*) causes bacterial blight on crucifers, which can reduce crucifer yields and has resulted in economic losses in the U.S. Prior to the late 1990s *Pca* was not distinguished from the pepper spot pathogen of crucifers, *Pseudomonas syringae* pv. *maculicola* (*Psm*), although these organisms have since been found to have distinct host ranges and to belong to different species. The objective of this research was to determine whether recent and historical reports of crucifer diseases attributed to *Psm* were in fact caused by *Pca*. Bacteria identified as *Psm* from disease outbreaks worldwide were compared to *Psm* and *Pca*. DNA fragment banding patterns generated by repetitive-PCR using the BOXA1R primer distinguished *Pca* from *Psm* and demonstrated that some of the pathogens previously identified as *Psm* were *Pca*. Additionally, the putative *Pca* strains and the pathotype of *Pca* were sensitive to bacteriophage PBS1 while *Psm* was not. The identity of the putative *Pca* strains was confirmed through host range evaluations. The putative *Pca* strains and the *Pca* pathotype were pathogenic on radish (cv Comet), rapini (cv Sorrento) and oats (cv Montezuma) but *Psm* was not. Correctly identifying and distinguishing these pathogens is crucial for developing effective management strategies and preventing pathogen spread. The outlined suite of assays represent methods effective in distinguishing these previously commingled pathogens.

Impact of soybean cyst nematode on Rhizoctonia root and crown rot of sugar beet

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Soybean cyst nematode (SCN; *Heterodera glycines*) penetrates sugar beet roots and likely creates small lesions. This damage might affect seedling disease caused by *Rhizoctonia solani*, an important pathogen of sugar beet in the Red River Valley. The objectives of this research were to determine if SCN penetrates sugar beet roots under field conditions and if SCN increases root disease caused by *R. solani*. Field soil free of SCN was infested with SCN eggs at three egg density levels and sugar beets were grown under field conditions and the roots harvested at 4, 8 and 16 weeks post planting. DNA was extracted and PCR analysis was performed to identify SCN in the roots. To determine if SCN increases disease 2 week old plants in growth chambers were either inoculated with *R. solani* or co-inoculated with *R. solani* and SCN eggs then rated for necrosis after 10 days. Results showed that SCN could be detected in sugar beet roots grown under field conditions throughout all time points using PCR analysis. Although there was no significant difference in necrosis between treatments with *R. solani* alone and *R. solani* plus SCN when data was averaged over four experiments, there were consistently higher ratings for necrosis in each experiment when SCN was added in with *R. solani.* This consistent increase in root necrosis observed in the presence of SCN warrants continued investigation to determine the overall impact SCN has on sugar beet seedling diseases.

Assessing the validity of diagnostic quantitative PCR assays for Phakopsora pachyrhizi and P. meibomiae

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There are 123 confirmed species in the genus Phakopsora worldwide, with 19 species reported in the continental United States. In 2002, a quantitative PCR (qPCR) diagnostic assay was developed by Frederick et al. and currently is being used for detecting Phakopsora pachyrhizi in spore trapping studies. Based upon these assays, spores of P. pachyrhizi have been reported in Ohio and other states where soybean rust has never been found. These reports may be based upon false positives. False positives are problematic because they may lead to unnecessary fungicide applications when there is no risk of disease development. In 2009 a new qPCR diagnostic assay was developed by Barnes et al. to eliminate false positive results. Both qPCR assays were tested against other rust pathogens; however, neither of these assays was tested against closely related Phakopsora spp. (other than P. meibomiae) that are known to occur in the continental United States. The species that we will test include P. arthuriana, P. crotonis, P. meibomiae, P. nishidana, P. pachyrhizi, P. tecta, and an unknown Phakopsora species. We will assess the two diagnostic assays against these Phakopsora spp.

Detection and identification of various *Clavibacter michiganensis* strains using a novel isothermal nucleic acid amplification

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Clavibacter michigenensis is the causative agent for diseases in several economically important crops. *Clavibacter michigenensis* subsp. *sepidonocus* (Cms) causes ring rot in potatoes while *Clavibacter michigenensis* subsp. *michiganensis* (Cmm) causes bacterial canker in tomatoes. We report here on a novel isothermal nucleic acid amplification method for the detection and identification of detection of Cmm and Cms, using bacteria in culture and infected plant material, within 30 minutes time. The estimated sensitivity of the Cms specific test is approximately $10^3 - 10^4$ cfu/ml. The Cmm specific test was evaluated against a culture collection of over 150 strains. Data demonstrating the sensitivity and specificity of the tests, as well as results of plant inoculation studies, will be presented.

First report of *Phyllactinia guttata* on almonds in Lebanon

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Almond, *Prunus dulcis* (Mill) Webb = *Amygdalus communis* L., has been cultivated for centuries in Lebanon, of a typical Mediterranean climate. In a recent survey of diseases occurring on almond species in Lebanon, the powdery mildew disease caused by *Podosphaera pannosa* was mainly observed. However a new record of a late season powdery mildew caused by *Phyllactinia guttata* (Wallr.) Lév, was detected on cultivated and wild almond species. In the coastal area, it was observed on the cultivated almond species *P. dulcis* and at the higher elevations was on the wild almonds *P. korschinskii* and *P. orientalis*. The signs of *Ph. guttata* showed as white mycelium on the lower side of leaves. The cleistothecia, 150-250 um in diameter, could be clearly seen with the naked eye. Each cleistothecium has 8 to 12 equatorial bristle-like appendages. Each appendage has a bulbous base 25-50 um wide and its length is 1 to 2.0 times the diameter of the cleistothecium ascocarp. Each ascocarp contains up to 20 asci and each ascus contains 2 ascospores ellipsoidal to ovoid in shape. The hyphae of its anamorph were thin and persistent, conidia clavate to rhomboid belonging to genus Ovulariopsis. These findings were consistent with those reported by Braun (1987) for the same species. This powdery mildew species has not been reported previously on Almonds in other countries of the Mediterranean region.

Host specificity in *Erwinia tracheiphila* (Smith): Evidence from rep-PCR and pathogenicity assays
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Bacterial wilt of cucurbits, caused by *E. tracheiphila*, impacts all cucurbits except watermelon. Although this disease causes severe yield losses throughout the eastern U.S., little is known about the biology of the pathogen. Genomic DNA from *E. tracheiphila* strains, isolated from symptomatic cucurbit crop plants obtained from seven states, was amplified with BOXA1R and ERIC1-2 primers. Banding patterns were observed after agarose gel electrophoresis and compared among hosts. Banding patterns from strains isolated from *Cucumis* spp. plants were distinct from those isolated from the genus *Cucurbita* regardless of geographic origin. Twelve *E. tracheiphila* strains isolated from muskmelon (*C. melo* L.), cucumber (*C. sativus*), or squash (*C. pepo)* were inoculated onto leaves of 2-week-old *C. melo* L. and *C. pepo*. Wilt symptoms were assessed over two weeks, strains were re-isolated, and rep-PCR banding patterns were compared to the inoculated strain. All strains were pathogenic to both hosts. *C. melo* plants expressed wilt symptoms 4 to 5 days sooner when inoculated with *Cucumis* spp. strains than when the same strains were inoculated onto *C. pepo. C. pep*o plants inoculated with *Cucurbita* spp. strains expressed symptoms 4 to 5 days sooner than when the same strains were inoculated onto *C. melo* L. Banding patterns from the re-isolated strains were consistent with the originally inoculated strains. Our results suggest that *E. tracheiphila* strains are genetically diverse and that this diversity may be specific to host genus.

Identification and characterization of a new ampelovirus infecting cultivated and wild blackberries
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Phytopathology 101:S158

A novel ampelovirus from blackberry was identified recently in Mississippi and characterized in the framework of NIFA-funded Specialty Crop Research Initiative (SCRI) Project on viruses affecting blackberries in the Southeastern United States. The virus sequence was obtained from high throughput sequencing (Illumina platform) using dsRNA as template and RT-PCR to fill in gaps. The genome organization of this virus resembles that of Grapevine leafroll-associated virus 3, the type member of the genus Ampelovirus (family Closteroviridae). Amino acid sequence identities between genomic products of the blackberry ampelovirus and GLRaV-3 varied from 35% (diverged coat protein) to 65% (RNA-dependent RNA polymerase), suggesting that this blackberry virus is a new member of the genus. Phylogenetic trees, independent of method used or genomic products compared, always placed this virus closest to GLRaV-3. Preliminary survey carried out on a limited number of samples, indicated the presence of this virus in several cultivated and wild blackberry specimens. Identification of its putative mealybug vector and evaluation of its incidence/importance in blackberry in the major blackberry-producing areas of the U.S. are the present focus of this research.

Molecular characterization of an endornavirus from Cucumis spp.
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Endornaviruses infect hosts in the kingdoms Plantae, Fungi and Chromista. They are efficiently transmitted vertically and generally do not induce visible symptoms. In this investigation high molecular weight dsRNA, representing the genome of an endornavirus, was isolated from an unknown melon (Cucumis melo) cultivar and used as a template for molecular characterization. The complete genome of the virus, provisionally named Cucumis endornavirus 1 (CucEV-1) consisted of ca 15 kbp, terminating with a stretch of cytidine residues and encoding a large precursor polyprotein containing domains characteristic of RNA-dependent RNA polymerase and glycosyltransferase. CucEV-1 is phylogenetically closely related to endornaviruses reported from cultivated and wild Oryza spp. Preliminary studies indicate the presence of variants of this virus, or closely related endornavirus species, in several other wild and cultivated cucurbit species.

Emaravirus and cryptovirus infection of Viburnum lantanoides in the Great Smoky Mountains National Park
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Mosaic/line pattern symptoms resembling viral infections were observed on Viburnum lantanoides Michx. (Witch-Hobble, Hobblebush or Moosewood) in the Great Smoky Mountains National Park. Double stranded RNA extraction revealed the presence of multiple faint bands that were used as a template for further molecular investigation. Shotgun cloning of reverse-transcribed dsRNAs revealed the presence of genomic sequences of two phytoviruses in the original sample. Genomic segments of a putative cryptic virus showed similarities with corresponding regions of Beet cryptic viruses 2 and 3 and several other cryptoviruses. The second virus identified in this study is related, but distinct, from European mountain ash ringspot-associated virus and Fig mosaic virus (ca 40–42% identical amino acid contents of putative nucleocapsid and glycoprotein precursor), recognized species in the genus Emaravirus. Considering that emaraviruses are an emerging group of viruses associated with diseases in cultivated and wild flora, the involvement of this virus in the observed symptomatology is very plausible. Further epidemiological and etiological studies are underway.

A putative novel carlavirus associated with the disease in Magnolia tripetala L.
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Mosaic symptoms were observed on a specimen of an Umbrella-tree (syn. Umbrella magnolia) present on the campus of the Highlands Biological Station, North Carolina, in summer 2010. Transmission electron microscope observations of partially purified preparations revealed the presence of slightly flexuous filamentous virus-like particles c 650-700 nm in length that prompted further study. RT-PCR performed on purified dsRNAs using general flexivirus primers indicated the presence of a virus closely related to several species in the genus Carlavirus, fam. Betaflexiviridae. The polyadenylated genome of this virus, a putative new species in this taxon tentatively denominated Magnolia mosaic virus (MagMV), is 8.6 kb long and contains six ORFs. MagMV shares ca 67% overall nucleotide sequences with the type isolate of BlScV. Common amino acid content of genomic products between the two viruses vary from 65% (NABP) to 80% (TGBp-3). Virus-specific RT-PCR was developed and used to investigate the presence of this virus in different magnolia genotypes.

Practical resistance to fenhexamid *Botrytis cinerea* isolates from grapevines in New York
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Fenhexamid is a fungicide used to control *Botrytis cinerea* on grapes worldwide. Resistance appears to be of a quantitative rather than qualitative nature, with minimum EC_{50} values that define a resistant phenotype proposed as exceeding 0.1 mg/L by some workers and 0.4 mg/L by others. However, little is known about the degree to which isolates of these sensitivities are controlled by the material when subjected to typical application rates in the field environments. In this study, a total of 388 *B. cinerea* isolates were collected from New York State vineyards and their sensitivity to fenhexamid was examined. Morphological, physiological and genetic characteristics of 12 strains with EC_{50} values greater than 0.1 mg/L were defined. Four isolates whose EC_{50} value of fenhexamid is 0.033, 0.105, 0.318 and 1.626 were used for *in vitro* inoculation tests to gauge whether they show practical resistance. Inoculation tests using grape berries showed that pre-inoculation spray with the field rate of 1.2 mg/L controlled *B. cinerea* more than post-inoculation spray with the same rate, regardless of EC_{50} value of four isolates tested.

Studies on *Maize streak virus* infection and yield attributes in F1 maize hybrids
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Cultivation of tolerant genotypes remains an effective strategy against streak disease caused by *Maize streak virus* (MSV; genus *Mastrevirus*) in sub-Saharan Africa. Yield attributes in 15 MSV tolerant F1 hybrids, MSV resistance gene donor Tzi3 and MSV susceptible Pool-16, were estimated under field conditions from June to September, 2010. Plants at 2-3 leaf stage were inoculated with viruliferous leafhopper vector, *Cicadulina triangula*. Non-inoculated plants of the same, protected by spraying at weekly intervals with lambdacyhalothrin served, as control. The inoculated plants of each entry were compared with respective uninoculated entry. MSV incidence was 100% in infected trial irrespective of the genotype, but severity differed significantly ($p < 0.01$). Plants of protected trial remained disease free. Area under the disease progress curve (AUDPC) indicated that 52.9% of the entire genotypes were moderately resistant and the remaining were moderately susceptible to MSV. In all, 75% of the moderately resistant hybrids recorded AUDPC value higher than the Tzi3. In contrast, AUDPC value of moderately susceptible hybrids were less than Pool-16. There were substantial differences among the entries in the extent of plant growth and yield parameters relative to the corresponding uninfected controls. MSV infection in the hybrids reduced plant height (17.7 to 35.9%), cob weight per plant (12.5 to 52.1%), grain weight per plant (15.4 to 58.8%), 100-kernel weight (3.6 to 29.4%) and kernel number per plant (13.8 to 61%). These results suggests that cultivation of high-yielding genotypes with adequate protection against streak disease would contribute to increase in maize productivity.

PGPR mediated IPM for tropical vegetables in South India

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In IPM for major tropical vegetable crops (tomato, egglant, okra, chillies and onion) in South India, PGPR (plant growth promoting rhizobacteria) viz., Pseudomonas fluorescens and Bacillus spp were used as one of the IPM components for managing pests and diseases. The Pseudomonas fluorescens (TNAU-Pf 1) biopesticide formulation as seed treatment (10 g/kg) followed by soil application (2.5 kg/ha) significantly reduced the incidence of major insect pests (both sucking and chewing insects) and damage caused by plant pathogens (fungi, virus and nematodes) occurring in major tropical vegetable viz., tomato, eggplant, okra, onion and chillies in various laboratory and field studies conducted. The major mechanism involved in Pseudomonas fluorescens (TNAU-Pf 1) mediated IPM was induced systemic resistance (ISR) in host plants. The ISR activity in plants was associated with increased in activity of defense related proteins viz., chitinase, glucanase, peroxidise and polyphenol oxidase. Also, the population of natural enemies (coccinellids and spiders) of insect pests was increased. In all the field trials with PGPR as one of the IPM components, the yield was significantly increased and good market quality of vegetable products was obtained.

New host record for *Pseudomonas syringae* on *Lomatium* spp.

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A leaf spot disease of *Lomatium spp*. [*L. triternatum*, *L. grayi and L. dissectum]* was observed during summer 2009 and 2010 in an experimental plot at Malheur Experimental station, OSU, Ontario, OR. Symptoms on leaves initiated as water-soaked or translucent lesions that soon expanded and turned brown to black. Infection was severe under arid [hot and dry] conditions resulting in leaf collapse or a blighting effect. The disease was randomly distributed throughout the plots affecting *L. triternatum* (25% plants) followed by *L. dissectum* (5–8%) and *L. grayi* (5%). Microscopic examination of the necrotic tissues revealed characteristic bacterial streaming and isolations consistently yielded a fluorescent pseudomonad from all the hosts. Pathogenicity was confirmed by spray inoculations on to healthy leaves of *L. triternatum*. Typical symptoms developed on spray inoculated cilantro (*Coriandrum sativum*) but not on celery (*Petroselinum crispum)*. The bacterium was identified as *Pseudomonas syringae*, based on carbon source utilization (Biolog, Inc., Hayward, CA) and MIDI microbial Identification System (Microbial ID, Inc., Newark DE) although pathovar conclusions differed and similarity coefficients were variable. To our knowledge, this is the first report of occurrence of *P. syringae* on *Lomatium* spp.

Overseas migration affects the status of insecticide resistance in domestic populations of the small brown planthopper, *Laodelphax striatellus*

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The small brown planthopper (SBPH), *Laodelphax striatellus*, in one of the serious pests of rice plants in many Asian countries. A large overseas migration of SBPH was reported in western parts of Japan in June 2008. The immigrant populations were resistant to imidacloprid but not fipronil, while domestic ones were resistant to fipronil but not imidacloprid. Insecticide resistance to imidacloprid and fipronil was compared among local populations in western regions in Japan after the overseas migration. In some populations collected near west coast, the resistance status coincided with that of the immigrant populations just after migration, i.e., resistance to imidaclorpid but susceptibility to fipronil. In other populations collected relatively far from the west coast, resistance was observed against not only imidacloprid but also fipronil. It is likely that the status of the latter populations resulted from intercrossing between immigrant and domestic populations. The resistance status in each of populations had been maintained until the nest spring after over wintering. Insecticide resistance was also assessed in other areas of northern and eastern parts of Japan. In general, these populations showed relatively low resistance, although resistance to fipronil was high in the eastern part of Japan where the density of domestic populations has recently increased.

Effect of fungicide and plant defense activator drench applications for controlling Fusarium wilt of watermelon

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Fusarium wilt (FW), caused by fungus *Fusarium oxsysporum* f. sp. *niveum* (FON), is a soil borne disease of watermelon that causes significant losses to United States watermelon growers every year. Seven years is the recommended crop rotation for fields where FON is severe, but lengthy rotations are not economically feasible for most growers and alternatives to crop rotation are needed. In this investigation, fungicide drenches and one plant defense activator drench were tested for the control of FW in a field trial at the Vidalia Onion and Vegetable Research and Educational Center in Lyons, Georgia. Plots (30 ft × 6 ft) were replicated six times in a randomized complete block design. The soil was inoculated prior to transplanting by applying 50 ml of a 1×10^5 microconidial suspension of FON race 1 to holes that watermelon transplants (cv 'Black Diamond') were planted into. Treatments were applied after watermelons were transplanted by drenching each plant with either a fungicide solution, a solution of the plant defense activator, Actigard (acibenzolar-s-methyl), or with a water control. One foliar application of each treatment was applied one month after planting. Disease incidence was assessed during the season. Plots treated with Actigard demonstrated significantly less FW incidence than the untreated control, and there was no significant difference between Actigard treated plots and plots treated with Proline (prothioconozole) or V10116 (metconazole).

Mycelial growth and sporangial production of *Phytophthora capsici* as affected by extracts from pecan tissues

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Pecan (*Carya illinoensis*) is an economically important nut crop in New Mexico and other regions in the U.S. Production of pecan generates several by-products including leaves, husks, shell, and wood. Previous work has demonstrated that various pecan tissues contain fungitoxic substances. Therefore, pecan by-products may be used in the control of plant pathogens. This study was conducted to examine the effects of pecan tissue extract on mycelial growth and sporangial production by *Phytophthora capsici*, a major oomycete pathogen of various vegetable crops in New Mexico and other regions within and outside the U.S. Aqueous extracts (5 and 10%, w/v) were prepared from ground tissue of leaf, husk, shell, and woody branches. Mycelium plugs from a 5–7 day-old V8 culture of an isolate of *P. capsici* were placed in 25 ml extract in 9-cm diameter petri dish, and incubated in a growth chamber at 26°C under continuous light. Plugs serving as control were placed in sterile distilled water. After 48 h, plugs were examined for mycelial growth and sporangial production. The greatest mycelial growth was recorded in extract of woody tissue. Mycelial growth was lowest in shell extract. No sporangia were formed in any of the extracts from pecan tissue. Abundant production of sporangia was observed on plugs incubated in distilled water. These results suggest that extracts from pecan tissue may reduce sporangial inoculum potential of *P. capsici*.

Genetic characterization of *Rhizoctonia solani* population isolated from sugar beet and dry bean

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Rhizoctonia solani causes Rhizoctonia root rot in sugar beet and dry bean, and crown rot in sugar beet. The diseases can cause up to 50% yield loss in both crops. Genetic diversity analysis in plant pathogen populations is necessary to understand co-evolution in plant pathosystems. The objectives of the present study were (a) to elucidate the extent of genetic variability present in R. solani isolates from the diseased sugar beet and dry bean fields in western Nebraska; (b) to determine the similarity/diversity among the isolates of sugar beet and dry bean. Twenty-eight and nine isolates of sugar beet and dry bean, respectively, were evaluated based on morphological characteristics and PCR-based DNA markers (ISSR, RAPD, and AFLP). Fungal colony colors growing in culture were variable and consisted of light tan, tan, dark tan, light brown, brown, and cream isolates. A high degree of genetic diversity among the isolates was observed based on DNA marker analysis using five ISSR primers. Total numbers of amplified bands varied from 1-20 within the range of 200 bp to 3 kb. We did not find any distinguishing DNA marker patterns between isolates of sugar beet and dry bean based on these five markers. All these isolates will be analyzed with about 50 different markers. The polymorphic DNA markers will be scored for each isolate and the data will be used for cluster analysis. This information may help in disease management and molecular pathotyping of the pathogen.

Detection of *Tomato ringspot virus* in rose and almond in Fars Province of Iran

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Field surveys were conducted to asses the incidence of *Tomato ringspot virus* (ToRSV, genus *Nepovirus*, Family *Secoviridae*) in rose (*Rosa chinensis* L.) and almond (*Prunus amygdalus* L.) in Fars Province of Iran during 2009–2010. A total of 100 leaf samples with viral disease symptoms were collected and analyzed by Double Antibody Sandwich Enzyme-Linked Immunosorbent Assay (DAS-ELISA) and Dot-Blot Assay (DIBA) for the presence of *Tomato ringspot virus* (ToRSV) with polyclonal antibodies. Serological diagnoses were confirmed by Immuno Electron Microscopy (IEM) and bioassay. Results indicate the presence of ToRSV in all surveyed gardens. Among the samples tested, ToRSV was found in 21% of roses and 10% of almond trees. By applying RT-PCR to ToRSV-infected rose and almond plants, the expected 330 bp DNA fragment for ToRSV was obtained from all the samples tested. To our knowledge, this is the first report of ToRSV infecting rose and almond plants in Iran.

Effect of microbial diversity on soil fungistasis, disease suppression and colonization by biological control agents

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Biodiversity strongly affects ecosystem functions such as productivity, stability and invasibility. Research efforts have been mostly focussed on terrestrial plant diversity, while little is known about soil microbial communities. This work aims to investigate the effects of microbial diversity on soil fungistasis, disease suppression and capability of non native microbes to colonize rhizhosfere. Synthetic microbial communities with species richness ranging from 1 to 8 were used in factorial experiments. Fungistasis was assessed by germination tests on various fungi (Aspergillus niger, Botrytis cinerea, Trichoderma harzianum), while disease suppression was assayed on the Pythium ultimum-tomato system. Invasiveness potential of Pseudomonas fluorescens was investigated using soil amended with crop residues or rhizospheres of three plants (wheat, tomato and alfa-alfa). Increasing levels of microbial diversity determined amount and stability increases of fungistasis and disease suppression. The capability of P. fluorescens to colonize soil and rhizosphere dramatically decreased with the species richness of the residential community. These results suggest that microbial diversity of communities affects their resistance to invasive species.

Survey on the distribution of Rhizoctonia spp. in European soils

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Phytopathology 101:S160

Soil samples from UK, France, Germany, and Poland and to a lesser extend from other countries were collected and analyzed for the presence of *Rhizoctonia* spp. Rhizoctonia was isolated using a bait method adopted from Paulitz & Schroeder (2005). Rhizoctonia was found to be present in more than 65% of the soils tested. DNA from around 200 isolates was extracted and pyro-sequenced to define the anastomosis groups (AG). Fourteen different AGs were isolated with AG -5 being the biggest groups. Until now the pathogenicity of 68 isolates against wheat was tested. Sensitivity of 173 isolates to Fludioxonil and Sedaxane was tested in vitro. Activity against Rhizoctonia attack on wheat was tested in inoculated field trials.

Evaluation of rotation crops for their ability to suppress plant-parasitic nematodes in strawberries

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Phytopathology 101:S160

To investigate the efficacy of crop rotation for nematode control, six rotation sequences (sweet corn/pumpkin; rapeseed/pearl millet; broccoli/pumpkin; broccoli/broccoli; oats/june clover/oilseed radish, continuous alfalfa, and fallow with tilling) were established in 2009 in a site infested with needle (*Longidorus* spp.), lesion (*Pratylenchus* spp.) and ring (*Criconemella* spp.) nematodes. Rye was planted in the fall in all annual plots. Needle and ring nematode populations were lowest in the broccoli/broccoli and corn/pumpkin rotations whereas the rapeseed/pearl millet rotation had the fewest lesion nematodes. Incorporation and tarping of broccoli residue after harvest suppressed nematode populations even more. In a replicated greenhouse experiment, potted marigold, squash, oat, rye, barley, pearl millet, sweet corn, hairy vetch, rapeseed, broccoli, soybean, june clover, sorghum-sudan grass, oilseed radish, buckwheat, switch grass, forage pea, pumpkin, and alfalfa were evaluated for their ability to suppress lesion (*P. penetrans*) and northern root-knot (*Meloidogyne hapla*) nematodes. The highest numbers of *P. penetrans* (30-50 per gram of root tissue) were recovered from the legumes whereas millet, squash, switch grass, marigold, barley, and pumpkin all yielded fewer than 2 lesion nematodes per gram of root. All grasses were non-hosts for *M. hapla* but pea was an excellent host and vetch, pumpkin, clover and soybean also were hosts. This study provides strawberry growers with alternative options for control of nematodes.

Antagonist *Cryptococcus flavescens* OH 182.9 3C colonization of wheat heads when applied with triazole fungicides and the effect on scab

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Integrated pest management (IPM) is the best available approach for reducing Fusarium head blight (FHB) and the mycotoxin deoxynivalenol (DON) in grain. Utilizing the effective FHB biological control agent *Cryptococcus flavescens* OH 182.9 (NRRL Y-30216) as part of an IPM approach against FHB is understudied. Trizole fungicides such as prothioconazole (PTC) used alone (Proline) or in combination with tebuconazole (Prosaro) are effective against FHB, but their use generally is not recommended after wheat anthesis to control late infections. A PTC-tolerant variant of OH 182.9 (OH 182.9 3C) in a tank mix with a fungicide or applied after flowering, could reduce DON by establishing populations that reduce late DON-producing infections by *Fusarium graminearum*. In a two year study, the colonization of glume and lemma tissues by OH 182.9 3C was determined when the agent was applied alone or in combination with a fungicide at or seven days after wheat flowering. For all treatment combinations, the population of OH 182.9 3C represented 50–95% of the total microbial population recovered from both glume and lemma tissues from 8 to 11 days after flowering, demonstrating the competitive success of the strain. While the application of strain OH 182.9 3C at times reduced ($P < 0.05$, FPLSD) FHB and/or DON, combinations of fungicide and antagonist were rarely significantly more effective than either component used alone for the doses tested.

Characterization of *Cylindrocarpon* populations associated with replant disease of almond and peach

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Growth and cumulative yield of replanted almond and peach orchards are often seriously compromised by Prunus replant disease (PRD), a poorly understood soilborne complex affecting successive plantings of *Prunus*. Previously, our culture-based (CB) and culture-independent (CI) examinations of fungal, bacterial, and stramenopile communities revealed that the fungus *Cylindrocarpon* (*Cyl*) was among organisms commonly associated with PRD, yet little is known of *Cyl* on *Prunus*. We examined these *Cyl* species using CB and CI methods. Eighty-eight cultured isolates, each from roots of a different

tree, were obtained from six California (CA) almond and peach orchards affected by PRD. The isolates were identified based on BLASTn searches of sequences of three loci (rDNA ITS, beta-tubulin, mtSSU rDNA). Also, from a subset of 17 of the trees among three of the six orchards, *Cyl* populations were examined using CI amplification and sequencing of ITS 2 rDNA fragments. Neighbor-joining cluster analysis and BLAST searches identified 87 of the cultured isolates as *C. macrodidymum*; one was *C. liriodendri*. Similarly, 117 of the 121 CI *Cyl* clones were *C. macrodidymum*; four were *C. destructans*. Our results indicate that *C. macrodidymum* is the most prevalent species of *Cyl* associated with PRD in CA. We are testing pathogenicity and aggressiveness of *Cyl* on Nemaguard rootstock for almond and peach; preliminary results indicate that at least some isolates of *C. macrodidymum* are pathogenic.

High planting combined with root collar excavation extends life of peach trees on Armillaria root rot infested replant sites

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Phytopathology 101:S161

Armillaria root rot is a serious disease of peach, killing thousands of trees every year in the Southeastern United States. In this study we investigated the survival of peach trees planted in 45 × 60 cm open-bottom SmartPots about 40 cm higher than the grower standard on two Armillaria root rot infested replant sites. The root collars were excavated after one growing season on half of the potted trees. After five years, almost all trees with excavated roots were still alive and productive at both locations, whereas 60% and 20% of control trees had died in locations 1 and 2, respectively. Trees left in pots for the entire 5 years were also significantly more resistant to Armillaria root rot compared to the grower standard. Across years root suckering of rootstocks was no different between treatments, however, potted trees were more susceptible to drought in the absence of irrigation the year of establishment. The results show that root collar excavation of peach trees planted high may be a suitable approach to lengthen the life span of trees on replant sites with high Armillaria root rot pressure. Research is underway to investigate commercialization of this system in commercial peach production areas.

RIFdb: An online database for the classification of plant-associated bacteria using the computationally derived RIF marker

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A DNA marker that distinguishes plant associated bacteria at the species level and below was derived using comparative genomics of six sequenced genomes of *Xanthomonas*, a genus that contains many important phytopathogens. This DNA marker comprises a portion of the *dnaA* replication initiation factor (RIF). Unlike the rRNA genes, *dnaA* is a single copy gene in 95.2% of the sequenced bacterial genomes, and amplification of RIF requires genus-specific primers. The RIF DNA marker was sequenced using genus-specific primers for 315 *Xanthomonas*, 210 *Ralstonia*, 11 *Pectobacterium*, 6 *Pantoea*, 7 *Dickeya* and 114 *Clavibacter* characterized strains in the Pacific Bacterial Collection and 43 *Xanthomonas* strains from the International Collection of Microorganisms from Plants. Genus-specific primers were also developed for *Xylella* and *Pseudomonas*, and RIF sequences were extracted from the sequenced genomes of five and twenty-three strains, respectively. The RIF sequence frameworks are available online at RIFdb, and can be queried in both chromatogram and FASTA format with RIF sequences obtained from unknown strains. This database provides an easy access point to help biologists classify plant-associated bacteria and compare local strains with a worldwide collection of phytopathogenic bacteria.

Effects of *Bacillus firmus* GB-126 on the Soybean Cyst Nematode mobility *in vitro*

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Cells and cell-free extracts of *Bacillus firmus* strain GB-126 were evaluated for their capacity to reduce mobility and cause paralysis of juveniles of the Soybean Cyst Nematode (SCN) (*Heterodera glycines*). Two *in vitro* assays were developed to examine mobility and paralysis of J-2s in 96 well plates containing 100 µl of GB-126 cells at $1 \times 10,^{7}$ and 1×10^{6} cfu/ml and cell-free extracts at 100%, 50%, and 25% concentrations. Juveniles were evaluated for mobility and paralysis with a Nikon TS100 inverted microscope at 0, 12, 24, and 48 hours. GB-126 cells at both concentrations significantly reduced mobility compared to Tryptic Soy Broth (TSB) and Sterilized Tap Water (STW) controls at 36 h after treatment. Mobility was reduced to 61% and 67%, respectively, in the 1×10^{7} and 1×10^{6} cfu/ml cell suspensions at 48 h of exposure. With cell-free extracts, mobility was significantly reduced 12 h after treatment with 100% and 50% concentrations compared to TSB and STW controls. Mobility of SCN J2s ceased completely to a paralytic form in the 100% cell-free extract concentration at 48 h. The 50% and 25% cell-free extract concentrations reduced mobility of SCN J2s by 95% and 54%, respectively, at 48 h. The results of the experiments indicate that both cells and cell-free extracts of GB-126 can have direct effect on SCN J2.

Survey of *Rhizoctonia* spp. from wheat soils in the U.S. and determination of pathogenicity on wheat and barley

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Rhizoctonia root rot and bare patch are chronic diseases of wheat and barley in the Pacific Northwest (PNW), but little is known about *Rhizoctonia* spp. in other cereal growing areas of the U.S. A survey was conducted in the fall of 2009 and 2010 to identify *Rhizoctonia* spp. from soils collected throughout the wheat growing regions of the U.S. Soils were collected from 114 fields in 14 states and *Rhizoctonia* isolates were baited from these soils using a toothpick baiting method. Recovered isolates were identified by sequencing the ITS region of the rDNA and comparison of the sequence with the sequence of previously identified reference strains. Isolates were recovered from 49 locations and 51 isolates were sequenced. *Rhizoctonia solani* AG-2-1 (27%) and *R. oryzae* (*Waitea circinata*) (39%) were the most common species found. *Rhizoctonia solani* AG-3, AG-4, AG-10 and AG-11; and *Ceratobasidium* sp. AG-A and AG-I were also found. Interestingly, *R. solani* AG-8 was not found outside of the PNW. In pathogenicity assays conducted in the greenhouse in pasteurized soil, most isolates caused significant plant stunting and displayed typical root disease symptoms compared to the non-inoculated control. The highest disease ratings (0-8 scale) were observed with AG-3 (2.2 on wheat, 2.7 on barley), AG-A (1.9 on wheat, 2.8 on barley), and AG-4 (1.7 on wheat, 2.1 on barley). This data suggests that other groups of *Rhizoctonia* may be capable of causing damage on wheat and barley.

Overview of the Onion ipmPIPE and the development of innovative disease diagnostic tools for onion diseases

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The overall goal of the Onion ipmPIPE Project is to incorporate existing onion pest management programs and pest risk assessment models developed at local and regional levels into an internet platform for national implementation and validation. This project will develop a systems based trans-disciplinary approach to onion production to improve the productivity and profitability for onion stakeholders. The Onion ipmPIPE Project will expand the innovative diagnostic tools and coverage for priority diseases of onion caused by fungal and bacterial pathogens and their complexes. The Onion ipmPIPE will link with weather impact management tools and recommendation systems. IYSV and thrips are among the top priorities for the Onion ipmPIPE components which will be monitored via sentinel plots. In addition, the complexes of bacterial and fungal diseases assume an important secondary priority in terms of developing monitoring and sampling protocols. A set of diagnostic cards have been developed to facilitate rapid and accurate visual identification of onion growth stages, diseases and pests. The national expert laboratories are in the process of developing high throughput molecular methods to aid in the diagnosis of diseases caused by fungal and bacterial pathogens. Educational on-line resources such as the expanded Alliumnet and Pest Image Gallery and Bugwood Wiki hosted by CISEH Bugwood on an Onion ipmPIPE infrastructure will be accessible via the Web.

Legume ipmPIPE—A real-time disease/pest monitoring and reporting network

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The Legume ipmPIPE objectives and accomplishments comprise a variety of programs and resources for common beans, cool-season legumes and warm-season legumes including: (a) established sentinel or mobile plots in as many as 25 states in the United States, Canada and Mexico through collaborations with in-country scientists; (b) identified priority diseases and pests for monitoring; (c) monitored fungal and bacterial diseases and insect pests throughout the growing season; (d) implemented sampling protocols to monitor viral disease prevalence and kit-based high output immunoassays for six common legume viruses for use by National Plant Diagnostic Network labs; (e) established communications between scientists specializing in legumes across the U.S.; (f) collated and archived data from across the U.S., (g) supported a web-based platform for access and information display to extension educators, research scientists, industry, and other stakeholders; (h) created a web-based portfolio of management and education tools; and (i) distributed a popular series of pocket sized cards (print and online versions) that improve the accuracy of pest and disease diagnostics. The ultimate goal of the Legume ipmPIPE remains identifying causes of loss in legumes and assisting producers in minimizing those losses by implementing timely and economical components of Integrated Pest Management for priority pathogens and pests.

Pyrethrum yield estimation by digital image analysis

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Pyrethrum is grown for the production of insecticidal pyrethrins. Production in Australia is constrained by a number of fungal pathogens, including *Stagonosporopsis ligulicola* and *Sclerotinia* spp. To accurately assess control strategies for these and other diseases, a practical method of yield assessment is required. Previously, yield assessment has relied upon destructive, manual harvesting, which is both costly and time consuming. As an alternative, yield estimation by digital image analysis was trialled at 29 sites in summer 2010, with replication. Images were taken at a height of 0.9 m above the crop canopy, encompassing a defined 0.7 × 0.7 m quadrat, placed level with flower height. Image capture occurred once over 50% ray florets were open within a crop. With the software package ImageJ, HSB color thresholding was used to isolate the yellow centres of individual flowers, from which the particle analyser function estimated the number of flowers per quadrat. Linear regression indicated a strong relationship between automated (AC) and manual (MC) flower counts, ($AC = 21.1 + 0.97*MC$; $R^2 = 0.95$, $P < 0.0001$) with an intercept not significantly greater than zero. A significant correlation was also obtained between automated flower counts and hand harvested dry weight of flowers (DW) from each quadrat ($AC = 109.1 + 3.45*DW$; $R^2 = 0.488$, $P < 0.0001$). These results highlight the potential for predicting yield using non-destructive image capture.

Disease incidence and race characterization of Fusarium wilt

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Cotton cultivars were examined for susceptibility to Fusarium wilt (FW); subsequently fungal isolates were collected to confirm *Fusarium oxysporum* f. sp. *vasinfectum* (FOV) presence and race. Symptoms of FW were initially observed June 23 on Stoneville 4288B2RF. By July 7, Deltapine 0949B2RF, DP1050B2RF, DP1028B2RF, Phytogen 375WRF, PHY485WRF, PHY565WRF and the susceptible Rowden were symptomatic. FiberMax 1740B2RF developed symptoms two weeks later. By Aug. 26, FW incidence culminated with 16% of the Rowden and < 1% of the resistant control M-315 plants dying. FOV was re-isolated on APDA from hypocotyls regions of all cotton cultivars except PHY367WRF, ST5458B2RF, and M-315. Morphologically, FOV colonies were white, loosely floccose with reverse pigments purple, intensifying over time. Phialids were monophialidic, short, and single. Microconidia were abundant ellipsoid, 1 celled, averaging 10.09 × 2.58 µm. Macroconidia were falcate with tapering apical and basal cells, 3-5 septate measuring an average of 18.40 × 4.86 µm. Chlamydospores were rough-walled, sub-globose, and 8.73 × 3.4 µm in diam. Genetic analysis of each FOV isolate, by variety, was characterized by partial sequences of the EF-1α gene, indicating very diverse clades of genotypes within the field. Combined analysis done in CA exposed genotype races 1, 2, 4, 8, and several undefined genotypes to be present. Results of this field test indicate resistance to FW does exist in our cotton cultivars, and FOV in Alabama appears to be extremely diverse genetically.

Integrated management of invasive mealybugs in brinjal

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Brinjal is widely grown in Tamil Nadu, India. Apart from shoot and fruitborer, mealybugs are the major concerns for farmers. Coccidohystrix insolita is the mealybug which occurs in the late stage of the crop growth. Two invasive mealybugs viz., Solenopsis mealybug, Phenacoccus solenopsis and papaya mealybug, Paracoccus marginatus were found heavily damaging the brinjal. Integrated management practices involving timely monitoring, the removal of affected plant parts, and weeds, conservation of predacious coccinellid and parasitoids and need based insecticides were recommended. Development of resistant variety will give a long term real solution in combination with parasitoids and natural enemies. Wild species, Solanum viarum is found to be free from damage by both the mealybugs and the mechanism of resistance is studied with cheap method of mass multiplication of Harmonia octamaculata and parasitoids. The non preference mechanism of resistance was exhibited in S. viarum high trichome length and density, more thickness of leaf and phloem region. Laboratory studies indicated that profenophos, dimethoate Pseudomonas fluorescens and Beauveria bassiana showed better ovicidal action against P. marginatus causing high per centage of egg mortality.

Characterization of *Phytophthora infestans* from Wisconsin in 2009 and 2010

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Late blight, caused by the oomycete *Phytophthora infestans* is the most limiting disease to potato production worldwide. After 2002, Wisconsin growers enjoyed a 6-year respite from this disease, until it appeared in 2009 and 2010. In these years, 33 isolates were collected from potato and tomato from across the state. Allozyme genotype was resolved using cellulose acetate electrophoresis at the *Glucose-6-phosphate isomerase* locus. This revealed 3 banding patterns: 100/122 (US-22), 100/100 (US-23), and 100/100/111 (US-24). Sensitivity to the fungicide mefenoxam was determined by measuring percent radial growth on Rye A media containing 100 ppm mefenoxam compared to an unamended control. US-22 and US-23 showed sensitivity, averaging 6.7 and 14.3% growth respectively, while US-24 showed partial insensitivity, averaging 42.4% of the control. This indicates that use of allozyme genotyping can aid in the selection of mefenoxam to control late blight. Mating type was determined by plating each isolate with a known A1 (US-1) and A2 (US-7) on Rye A and observing the presence of oospores. US-22 isolates formed oospores with US-1, indicating A2 mating type, and US-23 and US-24 isolates formed oospores with US-7, indicating A1 mating type. Isolates of opposite mating types were geographically isolated in the state, but potential exists for oospore formation in subsequent years, which could challenge current late blight management strategies.

Effects of temperature on potato zebra chip disease development

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Temperature has been shown to have significant impact on development of liberibacter species associated with citrus Huanglongbing disease. "*Candidatus* Liberibacter africanus" and "*Ca*. L. americanus" are both heat sensitive, whereas "*Ca*. L. asiaticus" is heat tolerant. The recently described "*Ca*. L. solanacearum" is associated with zebra chip (ZC), a newly emerging and economically important disease of potato worldwide. This psyllid-transmitted liberibacter species severely affects several other solanaceous crops and carrot. Experiments were conducted to evaluate effects of temperature on development of "*Ca*. L. solanacearum" and ZC disease. Potato plants were inoculated with "*Ca*. L. solanacearum" by briefly exposing them to liberibacter-infective potato psyllids at various temperatures under laboratory conditions. Following insect exposure, the plants were maintained at selected temperature regimes in growth chambers and monitored for ZC symptom development and later tested for liberibacter by polymerase chain reaction to confirm infection. Results indicated that temperatures below 17°C appear to slow development of "*Ca*. L. solanacearum" and ZC symptoms whereas temperatures above 35°C are detrimental to this liberibacter. Compared to Huanglongbing liberibacters, "*Ca*. L. solanacearum" appears heat tolerant. This sensitivity of this bacterium and its insect vector to temperature may partially explain incidence, severity, and distribution of ZC in affected regions.

Epiphytic yeasts for biocontrol of *Botrytis cinerea* on table grapes cv. Thompson seedless

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Botrytis cinerea, the causal agent of gray mould disease, causes severe economic losses on postharvest fruit and vegetables, particularly on table grapes in Chile. Traditionally, gray mould control has been based on application of fungicides. However, the growing public concern about fungicide residues in food and environmental risks associated with its use, and the development of resistant strains of these fungal pathogens have generated interest in developing non-chemical methods of control, like biological control. Yeasts (N = 125) isolated from grape and apple surface fruits were tested in a preliminary screening on agar plates, of which 8 inhibited the growth of mycelium of *B. cinerea*, in grade 2 to 4 on the scale proposed by Swadding and Jeffries (1996). Antagonist activity of these strains was also evaluated at different pH (4.2, 4.6, 5.0 and 5.4), resulting the pH 4.2 the most favorable. Two yeast isolates reduced the incidence of *B. cinerea* to 29–33%, on wounded grape berries, with a period of yeast colonization in the fruit of 24 hours prior to pathogen inoculation, and stored at 20°C for 7 days. Our results suggest that antagonist yeasts with the potential to control *B. cinerea* on table grapes can be found among microflora associated with the fruits. Research funded by FONDECYT 11080062.

The Beauty & the Smut: An examination of the evolutionary relationships of *Microbotryum* transmission with the Montiaceae family

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Microbotryum spp. represent a group of fungi that causes smut disease in a variety of flowering plant families. Here we present an initial inspection of evolutionary relationships among *Microbotryum* species that infect members of the Spring Beauty family (Montiaceae). Spring Beauties infected with *Microbotryum* develop anther smut disease, which disrupts fertility. To widen host taxonomic coverage for statistical analysis, we inspected preserved herbaria specimens within four genera (*Calandrinia*, *Claytonia*, *Montia*, and *Lewisia*) in the Montiaceae for the presence of the disease. To date, we have surveyed 11,864 sheets from 21 herbaria representing 206 species. Of these, 51 sheets showed signs of disease giving a 0.43% disease rate. However, smut disease seems to be very prevalent in a few species: the disease rate for the twenty species that do show signs of infection is 9.9% (mean # of specimens per species = 25.7). Additionally, molecular data gathered from 83 healthy plant herbarium samples and 50 diseased plant herbarium samples have been amplified using a set of six molecular markers for chloroplast (*rcbL*, *rps16*), mitochondrial (*matK*, *trnL/L*, *trnF/L*) and nuclear (*ITS*) regions. The 50 fungal spore herbarium samples were amplified with two separate markers (*NADH*, *ITS*). The resulting phylogeny will be used to analyze the evolutionary history of Microbotryum species associated with Montiaceae in the context of varying pathogenicity and host specificity.

Characterization of VvBsl-1 a R2R3-MYB transcription factor involved in response to *Botrytis cinerea* infection in *Vitis vinifera*

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Chile is one of the most important grapes-exporting countries in the world. For this reason, grape quality and pathogen control are always required. One of the most relevant grapevine pathogens is *Botrytis cinerea*, a necrotrophic fungi that produces the gray mould disease. The objective of this work is to understand this interaction at the transcriptional level. Using bioinformatic approaches, we identified a gene model with high identity to an Arabidopsis thaliana gene required for susceptibility to *B. cinerea*. This gene, named VvBsl-1, belongs to the R2R3MYB family of transcription factor. We found that VvBsl-1 is significatively induced by *B. cinerea* infection in Cabernet Sauvignon and *V. labrusca* leaves. Surprisely, the VvBsl-1 expressión in mature berries was constitutively high, even in *B. cinerea* infected berries. To understand the VvBsl-1 expresion profile, we analysed the promoter region this gene, and we detected Abscisic acid response elements over-represented, a very important hormone in berries development. We perfomed ABA treatments on grapevine leaves and found VvBsl-1 highly induced after few hours of treatment. Since many of the abiotic stress are regulated by ABA, we conduced a high salinity experiment with disc leaves and we obserced a high expression of this gene since 24 hours. Together, our data suggest that VvBsl-1 integrates both stress response in grapevine, and this is probably regulated by ABA.

Pathogenic and non-pathogenic fungi associated with longan (*Dimocarpus longan* L.) in Puerto Rico

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Longan (*Dimocarpus longan* L.) is a tropical fruit tree that belongs to the Sapindaceae family. From August to September 2008 and April to May 2009, diseased plant organs were collected at commercial longan farms located in western Puerto Rico. Plant tissue was superficially disinfected and plated onto acidified potato dextrose agar to isolate fungi associated with injuries. A total of 240 fungal isolates were obtained from leaves, inflorescences, and fruit. Isolates were characterized morphologically, and by amplification and sequencing of the rDNA ITS region. *Albonectria* sp., *Alternaria alternata*, *Bipolaris oryzae*, *Botryosphaeria* spp., *Cochliobolus lunatus*, *Colletotrichum gloeosporioides*, *Fusarium* sp., *Nigrospora oryzae*, *Pestalotiopsis* sp., *Phoma* spp., and *Phomopsis* sp., were identified from these isolates. The following fungi were pathogenic to the inflorescences of longan: *Albonectria* sp., *Botryosphaeria* sp., *Fusarium* sp., *Pestalotiopsis* sp., and *Phoma* spp. *Albonectria* sp., *Botryosphaeria* sp., *Fusarium* sp., *Pestalotiopsis* sp., and *Phoma* spp. produced symptoms on the inflorescences ranging from rachis wilt, rachis spots, inflorescence blight, flower abortion, canker, and mummification. Three days after inoculation, *Botryosphaeria* sp. and *Pestalotiopsis* sp., colonized 100% of the surface of longan fruits. None of the fungal isolates tested caused symptoms on leaves. Further characterization studies will allow for better disease management practices for production of longan in Puerto Rico.

Establishment of a TMV-based transient expression system for AMPs in plants and their *in planta/in vitro* activity against compatible pathogens

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There have been discovered several antimicrobial peptides (AMPs) in many plants and animals secreted in response to various pathogens via innate immune system. These include variety of relatively small, basic, cysteine rich peptides such as Defensins, Thionins, Lipid Transfer Proteins and Snakins. These AMPs have shown antimicrobial activity *in vitro* and some of them have been expressed in transgenic plants leading to higher resistance against different pathogens. Previously it was found that Arabidopsis contains hundreds of genes that potentially encode putative AMPs such as thionin like peptides. We have developed a TMV RNA based efficient transient expression system (pPZP5000) to express these peptides in Nicotiana benthamiana leaves by agroinfiltration. In a comparison assay of five different plasmid vectors by expressing markers (GFP and GUS), pPZP5000 came out as competent system for transient production of higher levels of recombinant proteins in plants therefore, we are using it to produce putative AMPs to test their activity in two ways: First, we will isolate the peptides for *in vitro* tests against bacteria and fungi. Second, activity will be tested *in planta* by infecting leaves that transiently express peptides with compatible pathogens.

First report of apple canker caused by Xanthomonas sp. from Iran

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A local apple cultivar showing canker symptoms in one commercial orchard of northern Iran were sampled and brought to the laboratory during spring and summer in 2010. Disease symptoms were gray to dark brown discoloration with the expanding oozing. A total of 50 samples were collected. Small pieces of twigs and branches were emersed in 5 ml of saline solution (0.80% NaCl) for 20 min to disperse the bacterial cells. Thirty microliters of the resulting suspension was separated on nutrient agar (NA) medium and incubated at 30°C for three days. Purification of cultures were repeated twice on this medium. Biochemical and physiological test carried out according to conventional test. Hypersensitivity reaction with infiltration of 108 CFU/ml of bacterial suspension in to the Geranium leaf epidermis was positive. In pathogenicity test detached fresh apple shoots were inoculated with needle charged of 108 CFU/ ml of the representative bacterial suspension. Inoculated and uninoculated (control) samples were placed in growth chamber with 80–90% relative humidity at 27°C. Symptoms that occurred 4 days after inoculation were the same as naturally infection, while samples inoculated with water as control, remained healthy. Based on biochemical, physiological and PCR amplification with RS21/RS22 primers all isolates were identified as Xanthomonas sp. This is the first report of apple canker caused by Xanthomonas from Iran.

A multiplex RT-PCR for detection of three Cucurbits-infecting poleroviruses

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The presence of three poleroviruses including Cucurbit aphid-borne yellows virus (CABYV), Melon aphid-borne yellows virus (MABYV) and Suakwa aphid-borne yellows virus (SABYV) in China were definitely confirmed in our preliminary surveys. Here we reported a multiplex RT–PCR method for rapid detection and differentiation of these three Cucurbits-infecting poleroviruses. The multiplex PCR assay was developed with a mixture of universal polerovirus primer PococpR and species-specific sense primers CA3414F, MA3639F and SA3133F selected to differentiate three viruses CABYV, MABYV and SABYV, respectively. The mRT-PCR products consisted of fragments of 700bp for CABYV, 450bp for MABYV and 950bp for SABYV. Amplification of three target viruses was optimized by increasing the PCR annealing temperatures. And, the sensitivity of the multiplex PCR detection for CABYV, MABYV and SABYV were also evaluated in the study. Detection limit for PCR products was 1pg for CABYV, 0.1pg for MABYV and 1pg for SABYV. The multiplex RT-PCR is found a specific, sensitive and cost-effective method to detect multiple poleroviruses in cucurbits. This detection technique may facilitate research on cucurbit-infecting poleroviruses epidemiology, outbreak monitor and host-virus interaction analysis. The research is supported by the fund from National Natural Science Foundation of China (31000840), the Natural Science Foundation of Beijing City (6082006) and the Science and Technology Nova Program of Beijing (2007B032).

Pathogenic and genetic diversity in *Alternaria brassicae* and *Alternaria brassicicola* causing black leaf spot of cauliflower in India

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Biological pathotyping of *Alternaria brassicae* (21) and *Alternaria brassicicola* (6) isolates collected from cauliflower grown in seven different states of India were evaluated against a set of six cauliflower cultivars under lab and greenhouse conditions. Based on the pathogenic reaction, following seven groups were formed : Pathotype I (all cultivars); Pathotype II (all except Pusa Kartik Shankar); Pathotype III (all except Pusa Meghna); Pathotype IV (all except Pusa Sharad and DC-41-5); Pathotype V (all except Pusa Deepali and Pusa Kartik Shankar); Pathotype VI (Pusa Meghana and DC-23000); Pathotype VII (only Pusa Meghana). Based on disease severity ten isolates of *Alternaria brassicae* were Less aggressive, eight Moderately aggressive and three including six of *A. brassicicola* were Highly aggressive. Genetic analysis was performed using 42 RAPD and 3 ISSR primers. Combined dendrogram produced clearly distinguished between *A. brassicae* and *A. brassicicola* populations at 0.48 similarity levels which when related with pathotyping data formed 13 and 3 lineage groups respectively though being geographically distinct belonged to same pathotype. Internal transcribed spacer sequences (ITS) within the ribosomal DNA (rDNA) region were targeted to explore genetic variability among above Alternaria isolates. Phylogenetic relationship based on the ITS sequence and PCR-RFLP of amplified rDNA sequences clustered the *A. brassicicola* distinct from *A. brassicae* similar to RAPD and ISSR analysis irrespective of pathogenicity.

Biological control of root rots of groundnut in Rajasthan, India

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Groundnut is an important oilseed crop predominantly grown in Rajasthan, India suffered from 55 to 85 percent root rot disease caused by multiple pathogen complex mainly *Aspergillus niger*, *Apergillus flavus*, *Thievaliopsis basicola*, *Rhizoctonia solani* and *Pythium aphanidermatum* infecting seed, soil and crop. Biocontrol technology using *Trichoderma harzianum* (Th3) was used against Groundnut varieties, GG-10, GG-20 and Local varieties in 2009 and 2010 at farmer's fields in twelve different villages of Jaipur district. Field trials on soil, seed and foliage treatment with powdered bioformulation (Th3 SD, SA) @ 4-5 g per kg seed/soil followed by the spray treatment of the liquid bioformulation (Th3 FS) @ 4-5 ml/l along with recommended IPM practices were conducted. The untreated crops were significantly low in yields with the diagnostic blackening symptoms travelling from roots to stem affecting the vascular system followed by shredding at root-stem internodes resulting in complete wilting and plant death while in treated crop blackening reduced and the root vascular system was free of disease. Maximum values of R.C. Index (0.15), C.F.U. (38.5 × 106), seed germination (85%), pod yield (40 Q/ha) and lowest root rot incidence (15%) was recorded. Participatory approach and interaction between researcher and farmers helped in quick adoption and dissemination of use of biocontrol agents for groundnut growers in Rajasthan state, India.

Characterization of silencing suppressor activity of NSs from Iris yellow spot virus (Genus *Tospovirus*)

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Iris yellow spot virus (IYSV), a *tospovirus*, causes serious losses to onion bulb and seed crops. The viral genome consists of three RNAs, large (L), medium (M) and small (S). The S RNA codes for a non structural protein (NSs) in sense direction which was shown to function as the viral suppressor of gene silencing in plants. To further characterize the suppressor activity of NSs, a series of constructs of various lengths of IYSV NSs gene were made in pCAMBIA vector. These constructs were mobilized into Agrobacterium and plants of Nicotiana benthamiama line 16c, expressing GFP were agro-infiltrated either with or without a GFP expressing pCAMBIA vector. The ability of various NSs constructs to relieve the suppression of GFP was evaluated. This approach facilitated a better understanding of the role of NSs as the silencing suppressor during tospovirus infection of plants.

Pathogenic variation in *Pyricularia grisea*, the causal agent of pearl millet blast and resistance in mini core collection to the pathogen

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Pearl millet blast caused by *Pyricularia grisea* (Cooke) Sacc. [teleomorph: *Magnaporthe grisea* (Herbert) Barr] has emerged as a serious disease during the past few years in several states in India. Pathogenic variation in *P. grisea* was studied through pearl millet blast variability nurseries (PMBVN) established at four locations (Gwalior, Anand, Dhule and Patancheru) in India during the rainy season 2010, and by seedling reaction of host differentials to *P. grisea* isolates in a greenhouse screen. Four genotypes (ICMR 0622, ICMR 06444, ICMB 97222 and ICMB 99444) of the 20-entry PMBVN showed differential reaction across the test locations indicating pathogenic variation in *P. grisea* in India. This was confirmed in a greenhouse screen by the reactions of ten pearl millet genotypes to 25 isolates of *P. grisea* collected from major pearl millet growing areas in India. Based on the differential reactions, 25 isolates formed ten distinct pathogenic groups/pathotypes. Sources of blast resistance were identified by evaluating 238 pearl millet mini core accessions (1% of entire collection representing most of the useful variation) against a Patancheru isolate of *P. grisea* under greenhouse conditions -ten accessions (IP 4291, IP 4488, IP 5964, IP 7358, IP 8913, IP 9692, IP 11044, IP 13636, IP 21503 and IP 22449) were found resistant. The mini core accessions will be further evaluated to identify resistance to multiple pathotypes.

Virulence diversity of international collections of the wheat stripe rust pathogen, *Puccinia striiformis* f. sp. *tritici*

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Virulence information in the wheat stripe rust (yellow rust, *Yr*) pathogen, *Puccinia striiformis* f. sp. *tritici* (*Pst*), is important for controlling the disease with resistant cultivars. A total of 236 *Pst* isolates from Algeria, Australia, Canada, Chile, China, Hungary, Kenya, Nepal, Pakistan, Russia, Spain, Turkey, and Uzbekistan were tested on 20 single-gene lines and the 20 wheat genotypes for differentiating U.S. races. Thirty U.S. isolates representing 15 major races in 2006–2009 were selected for comparison. The 236 isolates were identified as 115 races on the single-gene lines and 160 races on the U.S. differentials. None of the isolates were virulent to resistance genes *Yr5* and *Yr15*. Virulences to *Yr10*, *Yr24*, *Yr32*, *YrSP* and Moro (*Yr10*, *YrMor*) were low (<20%); those to *YrA*, *Yr2*, *Yr6*, *Yr7*, *Yr17*, *Yr26*, *Yr31*, *YrExp2*, *Yr21*, Produra (*YrPr1*, *YrPr2*), Stephens (*Yr3a*, *YrS*, *YrSte*), Lee (*Yr7*, *Yr22*, *Yr23*) and Fielder (*Yr6*, *Yr20*) were high (>80%); and those to *Yr1*, *Yr8*, *Yr9*, *Yr25*, *Yr27*, *Yr28*, Heines VII (*Yr2*, *YrHVII*), Paha (*YrPa1*, *YrPa2*, *YrPa3*), Druchamp (*Yr3a*, *YrD*, *YrDru*), Yamhill (*Yr2*, *Yr4a*, *YrYam*), Tyee (*YrTye*), Tres (*YrTr1*, *YrTr2*), Hyak (*Yr17*, *YrTye*), Express (*YrExp1*, *YrExp2*), Clement (*Yr9*, *YrCle*) and Compair (*Yr8*, *Yr19*) were moderately frequent (20–80%). Although races were generally different, most of the virulences were common in these countries. The virulence data indicated gene flow between some of the countries.

Effects of DMI fungicide applications on secondary metabolites in creeping bentgrass (*Agrostis stolonifera* L.)

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Geranylgeranyl diphosphate is the 20-carbon compound in the isoprenoid pathway that gives rise to the diterpene gibberellin (GA) compounds and the tetraterpene carotenoid pigments. Applications of DMI fungicides inhibit GA synthesis in plants. By blocking the GA pathway, we hypothesize that a shift of metabolic precursors from normal GA synthesis to the carotenoid pathway may occur. Carotenoids act as powerful antioxidants in plants; an increase in products of the carotenoid pathway, particularly the xanthophyll cycle, may result in improved stress tolerance. The objective of this study was to determine carotenoid and chlorophyll (chl) concentrations in creeping bentgrass following applications of propiconazole (PPZ) and tebuconazole (TBZ). Two applications of PPZ and TBZ were made at 7 day intervals at rates of 0, 976, and 1952 g a.i. ha^{-1}. Leaf blades were harvested 7 days after the last treatment. Carotenoid and chl pigments were extracted from frozen leaf blades and measured using HPLC. Violaxanthin concentrations increased approximately 20–25% while zeaxanthin concentrations dropped 21–28% compared to non-treated plants. Both fungicides significantly increased chl a, chl b, and total chl. Fluxes in xanthophyll pigments towards increased violaxanthin concentrations indicate that the plant may tolerate stresses better than non-treated plants. Further research is needed to determine if changes in xanthophyll pigments result in improved quality under stressful conditions.

QTL mapping of resistance genes for eyespot of wheat in *Aegilops longissima*

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Eyespot is an economically important disease of wheat caused by the soilborne fungi *Oculimacula yallundae* and *O. acuformis*. Resistant cultivars are the most desirable control method, but resistance genes are limited in the wheat gene pool. A wild wheat, A*egilops longissima* (2n = 14, S^lS^l), was evaluated as a new source of eyespot resistance. A recombinant inbred line (RIL) population developed from the cross PI 542196 (R) x PI 330486 (S) was used to construct genetic linkage map of the S^l genome with 169 wheat SSR markers covering 1261.3 cM in 7 groups. F_5 lines (189) were tested for resistance with GUS-transformed isolates of *O. yallundae*. Four QTL were detected in chromosomes $1S^l$, $3S^l$, $5S^l$, and $7S^l$ that explained 44% of the total phenotypic variation by GUS scores and 63% by visual disease rating. These results demonstrate that genetic control of *O. yallundae* in *Ae. longissima* is polygenic and is the first report of multiple QTL conferring resistance to eyespot. Markers *Xcfd6*, *Xwmc597*, *Xwmc415*, and *Xcfd2* are tightly linked to *Q.Pch.wsu-1S^l*, *Q.Pch.wsu-3S^l*, *Q.Pch.wsu-5S^l*, and *Q.Pch.wsu-7S^l*, respectively. These markers will be used in marker-assisted selection to transfer resistance genes to wheat and broaden the genetic diversity of eyespot resistance.

Integrating Sedaxane as part of a comprehensive seed care product for broad spectrum disease protection of small grains

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Seed and soil borne diseases have constantly challenged cereal small grain plant establishment and the consequent growth and development. These problems are also compounded when small grains are planted in no –till or low till conditions. Shifting trends of rotational crops in traditional small grain production areas have also increased the diversity and complexity of pathogens that affect grain seedlings. Seed Care solutions are now integral to overall crop protection strategies. The integration of Sedaxane as a new mode of action to existing seed care products expands the broad spectrum and sustained impact of seed applied fungicides. In wheat soil surveys new strains of *Rhizoctonia sp*. have been identified and the role of Sedaxane is evident. In addition Sedaxane seed treatment has also shown excellent activity against *Ustilago nuda* in barley. Field and greenhouse studies suggest that Sedaxane offers a unique advantage in preserving root health against constant challenge of seed and soil borne pathogens.

Biological characteristics regulated by *algU* in *Xylella fastidiosa*

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Xylella fastidiosa is a plant xylem-limited and Gram-negative bacterium that causes Pierce's disease in grapes through cell aggregation and vascular clogging. *algU* encodes an alternate sigma factor, AlgU, that confers tolerance to osmotic, oxidative, and the transcriptional regulation of function of pili in gram-negative bacteria including *Pseudomonas aeruginosa*. The biological characteristics of a *algU* mutant of *X. fastidiosa* were analyzed in the xylem sap, which includes nutrients within the host's xylem-vascular system. The *algU* mutant had reduced abilities to adhere to a glass surface and form biofilm compared with the parent. Additional, the colony of *algU* mutant reduced the characteristic of twitching motility. These results suggest that AlgU of *X. fastidiosa* might regulate many virulence factors, which might enhance the adaptation of *X. fastidiosa* to xylem-vascular environmental stresses and causing disease symptom in plants.

The effect of sodium hypochlorite on the control of bakanae disease of rice caused by *Gibberella fujikuroi*

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Sodium hypochlorite has been used as a disinfecting and sterilizing agent against a broad range of seedborne pathogens. In order to develop effective control method for rice bakanae disease caused by *Gibberella fujikuroi*, the effects of sodium hypochlorite on antifungal activity was investigated in vitro and greenhouse conditions. The spore suspended in 0.01% sodium hypochlorite for 30 minutes did not germinated on the medium. Treatment of household bleach (about 5% sodium hypochlorite) for four hours was more effective to eliminate fungus from rice seeds infected with bakanae disease compared with treatment for two hour or seed treatment with Prochloraz, a common rice seed disinfectant in Korea. The elimination effect in the treatment of household bleach followed Prochloraz was significantly higher on the diseased rice seeds than Prochloraz treatment or household bleach only. When the diseased rice seeds were soaked into the twenty fold solution of household bleach for twelve hours, the disease incidence of rice seedling was remarkably reduced up to 4.7% compare to 97.3% of non-treatment control. And the emergence rate of seed was higher at household bleach treatment compare to non-treatment control.

VCG and AFLP analysis of *Fusarium oxysporum*, the causal agent of koa wilt in Hawaii

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Acacia koa (koa), a tree native to Hawaii, has important roles in Hawaii. However, a serious dieback caused by *Fusarium oxysporum* (FO) makes the establishment of koa plantations difficult. The objectives of this study were: 1) to distinguish pathogenic and non-pathogenic strains of FO isolated from koa, and 2) to develop a rapid, economical method to detect pathogenic strains of FO in the soil. Pathogenicity tests were conducted with 46 *Fusarium* isolates collected from dieback koa specimens by adding 10^6 spores of a fungal isolate to each of 10 koa seedlings. After 3 months, koa mortality ranged from 0–85% with 18 isolates killing no seedlings. Vegetative Compatibility Group (VCG) tests grouped the isolates into 16 VCGs with 2 major groups, VCG1 and VCG2, containing 8 and 16 isolates, respectively. Of the 46 isolates, 14 killed 50% or more of the seedlings tested, and of these 14 isolates, 12 belonged to VCG2. In AFLP analyses, isolates from the same VCG cluster with one another. Thus, strains of FO from dying koa trees may be pathogenic or non-pathogenic. By pairing representative strains from the VCGs with FO isolates collected from soil, the presence of pathogenic field strains can be tested. Alternatively, AFLP analyses of a larger set of strains may lead to the identification of conserved AFLP fragments that can serve as the basis for a diagnostic test for this pathogen. Testing the soil prior to planting will greatly increase the likelihood of establishing healthy koa plantations.

A volatile substance from *Talaromyces* sp. promotes the plant growth and blocks the disease development on several plants

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Talaromyces sp. a plant growth-promoting fungus was isolated from an agricultural field at Okayama, Japan. An isolate FS2 was identified as *T. wortomannii* based on ITS1 sequence and morphology. FS2 enhanced seed germination, root elongation and leaf growth of *Brassica*. The growth was also accelerated by volatile substance(s) emitted by FS2. GC-MS analysis of

the volatiles indicated that FS2 emitted at least seven terpenoids including β-caryophyllene that promotes the growth of *Brassica*, *Crucifer* and *Nicotiana*. Thus a part of the plant growth promoting effect of FS2 seems to be attributed to this terpene. Interestingly, we also found that *Brassicae*, *Crucifer* and *Nicotiana* pretreated with β-caryophyllene became tolerant to diseases caused by *Colletotrichum higginsianum*, *C. lagenarium* and *Botrytis cinerea*, respectively. Pretreatment with β-caryophyllene for 24 h decreased lesion expansion and infection rate by *C. higginsianum* on *Brassica* leaves. Similar results were obtained with the combination of *Arabidopsis thaliana* and *C. higginsianum*. Additionally, the yield of cucumber fruits increased significantly by treatment with β-caryophyllene. Taken together with an analysis of transcriptional activation of defense-related genes by RT-PCR, the results indicate strongly that β-caryophyllene may act not only as a plant-growth promoting substance but also as an inducer or a priming compound for ISR. Based on these findings, we discuss the availability of PGPF-products for crop cultivation.

Risk analysis of native and ornamental plants for root infection and inoculum production from roots by *Phytophthora ramorum*
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Due to concern about possible spread of *P. ramorum* in the east through dispersal of inoculum in streams, over 45 species of plants were inoculated with *P. ramorum* and screened for root colonization and production of inoculum from colonized roots. All plants were compared to *Viburnum tinus*, which served as a positive control. Plant species were considered for further testing if the initial screen showed root colonization to be at least 10% and if roots gave off at least 10% of the inoculum produced by *V. tinus* plants inoculated at the same time. The ecological and commercial importance of the plant was also taken into account, as well as range and habitat. 16 plants were chosen for further tests: *Arctostaphylos uva-ursi*, four species of *Camellia*, *Cornus sessilis*, *Ilex glabra*, *Kalmia latifolia*, *Lonicera dioica*, *Nyssa sylvatica*, *Persea borbonia*, four species of *Quercus*, and *Rhododendron* 'Cunningham's White'. These more formal tests analyzed inoculum over time using mixed-model regression analysis. Root colonization and total inoculum produced over the course of the experiment (corrected for dry root weight) was analyzed by a General Linear Models analysis. In these tests, *Rhododendron* 'Cunningham's White', *Camellia sinensis*, *Quercus prinus* and *Persea borbona* showed relatively high levels of inoculum production and root colonization, although no plant ranked as high as *V. tinus*.

Suppression of bacterial panicle blight of rice by pretreatment with various chemical compounds
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Bacterial panicle blight (BPB), caused by Burkholderia glumae and B. gladioli, is a serious disease of rice, which causes sterility and, consequently, failure of grain-filling. In spite of its economic importance, there are few effective control measures for this disease. Because numerous chemical compounds, including salicylic acid (SA), jasmonic acid (JA) and 2,6-dichloroisonicotinic acid (INA), are known to induce physiological immunity and acquired disease resistance in many plants, we tested various chemical compounds for their ability to induce rice resistance to BPB in an attempt to develop a new control method for this disease. At 30% heading stage, panicles of the disease susceptible variety Trenasse were sprayed with SA, JA, ethephon, ascorbic acid (AA) and INA 24 h prior to inoculation with B. glumae. BPB symptoms were rated using a standard rating scale, 0 – 9, at 10 days after inoculation. Among the tested chemical compounds, pretreatment of AA resulted in significant suppression of disease development and minimal yield reduction. These results suggest that AA could be useful for management of BPB in rice production. Currently, the mechanism of this protective effect of AA against BPB is being investigated and additional materials are also being tested.

Microarray reveals the role of auxin in mediating the interactions between *Macrophomina phaseolina* and *Medicago truncatula*
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Macrophomina phaseolina is a soil born necrotrophic fungus that causes charcoal rot disease in a wild range of plant hosts. Unlike most of fungal pathogens, *M. phaseolina* prefers hot and dry conditions. Current management approaches are very limited to effectively control this pathogen. The possibility of genetic engineering of disease resistant crop is hindered by our limited knowledge on charcoal rot disease. To better understand the molecular interactions between *M. phaseolina* with its plant hosts, we established a model pathosystem using *Medicago truncatula.* Using Affymetrix Medicago Gene Chip, we conducted a gene expression profiling experiment using RNAs isolated from plants that are infected with *M. phaseolina* at three different time points, namely, 24 hour, 36 hour and 48 hour. The array data revealed up-regulation of genes in jasmonic acid and ethylene biosynthesis and signaling pathways. These results matched what we have found in our previously study. Interestingly, we also identified genes in auxin transport and signaling pathways that are regulated during the disease development. The expression of these genes is verified by quantitative real-time PCR. This finding has pointed to a new direction to better understand the compatible interaction between *M. phaseolina* and its plant hosts.

Mutation range leading to resistance to SDHI fungicides
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SDHI fungicides have a strong binding affinity towards a broad range of fungal succinate dehydrogenase enzymes, which inhibit electron transfer from succinate to ubiquinone and result in the inhibition of respiration. The current studied focused on the resistance occurrence in Alternaria alternata on nut trees in California. More than 330 monoconidial isolates from different treatments (before fungicide treatment, untreated, SDHI treated, SDHI plus QoI treated and anilinopyrimidines (AP) plus triazoles (DMI) treated) have been tested for the sensitivity to different SDHI fungicides (Boscalid, Isopyrazam, Fluopyram) and for point mutations occurring in the sdh genes b, c, and or d. The use of SDHI or SDHI+QoI increased the frequency of resistant isolates, whereas resistance in the AP+DMI treatment was comparable to before treatment and untreated. Eight different mutations (6 single amino acid and 2 combinations) have been detected among the 330 isolates. The frequency of mutations is dependent on the treatment: SDHI treatment increases the frequency and SDHI+QoI select also for the highest diversity. Some mutations confers cross resistance among all the SDHI tested, other mutations have a differential response. These findings suggest that SDHI selection increases the diversity of mutants in fungal populations and subsequent selection increases the frequency of the most adapted (resistant) mutants in the treated pathogen populations.

Species diversity, phylogeny and genetic structure of begomovirus populations infecting leguminous weeds in Northeastern Brazil
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Phytopathology 101:S166

Begomoviruses are whitefly-transmitted plant viruses with a circular, ssDNA genome. Begomovirus diseases are a serious constraint to crop yields in most tropical and subtropical regions of the world. In Brazil, begomoviruses affect mostly common bean and tomato production. Weeds are considered to be begomovirus reservoirs as well as primary inoculum sources for epidemics in crops. We have carried out a survey of leguminous weeds (family Fabaceae) in four states of the Brazilian Northeast. A total of 59 samples were collected, and 26 full-length begomovirus genomes were RCA-amplified, cloned and sequenced. Sequence analysis indicated the presence of six distinct viruses, including four novel species. *Macroptilium lathyroides* was revealed as a common host for several of these viruses, and could act as a mixing vessel from which recombinant viruses could emerge. Phylogenetic analysis indicated that five of the viruses cluster with other Brazilian begomoviruses, but one of them (*Euphorbia yellow mosaic virus*, EuYMV) clusters with viruses from Central and North America. Strong evidence of recombination was found among isolates of *Macroptilium yellow spot virus* (MaYSV). The genetic structure of the MaYSV population indicates a high degree of genetic variability. Our results indicate that leguminous weeds are reservoirs of several begomoviruses, and could play a significant role in begomovirus epidemics both as inoculum sources and as sources of emerging novel viruses.

Diversity of plant pathogenic fungi associated with native Amazon forest species
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There is growing demand for native plant species for reforesting in Amazon region and as a result the number of nurseries have increased. The incidence

of leaf diseases are the major limiting factors for the successful raising of nurseries. The main objective of this investigation was to identify associated microorganisms and fungal pathogens causing leaf spots on native forest species of Amazon. The plants showing symptoms were collected during 2007–2009 in the nurseries of Urucu- Coari- AM/Petrobrás. The samples were kept in humid chamber and later isolations of fungi were made by direct and indirect methods. Sixteen fungi associated with disease symptoms were identified. Of these, 10 fungal pathogens were identified based on the pathogenicity tests. All fungi including pathogenic ones belong to group mitosporico, 44% being Hyphomycetes and 56% Coelomycetes. The most frequent genera were Pestalotiopsis (21,4%), Colletotrichum (17,9%), Beltrania (10,7%), Curvularia (7%), Heterocephalum (3,6%), Phomopsis (3,6%), Stachylidium (3,6%), Bipolaris (3,6%), Lasiodiplodia (3,6%), Cytosporella (3,6%), Phyllosticta (3,6%), Meliola (3,6%), Myrothecium (3,6%) e Wardomyces (3,6%). Colletotrichum sp. and Lasiodiplodia theobromae were pathogenic to Bellucia grossularioides. In Euterpe precatoria the lesions were caused by Colletotrichum sp. and Pestalotiopsis sp. The leaf spots in Aniba rosaeodora were caused by Mirotecium.

Inhibition of *Magnaporthe oryzae* and *Rhizoctonia solani* by *Sarocladium oryzae*, the causal agent of sheath rot in rice

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Phytopathology 101:S167

Sheath rot of rice (*Sarocladium oryzae*) has been increasing in significant proportions in different rice growing States of Brazil. The antagonism of 16 isolates of S. oryzae obtained from infected grain and sheath was studied utilizing *Magnaporthe oryzae* as test organism. A clear inhibition zone was formed between the two rice pathogens in a dual culture test in Petri plates containing PDA. The isolates showed significant differences in relation to the inhibition of *M. oryzae*. Similar results were obtained in tests conducted with culture filtrates. The pathogenicity of isolates was tested on rice cultivar Metica-1 in the green house. The isolates were multiplied in autoclaved sorghum grains and 69 days old plants were inoculated by inserting a single grain infested with mycelium and spores between the top most leaf sheath and culm. The disease was assessed seven days after inoculation utilizing lesion length as a criterion for determining the differences in aggressiveness of the test isolates. The isolates exhibited significant differences in lesion length on leaf sheaths of inoculated tillers. There was, however, no correlation between the degree of inhibition of *M. oryzae* in vitro and aggressiveness of *S. oryzae* isolates in inducing sheath rot symptoms. Several isolates of *S. oryzae* also showed inhibition of mycelium and sclerotia of *Rhizoctonia solani* on PDA in dual culture method as well as by using culture filtrate in repeated laboratory assays.

Vermicompost tea for control of *Phytophthora nicotianae* in pineapple

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Phytopathology 101:S167

Heart rot of pineapple, caused by *Phytophthora nicotianae*, can be devastating to low-acid pineapple hybrids which are more susceptible than the Smooth Cayenne clones. In separate tests, hybrid 73-50 and 73-114 crowns were dipped in 100 ppm Acibenzolar-S-methyl (BTH), 0.29 kg Aliette/100 l water or water and planted into soil infested with *P. nicot ianae*. Vermicompost tea or water was applied as a 500 ml/crown drench after planting. Crown death was recorded 3, 6, 9, and 12 weeks after planting. In the untreated plots, 66% and 58% of the 73-50 and 73-114 plants, respectively, died within 12 weeks, whereas only 24% and 17% death was observed in the respective Aliette treated plots. Heart rot in pineapple treated with vermicompost tea was similar to the untreated control in 73-50 plants but greater (66%) in 73-114 plants drenched with vermicompost tea. BTH performed similar to Aliette during the first weeks after planting but suffered high plant loss by week 12 (52% death in 73-50 and 34% death in 73-114). The vermicompost tea was not an acceptable alternative to Aliette for the control of heart rot. BTH might prove to be an alternative for *P. nicotianae* control in pineapple.

Scale-dependent landscape epidemiology

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What is the effect of spatial heterogeneity, and scale of heterogeneity, on the spread of plant disease epidemics? Models are often constructed for particular pathosystems at specific scales, but a suitable modeling framework that can accommodate a wide range of pathosystems and scales is yet to emerge. To address this problem, we present a new class of neutral landscape model – the foveated landscape. Foveated landscapes can be used to quantify simultaneously the effects of spatial heterogeneity and scale on multiple spatial phenomena. To demonstrate this new tool, we employ a graph-theoretic perspective to investigate how the connectivity of landscapes for epidemic spread scales under various population and landscape scenarios, for: (i) a pathosystem characterized by biotic dispersal of disease vectors; and (ii) a pathosystem characterized by abiotic (atmospheric) dispersal of plant pathogen propagules. We found clear domains of scale in landscape connectivity, as well as scale-dependent effects of population and landscape characteristics on landscape connectivity, which have profound implications for disease management. We use our results to generate a new set of general hypotheses relating to disease spread processes, how they scale, and how they can be managed. Our approach could provide an overarching framework for investigating scaling issues and cross-scale interactions in other pathosystems characterized by physical or biological dispersal.

The heritability of virulence to pine in *Gibberella circinata*

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Gibberella circinata causes pitch canker, an important disease of *Pinus* species worldwide. A cross between native California isolates of *G. circinata* produced progeny with a nearly continuous range of variation in virulence, as determined by lesion lengths induced on seedlings of *Pinus radiata*. Through a series of sibling crosses, it was possible to rapidly alter the virulence of progeny in subsequent generations by selecting parents based on lesion lengths. Thus, an F2 generation produced by crossing two short lesion F1 progeny, had a mean lesion length 80% shorter than that of the F1 generation. Likewise the mean lesion length of F2 progeny increased relative to the F1 generation when long lesion F1 progeny were used as parents. A similar effect was observed when the same mate selection strategy was used to produce an F3 generation. These results and the distribution of virulence phenotypes suggest that virulence is a quantitatively inherited trait. Using AFLP-PCR, five bands were shown to have a strong association with the long lesion length phenotype. This should make it possible to identify individual genes that influence virulence to pine in *G. circinata*.

Effect of bed height and soil amendments on survival of southern highbush blueberry cultivars in *Phytophthora* spp. infested soils in Mississippi

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Phytophthora root rot is an important disease of blueberry and is most severe when plants are grown in wet soils with poor drainage. Symptoms include small, yellow or red leaves, lack of new growth, root necrosis, and a smaller root system than that of healthy plants. Four studies were conducted in south Mississippi to evaluate the effect of bed height (flat or raised bed) and soil amendment (none, peat moss, or pine bark) on the survival of 19 southern highbush blueberry cultivars transplanted into soils infested with the root rot pathogen, *Phytophthora cinnamomi*. Plants were rated twice a year for overall vigor. The most vigorous cultivars were: Southmoon (2005 study), Gulfcoast (2006 study), and Springhigh (2008 study). In the 2005 and 2006 studies, plants grown on raised beds were more vigorous than those grown on flat beds and those grown in peat moss amended soil were more vigorous than those grown in soil with no amendment. In the 2008 study plants grown in pine bark amended soil were more vigorous than those grown in peat moss amended soil. However, in each study plant vigor declined each year, and most plants died within three years whether they were planted on raised or flat beds and whether they received any soil amendments or not. No cultivar thrived in any study. These studies demonstrate that southern highbush blueberries should not be planted in soils known to be infested with *P. cinnamomi*.

Molecular and biochemical characterization of resistance to *Botrytis cinerea* among the Solanaceae

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Grey mold caused by the necrotrophic fungal pathogen, *Botrytis cinerea*, is a major economic concern for tomato (*Solanum lycopersicum*; *Sl*) production. Grey mold can result in lesions on stems, leaves, and flowers of infected tomato plants as well as fruit rot both pre and post-harvest. Although all known cultivars of tomato are susceptible to grey mold, *Solanum lycopersicoides* (*Slo*), a wild relative of tomato, is extremely resistant. The objectives of this study are to characterize the molecular and biochemical basis of resistance of *Slo* to grey mold as well as to gain new insight into host responses to necrotrophic pathogens. To this end, next generation sequencing

(454) was used to generate transcriptomics data from *Slo* prior to and 24 and 48 hours after inoculation with *B. cinerea*. Bioinformatics analysis of the transcriptomics data revealed numerous genes in *Slo* that are upregulated in response to infection by *B. cinerea*. Additionally, unbiased metabolic profiling techniques utilizing mass spectrometry were developed to identify defense-related compounds differentially expressed in *Sl* and *Slo* before and during infection. This research will help to uncover the molecular and biochemical mechanisms underlying resistance to necrotrophic pathogens.

Screening of a Valencia peanut core collection for resistance to *Sclerotinia sclerotiorum*

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Peanut, *Arachis hypogaea*, is grown in the U.S. with four market types, Spanish, Runner, Virginia, and Valencia. New Mexico peanut production is largely based on Valencia type. In 2008, a Valencia core collection of 78 accessions was developed based on morphological and agronomic traits. However, this core has not been evaluated for diseases. This study was conducted in a controlled environment to assess the reaction of the core to *Sclerotinia sclerotiorum*, causal agent of Sclerotinia blight in New Mexico. Stems and detached leaflets were inoculated with mycelium plugs of a 3-5 day-old PDA culture of an isolate of *S. sclerotiorum.* Disease incidence, number of days until plant collapse, and diseased leaflet area were measured. All accessions had disease symptoms. The number of days before plant collapse varied from 3 to 19, diseased leaflet area spanned from 17% to 100%, and disease incidence ranged from 6% to 81%. Accessions were categorized in groups with highest number of days before plant collapse (>15 days), lowest leaflet area affected by disease (<25%), and lowest disease incidence (<25%). Five promising accessions were identified based on highest number of days before plant collapse. Three of these had the lowest disease incidence (<10%), and one had the lowest diseased leaflet area (<10%). These results provide background information for further evaluation of promising Valencia core accessions for Sclerotinia blight under field conditions.

Assessment of resistance pathways induced in *Arabidopsis thaliana* by hypovirulent *Rhizoctonia* spp. isolates

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The study evaluated the involvement of induced resistance pathways [systemic acquired resistance (SAR), induced systemic resistance (ISR) and phytoalexins] in plants protected against pathogenic *Rhizoctonia* by colonization with hypovirulent *Rhizoctonia* isolates. Changes in protection of *Arabidopsis thaliana*, mutants defective in defense-related genes (*npr1-1*, *npr1-2*, *ndr1-1*, *npr1-2/ndr1-1*, *cim6*, *wrky70.1*, *snc1*, *pbs3*) and colonized with hypovirulent *Rhizoctonia* compared to that of the wild type plants colonized by the same isolates confirmed involvement of induced resistance in protection against the pathogen. Colonization by hypovirulent *Rhizoctonia* isolates induced expression of genes: *pr5* (SAR), *pdf1.2*, *lox2*, *lox1*, *cori3*, (ISR) and *pad3* (phytoalexins) confirming the induction of these pathways. Induction of SAR or ISR by application of chemical inducers: Bion or methyl jasmonate (meJA) respectively, only ISR protected (although minor) against the pathogen. In conclusion, plant colonization by the hypovirulent isolates significantly induced genes involved in SAR, ISR and phytoalexins production pathways. SAR probably did not play a major role in the protection against Pathogenic *Rhizoctonia*. However, it may play a more important role in protection against other pathogens.

Kermes scale (*Allokermes* sp.) and the drippy nut pathogen (*Brenneria quercina*) associated with a decline of red oak species in Colorado

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Northern red oak (*Quercus rubra*) and pin oak (*Q. palustris*), while not native to Colorado, are a small, but important component of the urban tree landscape. The kermes scale (*Allokermes* sp.) is a common insect pest associated with these tree species. Scale feeding results in reduced tree vigor, twig dieback and witches' brooms, and in some cases tree mortality. Historically, tree damage associated with kermes scale infestations has been attributed exclusively to insect feeding. However, we have noted the presence of small cankers and extensive gummosis at many of the scale feeding sites for several years. Gummosis is so copious in some years that it drips from branches onto sidewalks and parked cars creating a nuisance. In 2010 the bacterium *Brenneria quercina* was consistently isolated from canker margins and bacterial ooze. Identification of the bacterium was confirmed by sequence analysis of a 1.5KB region of 16s rDNA. This bacterium was first described in California in 1967 as causing a disease of acorns of two native California oaks. The disease was named drippy nuts because of the copious bacterial ooze that leaked from infected acorns. This bacterium has not previously been associated with kermes scale feeding or dieback of northern red and pin oaks, nor has it been reported outside of California or Oregon in the United States, although it is associated with an oak decline in Europe.

Development of molecular diagnostic markers for *Xanthomonas translucens*

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Xanthomonas translucens (*Xt*) is the cause of bacterial streak and black chaff in small grains. The bacterium is seed borne and there is need for reliable diagnostic methods to detect and differentiate pathovars that may contaminate seed lots. Also, the use of pathovar designations within *Xt* have been inconsistent and further characterization of this species and its pathovars is warranted. We developed draft genome sequences of 12 *Xt* isolates representing four different pathovars using Illumina sequencing technology. Genomes were assembled with the short read sequence assembler Velvet. The gene finder GLIMMER and MAKER genome annotation pipelines were used for gene prediction and annotation in each genome. Isolates of *Xt* pvs. *translucens* and *undulosa* were distinct from isolates representing pvs. *poae* and *cerealis* based on multilocus sequence typing. Eight PCR primer sets were developed from unique regions in the genome. These primers amplified geographically diverse strains of *Xt* pvs. *translucens* and *undulosa* collected from wheat and barley, but not other bacterial plant pathogens including *Xanthomonas*, *Pseudomonas*, *Burkholderia* and *Erwinia* spp. Several of the primer sets have been successfully incorporated into a multiplex PCR design to expedite identification of *Xt*.

***Chromobacterium sensu latto* isolated from native and commercial cranberry with potential for biological control of *Phytophthora* root rot**

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Disease and pest suppression with natural strains of bacteria has the potential for decreasing agricultural reliance on pesticide inputs. A primary screen of cranberry (*Vaccinium macrocarpon*) rhizosphere bacteria from both native and commercial cranberry beds in eastern Massachusetts yielded a new type of *Chromobacterium* (*Neisseriaceae*, β-proteobacteria) which produces the dark-purple pigments violacein and deoxyviolacein. *Chromobacterium* sp. was tentatively identified by pigment production, 16S ribosomal gene sequence, and phenotypic characteristics. However, unlike previously described strains, cranberry root-associated *Chromobacterium* were isolated from a temperate climate plant, are genetically stable for pigment production, actively secrete violacein and deoxyviolacein when grown planktonically, and cannot use citrate as a carbon source. Violacein has broad-spectrum antimicrobial activity, but has not been tested for its potential as an agricultural biocontrol agent. We have shown that mycelial growth of the root rot pathogen *Phytophthora cinnamomi* is significantly inhibited in as low as 0.05 µg/mL violacein (ID_{50} <0.1 µg/ml), indicating that this root-associated organism may naturally play a role in protecting cranberry plants, and is a potential biocontrol agent for fungal and oomycete root diseases.

AWR effector proteins from *R. solanacearum* play a role in virulence and plant recognition

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R. solanacearum is a devastating bacterial pathogen that infects *Solanaceae* spp. such as tomato, eggplant or banana. A functional T3SS is required for virulence and more than 70 putative effectors have been described, although only few have been studied. The present work focuses on a five-member gene family of effectors named *awr*. Virulence of a *Ralstonia* mutant strain devoid of all *awr* genes was tested on tomato, eggplant and *Aradidopsis*. Plant growth of quintuple mutant strain was considerably reduced in natural hosts, indicating a role in virulence, but remained unchanged in *Arabidopsis*. Col-0 infection with *Pseudomonas syringae* DC3000 heterologously expressing each AWR was also performed. While presence of some AWRs in

Pseudomonas did not have an effect on plant growth, others (like AWR5) dramatically reduced the pathogen multiplication. When AWR proteins were transiently expressed in non-host *Nicotiana* spp., necroses took place to different extents. AWR5 induced the strongest necrosis, resembling an HR phenotype which was confirmed by TB/DAB staining and by RT-PCR of specific HR marker genes. In summary, AWR effectors play an important role in pathogenesis and some might be also recognised as they reduce *P. syringae* virulence and trigger an HR-like phenotype in non-host plants. Deciphering effector function will open promising avenues towards the design of new strategies to control *R. solanacearum*.

Formation of chlamydospore-like structure in the ascomycete fungus *Gibberella zeae*

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The homothallic ascomycete fungus *Gibberella zeae* is an important pathogen on major cereal crops. In this study, we found that conidia of *G. zeae* were readily changed to chlamydospore-like structures in minimal conversion medium supplemented with mannitol. Chlamydospores are enlarged, thick-walled vegetative cells with various forms and are produced from many fungal species. These structures accumulated high level of glycogen, lipid, and chitin which might be functional for stress resistances including UV, heat, and drought. We also found that various biological processes, including signal transduction, acetyl-CoA production, and chitin synthesis, are involved in chlamydospore-like structure formation. Based on these characteristics of chlamydospore-like structures, these structures might be produced in field condition at mild temperature and could be used for survival in hot and drought field conditions.

The occurrence and management of brown planthopper, *Nilaparvata lugens* (Stål), in Korea

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The brown planthopper (BPH), *Nilaparvata lugens*, is a migrant pest from tropical into subtropical and temperate areas every year and presents a great threat to rice production in Asia. A computer model was developed to simulate the migration waves and the impacts on the temperate rice ecosystem in Korea. The simulations showed that 1) each migration wave had different mass distribution and immigration areas; 2) the vertical air current value in the planthoppers' take-off and landing area was only several centimeters per second; 3) the main migrant source into southern Korea was from the eastern part of Guangdong Province and south-eastern part of Fujian Province in China. The population dynamics of the BPH after migration were closely examined in both sprayed and unsprayed rice fields. Early immigration contributes more to the population build-up and damage in late season than the amount of migration. Severe BPH resurgence occurred in the sprayed rice fields in both years of 2005 and 2006. The peak density was 4 to 20 times higher in the sprayed field than in the unsprayed field. The effect of root zone application of some systemic insecticides was tested as one of the BPH management options. Carbofuran root zone treatment was the most effective in increasing BPH mortality and reducing the percentage of eggs hatched. The treatment had not been effective on spiders while the broadcasting and foliar spray killed all ambush and hunting spider groups within one day after application.

Eradicating grapevine disease with minimal economic impact

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Eradicating exotic grapevine diseases using current strategies, which include complete removal of affected and suspected vines, can incur significant costs to growers and the industry. An alternative strategy has been developed through collaboration between Australia and the U.S.A. which optimises the process of eradicating a pathogen while minimising the economic cost of returning the crop to its previous quality and production levels. In Australia, black spot disease (grapevine anthracnose, *Elsinoe ampelina*) was used as a model to evaluate a drastic pruning eradication strategy developed for the fungal disease black rot (*Guignardia bidwellii*), exotic in Australia but endemic in eastern U.S.A. The protocol involved cutting off vines at the top of the trunk and removing low water shoots; removing debris from the ground beneath and between vines; mulching the vineyard floor; and applying a targeted fungicide program. The same protocol was evaluated in a black rot-infested vineyard in New York. Following two seasons of weather conditions conducive to black rot development, no disease was detected on treated vines, whereas leaf and fruit infections developed on the control vines. These results confirmed the efficacy of the protocol for eradicating black rot from vineyards. The protocol provides an alternative to razing a vineyard following an incursion of black rot in Australia. This general strategy may have potential for use on other diseases of grapes and other perennial crops.

Isolation of double-stranded RNA mycoviruses in *Macrophomina phaseolina* isolates in Iran

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Macrophomina phaseolina, the causal agent of charcoal root rot disease, is one of the soil borne plant pathogens in the tropical and subtropical regions. This pathogen has a wide host range. Biological control of some fungal pathogens by mycoviruses has been reported in the world. Thus, this study was conducted to explore the possibility of using mycoviruses as a new biocontrol agent of charcoal root rot disease. For this purpose, the presence of dsRNA was surveyed in 66 isolates of *M. phaseolina* collected from different regions of Iran. At first, the isolates were grown on potato dextrose broth (PDB) and stirred at 25°C for two weeks for maximized mycelial mass production. DsRNA fragments were extracted from the mycelial mass using STE buffer and CF-11 cellulose chromatography. DsRNA fragments ranging from 0.9 to 12 kbp in size were detected in 18 out of 66 surveyed isolates. These isolates had been isolated from sugar beet, soybean and sesame hosts. The presence of dsRNA was confirmed by RNase-A at high and low SSC buffer concentration. This is the first report on the detection of viral dsRNAs from *M. phaseolina* in Iran.

The effect of dsRNA mycoviruses of *Macrophomina phaseolina* on pathogenicity, laccase activity, mycelial growth and microsclerotia production

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Various viral genomes from phytopathogenic fungi have been reported world-wide. The most common of these genomes are dsRNAs that in some fungi are associated with hypovirulence and have been used or proposed as biological control agents. In this study 66 Iranian isolates of *Macrophomina phaseolina*, the causal agent of charcoal root rot disease, were studied for dsRNA infection and investigated the effect of these viral genomes on some phenotypic characteristics. Extraction and purification of dsRNA was performed using CF-11 cellulose chromatography and confirmed by digestion with specific nuclease (RNase A). In 27.2% of these isolates dsRNA fragments ranging from 0.9 to 12 kbp in size were detected. Partial curing of dsRNA fragments in 11 isolates out of 18 dsRNA containing isolates using cycloheximide (100 µg/ml) was successful. Eleven isolates having dsRNA and 11 dsRNA-free isolates were evaluated in more details. The results indicate that dsRNA can have different effects on morphology and biology of the fungus. In some isolates dsRNA increased the mycelial growth, laccase activity and pathogenicity and in the others dsRNA decreased the above characteristics. In a few of them, there was no correlation between the presence of dsRNA and mentioned characteristics. DsRNA had no effect on microsclerotia production. In 4 isolates dsRNA reduced virulence towards sugar beet. These results indicate an association between the presence of dsRNA and hypovirulence.

Efficacy of silk channel injections with insecticides for management of lepidopteran pests of sweet corn

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The primary pests of sweet corn in Georgia, U.S.A., are the corn earworm, *Helicoverpa zea*, and the fall armyworm, *Spodoptera frugiperda*. Control of these pests requires multiple insecticide applications from silking to harvest, with commercial growers frequently spraying daily for 16 to 18 days. Injection of oil into the silk channel 5 to 8 days after silking initiation has been used to suppress damage by these insects. Initial work with this technique in Georgia provided poor results. Subsequently, a series of experiments was conducted to evaluate the efficacy of silk channel injections

as an application methodology for insecticides. A single application of spinosad or chlorantraniliprole, at reduced rates as compared to common foliar applications, provided excellent control of lepidopteran insects attacking the ear tip and suppressed damage by sap beetles. Oil and oil plus a *Bacillus thuringiensis* insecticide provided minor suppression. The use of water plus a surfactant as the carrier also showed promise and avoided potential adverse effects of oil on pollination. This methodology is labor intensive but requires a single application of insecticide at reduced rates applied approximately two weeks prior to harvest. This methodology is not likely to eliminate needs for foliar applications because of other insect pests but would greatly reduce the number of applications required and may prove particularly useful for small acreage growers.

Refined empirical models for predicting Fusarium head blight epidemics in the United States

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Fusarium head blight (FHB) of wheat causes significant yield and grain quality losses in U.S. wheat. A web-based FHB forecasting system (http://www.wheatscab.psu.edu/) was developed in 2006 to predict outbreaks early enough to apply preventative fungicides. Further enhancements to existing models and the development of new models are underway using an expanded dataset from 1982 to 2009 containing 527 observations over 15 states. Multiple imputation was used to fill missing values in the data matrix. Current model parameters have been updated using a bootstrap sample of the imputed datasets. This analysis suggests that a model currently used for spring wheat may also perform well for winter wheat. Subset selection algorithms identified several other weather-based predictors that may be of potential use for improving prediction models for FHB. A comparison of the models currently in use, updated models, and newly developed models will be presented.

Diversity of vegetative compatibility groups in Michigan populations of the chestnut blight fungus, Cryphonectria parasitica, 1996 to 2009

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Hypoviruses change virulence of Cryphonectria parasitica allowing infected chestnuts to recover. Hypovirus spread is limited by vegetative incompatibility in the pathogen, which is governed by a bi-allelic, six locus system. Mismatches of alleles cause apoptosis where hyphae fuse, inhibiting the spread of hypoviruses. Vegetative compatibility (vc) diversity was characterized in seven Michigan C. parasitica populations in both 1996 and 2009. Four populations are causing major epidemics and lack hypoviruses. The three remaining populations have hypoviruses and chestnuts are recovering. Thirty single-spore isolates from each population were scored for vegetative compatibility. Each recovering population contained vc groups that were unique to the site. Two recovering sites were dominated by a single vc group which was stable over time. A third site, Frankfort, was more variable; increasing in diversity from 1996 to 2009. Epidemic sites were more variable for vc diversity, and five vc groups were shared among sites. Shared vc groups did not display a discernable spatial pattern. Low diversity and stability at recovering sites is probably due to hypoviruses preventing sexual reproduction in C. parasitica. Epidemic sites may be more variable both spatially and temporally because ascospores are likely to migrate among populations and may cause regular recombination.

A new selective medium for isolation of *Rhizoctonia* spp. from soil

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Several assay methods, including elutriation, toothpick, and soil pellet, were evaluated for isolation of *Rhizoctonia* spp. from rice, cotton, and soybean fields in Arkansas. Growth of *Trichoderma* spp. and Mucorales made isolation and quantification of *Rhizoctonia* spp. difficult in Ko and Hora medium. Ko and Hora (KH), ethanol potassium nitrate medium with 2% (EPN_2) and 5% (EPN_5) ethanol, and various chemicals in water agar were used to evaluate average daily growth of *Rhizoctonia* spp. and other fungi. *Trichoderma* spp. were the fastest growing isolates in KH, while growth did not differ between *Rhizoctonia solani* AG4 and an isolate of *Rhizomucor variabilis*. Average daily growth of all isolates in EPN_2 and EPN_5 were non-significant although one *Trichoderma* sp. was completely suppressed. A new selective medium, "TS", based on chemicals that improved inhibition of *Trichoderma* spp. and *R. variabilis* was developed consisting of 7 g/L Moorehead agar, 0.09 g ai/L metalaxyl (Apron 50WP), 0.229 ml ai/L potassium phosphite (Alude), 100 µl of a rifampicin solution in dimethyl sulfoxide (10 mg/ml), 0.25 g/L ampicillin salt, and 0.4125 µl to 2.0625 µl ai/L thiophanate-methyl (3336F). The level of thiophanate-methyl depended on the level of suppression needed for *Trichoderma* spp. and desired growth of *Rhizoctonia oryzae*. The "TS" medium has been effective in assaying populations of *Rhizoctonia* spp. over fields and methods.

Identification of potential virulence genes of *Candidatus* Liberibacter asiaticus differentially expressed in citrus and psyllids, using real-time PCR

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Citrus greening is a destructive disease of citrus in the United States, and is caused by the bacterium, *Candidatus* Liberibacter asiaticus. This disease is transmitted by the Asian Citrus Psyllid, *Diaphorina citri*. The pathogen causes severe symptoms in plant compared to the psyllid. We hypothesized that a number of pathogenicity/virulence related genes of the bacterium would be overexpressed *in planta*, compared to the psyllid. To test this hypothesis, quantitative real-time PCR assays using total RNA isolated from infected plants and psyllids were conducted. Gene specific primers were used to check the expression of 560 genes in *Ca*. L. asiaticus. The genes showing a differential expression of two fold or more in either the plant or psyllid were categorized into Clusters of Orthologous Groups of protein functional categories. Potential virulence related genes including hypothetical genes, which were overexpressed *in planta*, were selected. Differential expression of these selected genes were also evaluated in susceptible and tolerant varieties of *Ca*. L. asiaticus infected citrus. Potential virulence related genes were then screened on *Nicotiana benthamiana* plants for symptom expression, using transient assays. The results from this study will be useful in identifying the potential virulence genes involved in symptom expression and survival of this pathogen *in planta*.

Assessing the genetic basis of resistance to rice sheath blight

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Sheath blight (ShB) caused by *Rhizoctonia solani* is one of the most important diseases of rice. Resistant rice varieties would represent the best possible disease control option, both environmentally and economically. To date, no immune rice accession has been reported, but some cultivars with partial resistance have been identified. Resistance to ShB is a complex, quantitative trait, determined by polygenes/QTLs. Plant height, heading date, and other canopy traits play an important role in field resistance to ShB. QTL for ShB resistance have been identified and mapped, some of them being co-localized with QTL governing morphological traits and heading date. Whether or not such colocalizations are due to pleiotropy or tight linkage remains a question. Here, we report a study aiming at identifying sources for ShB resistance and at further analyzing the genetic basis of resistance to the disease. We phenotyped 163 rice accessions of *Oryza sativa* L., many of which have been reported to be partially resistant to ShB. Accessions were screened using two complementary methods, in microfield tests and with a detached tiller assay. Morphological traits were also assessed. The accessions were further genotyped with SSRs reported to be linked with ShB resistance and candidate genes associated with processes involved in resistance. The results of this study are discussed with respect to the genetic basis of resistance to ShB, and implications on breeding for ShB resistance.

An in vitro evaluation of chemical and biological agents for control of Botryosphaeria species

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A major limiting factor in the successful culture of fruits such as pome fruit (apple, pear), stone fruit (peach, nectarine, plum), blueberries and persimmon in Florida are the diseases incited by the fungal genus Botryosphaeria. Botryosphaeria have a host range encompassing at least 100 plant species, and for most plant species there is currently no adequate chemical control. The fungicides Azoxystrobin, Chlorothalonil, Copper Hydroxide, Flutriafol, Propiconazole, Pyraclostrobin, Tebuconazole, Tetraconazole and Thio-

phanate-methyl were tested for efficacy against B. rhodina, B. obtusa, B. dothedia and Neofusicoccum ribis in vitro. The fungicides tested generally offered only partial reduction in fungal growth even at relatively high (10 to 25 mM) concentrations. Naturally occurring phenolic compounds from plants were also tested, including vanillic acid, syringic acid, catechol, vetaric acid, 2,6-dimethoxy benzoic acid, ferulic acid, benzoic acid, 2,6-dimethoxy phenol, p-courmaric acid and guaiacol. The efficacy of the phenolic compounds varied with the specific compound and with Botryosphaeria spp. Inhibition of mycelial growth was dose-dependant ranging from 48–100% inhibition with benzoic acid and 69–88% with guaiacol in different Botryosphaeria spp. Biocontrol agents (Bacillus spp.) were also tested in vitro and exhibited potential activity in limiting the establishment of the Botryosphaeria spp. in vitro.

Discovery of key pathogenesis-associated genes among predicted transcription factors in the plant pathogenic fungus, *Alternaria brassicicola*
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Phytopathology 101:S171

The necrotrophic fungus *Alternaria brassicicola* causes black spot disease of *Brassicaceae* family. Several physiological and morphological characteristics have been hypothesized to be involved in fungal pathogenesis, such as production of cell wall-degrading enzymes, proteases, secondary metabolites, and a fast growth rate. These characteristics are believed to be coordinately regulated by transcription factors and little is known about their regulatory mechanism. In this study we created knockout mutants of the genes encoding for 173 of 380 predicted transcription factors of *A. brassicicola*. Our bioassays on green cabbage (*Brassica oleracea*) leaves identified 11 genes strongly associated with pathogenesis. One of the genes caused a loss of pathogenicity, another caused 100% increase in virulence, and the others caused a reduction in virulence of up to 90% compared to the wild type, as measured by lesion size. Ten of these eleven genes were novel virulence-factors. Only one of the genes has been previously identified as a virulence factor in other fungi. The functional genomics approach by systematic targeted gene knockout proved to be useful in discovering transcription factors associated with pathogenesis. Detailed characterization of the functions of these transcription factors will shed light on the regulatory mechanisms of pathogenesis and allow the design of efficient strategies specific to the management of necrotrophic fungi.

Role of rhizosphere microbial communities and nematodes in SDS development and/or suppressiveness in soybean cultivated fields
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Biotic and abiotic characterstics of the soil were being evaluated in relation to the incidence and severity of Sudden Death Syndrome (SDS), caused by *Fusarium virguliforme*, in soybean fields. In order to compare soil biota across a large number of samples and to overcome the difficulty of culture dependent techniques, we have used a metagenomic approach employing PCR-DGGE targeting 16SrDNA, 18srDNA and internal transcribed regions (ITS) in total rhizosphere DNA. Soil was collected from fields with high SDS incidence in IL, IA and MN. The samples were collected from areas of high SDS incidence (hot-spots) and areas of low SDS incidence in the same fields. Fingerprint analysis of Fungi-DGGE showed a distinguishable pattern of banding separating samples from hotspots from those originating from non-hotspots in the same field. Quantitative PCR was used to assess the extent of *F. virguliforme* presence in tested soil samples. Soil samples from hot-spot areas had significantly higher levels of *F. virguliforme* than those from low SDS areas in the same field. This suggests that DGGE banding patterns may be used as indicators of SDS incidence and aggressiveness in soybean fields. DGGE followed by Illumina sequencing of small ribosomal DNA (16S, 18S and ITS) will enable us to establish a bank of information about microorganisms communities structures and functions in soil, their interaction with *F. virguliforme* and their possible effect on the incidence of SDS.

***Homalodisca vitripennis* reovirus polymorphism validates timing and limited introduction of glassy-winged sharpshooter to California**
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Homalodisca vitripennis reovirus (HoVRV) is a phytoreovirus species infecting the glassy-winged sharpshooter (GWSS), an invasive insect introduced to California. Complete genome sequences of five Californian and four southeastern U. S. isolates of HoVRV were evaluated for polymorphism. Nucleotide sequence diversity was 10-fold less for HoVRV in California compared to HoVRV from the southeastern U. S. Phylogenetic analysis of each dsRNA segment indicated that the Californian isolates grouped as a monophyletic lineage. To sample diversity at single locations, dsRNA segment 11 was sequenced for nine additional isolates each from Riverside, CA and Johnston Co., NC. Whereas 9 of 10 Riverside isolates were identical (the tenth varied at one position), Johnston Co. isolates varied by up to 1.5%. Coalescent analyses estimated median population age at 11.6 to 26.3 years, with the most appropriate model (exponential growth) yielding a median age of 19.9 years. Estimates of median molecular clock rate for the Californian population translated to 0.4 to 1.4 substitutions/genome/year. Collectively, the results indicate that HoVRV diversity in the native range (southeastern U. S.) was high relative to a newly established population (California), and that the Californian population of HoVRV was subjected to a bottleneck coinciding with introduction of GWSS circa 1988.

Evolutionary history and species boundaries of the citrus brown spot fungus
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Traditional species concepts are difficult to apply to closely related, asexual taxa because of the lack of an active sexual phase and a paucity of morphological characters. Phylogenetic species concepts such as genealogical concordance phylogenetic species recognition (GCPSR) have been extensively used; however, methods that are able to incorporate uncertainty of gene trees into species tree estimation may more accurately and objectively delineate species groups. Using a worldwide sample of the citrus brown spot fungus (*Alternaria alternata sensu lato*), the evolutionary histories of an *endo-polygalacturonase* gene, two SCAR loci, and two microsatellite flanking regions were estimated using the coalescent. Species boundaries were compared using four methods: concatenation, GCPSR, and two methods that incorporate gene tree uncertainty, the "minimize deep coalescence" (MDC) and the Bayesian Estimation of Species Trees (BEST) methods. Coalescent analyses showed patterns of divergence influenced by incomplete lineage sorting and recombination among four phylogenetic lineages. Divergence of the citrus 2 lineage from the other lineages was well-supported with divergence times estimated between 1- 12 my before present, predating the migration of citrus from SE Asia. Two species were identified using concatenation and GCPSR, but only a single species was detected using BEST and MDC.

Virulence of Fusarium root-disease pathogens (*Fusarium oxysporum* and *F. commune*) to Douglas-fir (*Pseudotsuga menziesii*)
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Phytopathology 101:S171

Fusarium species can cause damping-off and root rot of young conifer seedlings, resulting in severe crop and economic losses in forest nurseries. Management of Fusarium disease in forest nurseries could be greatly enhanced by accurate identification of the *Fusarium* species, especially highly virulent isolates of *F. commune*. The primary objective of this study was to test the roles of *F. commune* and *F. oxysporum* in disease of Douglas-fir (*Pseudotsuga menziesii*) using unknown *Fusarium* isolates under *in vitro* and greenhouse conditions. *Fusarium* isolates were collected from healthy and diseased seedlings of Douglas-fir and western white pine (*Pinus monticola*) from a nursery in Idaho, U.S.A. *In vitro* and greenhouse virulence tests were completed on Douglas-fir germinants and seedlings. The virulence tests demonstrated that *F. commune* is a highly virulent pathogen, whereas *F. oxysporum* is mildly virulent to Douglas-fir germinants and seedlings. In addition, a species-specific diagnostic primer set was developed to detect and identify isolates of *F. commune*. With this information, nursery managers could more effectively deploy an appropriate disease-management strategy. This is the first report of direct evidence that *F. commune* can cause damping-off disease on Douglas-fir seedlings under greenhouse conditions.

Use of massively parallel sequencing as a diagnostic tool
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Current methods for the detection of unwanted pathogens in plants include hybridization probes. Strict physical requirements limit the number of probes used in a single test. To alleviate this limit, a new detection method is being developed, using massively parallel sequencing (MPS) for detection. In this method, pathogen-specific sequences are used in BLAST searches of a database of reads attained from a MPS run. Without physical limits, dozens of queries are possible for virus pathogens, and thousands for bacteria. Mock sample databases were generated to find optimal parameters for the searches. The databases were generated using a MPS simulation program, and contained both host and pathogen sequences. The proportion of pathogen reads in the databases was varied from 25% to <0.5%. Queries were designed with various lengths (20 – 140 nt). Three criteria were considered to find an optimal length; the number of queries generated of a given length, the number of hits per query, and the number of queries that received one or more hits. An optimal length of 60-80 nt was determined. A cut-off E-value of 1e-3 for calling hits was determined by looking at the true/false hit ratio. The specificity of this method was also tested by querying databases that include near neighbors to the target pathogens. The number of matches from these searches was similar to the number of matches found when querying a pathogen free database, as opposed to the target pathogen database.

Factors influencing efficacy of plastic shelters for control of bacterial blight of lilac

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Plastic shelters are thought to reduce bacterial blight by protecting plants from rain and/or frost. In February to April 2008 and 2009, we studied the contribution of frost protection to efficacy of this cultural control practice. Lilacs in 1-gallon pots were exposed to four treatments: 1) plants grown with no shelters, 2) plants grown under plastic shelters, 3) plants grown with no shelters, but placed under shelters when frost was predicted, and 4) plants grown under shelters, which were removed when frost was predicted. Freezing events were frequent (>20/season), but average low air temperatures did not differ significantly inside vs. outside of shelters. Plants with no shelters were exposed to frequent rain, whereas, plants under shelters remained dry. Populations of *P. syringae* exceeded 10^6 cfu/leaf on plants with no shelters vs. $<10^2$ cfu/leaf on plants under shelters. Bacterial blight symptoms were observed on >80% of the leaves of plants with no shelters vs. <34% of leaves of plants under shelters. Disease severity was similar between treatments 1 and 3, and between treatments 2 and 4, indicating that cover during frost events alone was not a major factor influencing efficacy. These results demonstrate that lilacs grown under plastic shelters exhibit few symptoms of bacterial blight and support low population sizes of *P. syringae*. Limiting free moisture on leaf surfaces appears to be important in the disease control provided by plastic shelters.

Fluxapyroxad: A new broad-spectrum fungicide

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Fluxapyroxad is a new broad-spectrum fungicide under development by BASF Corporation. Fluxapyroxad inhibits respiration of fungi by blocking production of succinate dehydrogenase and will be classified in FRAC group 7. It has both excellent preventative and curative activity through the inhibition of fungi at several stages of the fungal lifecycle including spore germination, germ tube growth, appresoria formation and mycelial growth. Research has demonstrated fluxapyroxad is highly active on several major plant pathogens from the Ascomycete, Basidiomycete, Deuteromycete and Zygomycete classes of fungi. It is effective for use on a wide variety of crops, including cereals, corn, soybean, fruiting vegetables, tuberous and corm vegetables, pome fruits and stone fruits with excellent crop safety. Research use rates where effective ranged from 45 – 200 g ai/ha. The compound has a favorable toxicological and ecotoxicological profile. The active ingredient trade name for fluxapyroxad is Xemium® Fungicide. EPA registration is expected in 2012.

Influence of rhizoctonia-bacterial root rot complex on storability of sugar beet

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The root rot complex, caused by *Rhizoctonia solani* and *Leuconostoc mesenteroides*, can lead to yield loss in the field but may also lead to problems with sucrose loss in storage. Thus, studies were conducted to investigate if placing sugar beet roots suffering from root rot together with healthy roots could compromise the ability of the healthy roots to retain sucrose. Over a 3 year period, root samples from 3 commercial cultivars were compared in storage as a healthy (8 healthy roots) or rotted (8 healthy roots + one rotted root) treatment inside an outdoor storage pile. The experiment was arranged as a split block (healthy in one half of block and rotted in the other) with the whole blocks arranged in a RCBD with 4 reps. Samples were retrieved from storage in Dec, Jan, and Feb and evaluated for discolored and frozen root area, weight loss, and sucrose reduction and recovery. When comparing the healthy to the rotted treatment over the 9 year × sampling date combinations, the Wilcoxon signed-rank test indicated the median change for discoloration (7% increase), frozen area (14% increase), sucrose loss (5% loss), and recoverable sucrose (689 kg/ha less or 8% reduction) were significantly different from zero (P = 0.008, 0.031, 0.007, and 0.008, respectively). These data indicate that the root rot complex not only leads to yield loss in the field but can also negatively affect neighboring healthy roots in storage leading to additional sucrose losses.

Seed treatment and drench with *Reynoutria* sp. in controlling damping off caused by *Rhizoctonia solani* and *Pythium ultimum* in soybean or cotton

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Regalia®, an extract of giant knotweed *Reynoutria sachalinensis*, induces resistance in plants by increasing production of phytoalexins and other compounds that are toxic to wide spectrum of pathogens. In this study, Regalia® was applied as seed coating or drenched in soil to control soilborne disease caused by *Rhizoctonia solani* or *Pythium ultimum* in soybean and cotton. Regalia® alone, or mixed with azoxystrobin (Quadris®), fludioxonil (Scholar®), or mefenoxam (RidomilGold®) were coated with Sepiret® 1171-O on soybean or cotton seeds. The treated seeds were seeded in soil infested with *R. solani* or *P. ultimum*. Results show that soybean or cotton seeds coated with Regalia® had greater or significantly greater emergence than that of the untreated control. Regalia® showed synergy when mixed with the synthetic fungicides. Drenching with Regalia® also significantly increased emergence and growth of soybean planted in soil infested with *R. solani*.

Diversity and distribution of Iris yellow spot virus (genus *Tospovirus*) infecting onion in Eastern Africa

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Iris yellow spot virus (IYSV; family *Bunyaviridae*, genus *Tospovirus*), transmitted by thrips (*Thrips tabaci* Lindeman), is an important viral pathogen of onion in many parts of the world. In Africa, IYSV was reported from South Africa, and Reunion Island. Our recent surveys found that IYSV is present in most onion-growing regions in Kenya and Uganda and its incidence ranged between 27.38 – 72.03%. IYSV was confirmed with specific DAS-ELISA tests and RT-PCR with IYSV specific primers followed by cloning and sequencing of the amplicons. The IYSV-Kenya isolate (HQ711616) had the highest nucleotide sequence identity (97%) with the corresponding region of IYSV-Sri Lanka isolate (GenBank # GU901211) and the IYSV-Indian isolates (EU310287 and EU310290). The IYSV-Uganda isolate (HQ711615) showed the highest nucleotide sequence identity (95%) with IYSV- Sri Lanka (GenBank # GU901211) and IYSV-India isolates (95% with EU310274 and EU310297). The maximum entropy method (Maxent2) was used to model the IYSV geographic distribution based on the Bioclimatic data layer from the WorldClim (www.worldclim.org) version 1.4 dataset and the presence of IYSV in Eastern Africa, South Africa and Reunion Island. Results from the Maxent2 showed that most of the regions in Eastern Africa and Ethiopia are conducive for IYSV occurrence and outbreaks.

A quantitative PCR assay for the detection of phytoplasmas causing almond brownline, peach yellow leafroll, and pear decline diseases in California

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Phytoplasmas cause several important diseases in California's almond and fruit tree orchards. A phytoplasma associated with almond brown line (ABL) disease, peach yellow leafroll phytoplasma (PYLR-P) and pear decline phytoplasma (PD-P) are genetically related based on homology in the 16S-23S rDNA spacer region. In an attempt to detect these three phytoplasmas, a

primer pair was designed to amplify a 530-bp product from the 16S-23S rDNA spacer region. Using this primer pair and SYBR Green, a quantitative PCR (qPCR) assay was developed to facilitate detection and analysis from multiple samples. A detection limit of 100 copies was achieved in assays using nucleic acid extracts from almond leaves spiked with cloned target DNA from PYLR-P. The qPCR assay detected PYLR-P in almond and peach trees, PD-phytoplasma in pear trees, but not a genetically different X-disease phytoplasma in cherry trees. This new assay was also able to detect PYLR-phytoplasma in dormant peach buds from infected trees and is expected to aid in the detection and amplification of ABL-P, PYLR-P and PD-P in almond, peach and pear trees, respectively.

Genetical, biological and pathological characters of Japanese potato strains of *Ralstonia solanacearum*

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Strains of potato bacterial wilt pathogen *Ralstonia solanacearum* (Rs) in Japan, were surveyed about biovar, phylotype, and DNA fingerprints (rep-PCR), and/or endoglucanase gene (egl) sequence. Rs were isolated from potato fields in southwestern, warm, temperate regions. Of the 188 isolates, 74 belonged to biovar N2 (39%), 44 to biovar 3 (24%), and 70 to biovar 4 (37%), respectively. Biovars N2 and 4 strains were widely distributed, from north (Hokkaido) to south (Okinawa) in Japan. Based on the results of multiplex-PCR analysis, every potato strains belonged to either phylotype I or IV. Phylotype I comprised both biovars 3 and 4 strains. On the other hand, phylotype IV included biovar N2 strains. None of the strains belonged to phylotype II or III as well as biovar 1 or 2. Phylogenetic analysis based on DNA fingerprints and endoglucanase gene sequences clarified the genetic diversity of the Japanese potato strains and the close genetic relationship between the Japanese strains and the Asian strains in phylotypes I and IV. When we inoculated these strains to potato, tomato, tobacco, and eggplant, the disease severity varied and depended on the Rs strains as well as the plants tested. Strains of phylotype IV, biovar N2 were weakly virulent or avirulent to eggplant (cv. Tikuyou), whereas strains of phylotype I, biovar 3 or 4 wilted the plants severely.

Pythium root rot of corn in Japan; unique symptom climb up the mature stem, and possible drift of the major species in causal *Pythium* flora

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Root rot of corn (*Zea mays*) caused by *Pythium* spp. is observed in many part of the world as a serious restriction factor of the plant production. In Japan, it has been recognized long as a disease caused thoroughly by *P. graminicola*, and known to develop into stalk rot like symptom in late maturing stage, which is not common with this fungus in other part of the world, maybe due to the climate condition unique to the country. In addition to its damage to seedlings to cause damping-off, the damage to the matured stalk is also troublesome especially in its whole crop forage use, as the plant may fall down, or becomes too soft for whole crop harvest and processing with machines as forage. Recently, another *Pythium* species, *P. arrhenomanes* has recognized to be causing the same symptom in Japan, and reported cases have been increasing across the country. The fungus has been reported only as a sugar cane pathogen of the southern semitropical part of the country, so that the increased reports with broader host range are calling for intense study. We have been seeking resistant cultivars for the pathogens, but host response to the same fungal strain in seedling stage and mature plant not the same all the time, which made a problem for screening. Among 19 forage corn cultivars/strains evaluated in field as matured plants and artificial soil inoculation as seedlings with *P. graminicola* in pots, only half of them showed similar response in field and pots for the pathogen.

Effect of soil amendment with seeds of Vernonia anthelmintica on soilborne diseases and growth of okra

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Seeds of Vernonia anthelmintica has great medicinal value and are traditionally used for the treatment of various diseases particularly worms infestation. In this study, water extract of seeds caused significant nematicidal effect by killing 2nd stage juveniles of Meloidogyne javanica in vitro. Soil amendment with Vernonia seeds alone or with Pseudomonas aeruginosa, the plant growth promoting rhizobacteria (PGPR) and Paecilomyces lilacinus, the egg parasite of root knot and cyst nematode showed significant suppressive effect against root rotting fungi Fusarium solani, F. oxysporum and root knot nematode M. javanica on okra (Abelmoschus esculentus) roots. In field plot experiment, soil amendment with Vernonia seeds or asafoetida, gum from Ferula assafoetida alone or with PGPR caused similar suppressive effect on soilborne pathogens. Vernonia seeds, asafoetida and PGPR caused positive impact on plant growth by producing taller plants and greater fresh shoot weight. However, mixed application of PGPR with Vernonia seeds or asafoetida resulted in highest yield as compared to other treatments. Soil amendment with Vernonia seeds seems to be a good alternate of chemical pesticides for managing the okra root diseases.

Detection of latent infection of wheat leaves caused by Puccinia striiformis f. sp. tritici using single- tube nested PCR

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Phytopathology 101:S173

Wheat stripe rust, caused by Puccinia striiformis f. sp. tritici (Pst), is one of the most important diseases of wheat worldwide. Early detection of latent infection of wheat seedling is critical to estimate initial inoculums potential of epidemics and effective control. In order to improve the sensitivity and facilitate the procedure of common nested PCR Two pairs of Pst species-specific primers including external and internal primer pairs with different annealing temperatures for single-tube nested PCR were designed according to the beta-tubulin gene sequence of Pst. The annealing temperature of external primer pairs was 12°C higher than the internal ones. Sensitivity test demonstrated that the single-tube nested PCR could detect as low as 20 fg template DNA and about 100 times more sensitive than the standard PCR. And latent infections from wheat seedlings could be detected as early as 24 h after inoculation. This study provides a rapid and simple method to detect and estimate latent infections level of seedlings in the field.

Fungal communities on strawberry roots and in soils amended with mustard meal (MM)

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Phytopathology 101:S173

Community analysis of soil and root fungal pathogens is critical for understanding pathogen ecology and the impact of MM amendments on pathogen communities. The dynamics of microbial communities was monitored in soils amended with MM or deactivated MM (dMM) and non-treated plots (C). Soil samples and roots were assessed in October (before planting), December (2 months after planting), March (early spring) and April (before bloom). Based on dilution plating, the populations of *Pythium*, *Fusarium*, *Rhizoctonia*, *Phytophthora*, and *Trichoderma* were not significantly different in soil before planting. At the Dec sampling populations of *Fusarium* and *Rhizoctonia* were not significantly different in the soil. However, the populations of *Pythium*, *Trichoderma* and fungi were higher in dMM [(8.7 × 104 CFU/gds (*Pythium*); 3.7 × 104 CFU/gds (*Trichoderma*); 6.2 × 106 CFU/gds (fungi)] than MM [(8.4 × 104 CFU/gds (*Pythium*); 4.3 × 103 CFU/gds (*Trichoderma*); 2.9 × 106 CFU/gds (fungi)] and C [(1.5 × 104 CFU/gds (*Pythium*); 1.7 × 103 CFU/gds (*Trichoderma*); 2.6 × 106 CFU/gds (fungi)]. At the same time, average *Fusarium* and *Rhizoctonia* colony numbers on strawberry roots in were higher in C and dMM than MM on strawberry roots. Denaturing gradient gel electrophoresis is in progress to characterize fungal diversity in soils and roots. Information about the biology and ecology of microbial communities will lead to informed root disease management strategies for growers.

Transcriptional regulation of complementary sense genes in gemini-viruses

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Geminiviruses of the genus *Begomovirus* express a multifunctional protein, AL2, which modulates metabolism, regulates transcription and suppresses RNA silencing. Analysis of promoter-reporter deletions has identified sequences necessary for expression of *Tomato golden mosaic virus* (TGMV) AL2 in *Nicotiana benthamiana* protoplasts. Transcription of AL1629 mRNA is dependent on binding of nuclear factors to a 9 bp viral sequence and mutations in the binding site result in a 2 to 6-fold reduction in RNA accumulation. We have isolated a full-length cDNA clone using a 20bp element within the TGMV *AL1629* promoter as a target in a yeast one-hybrid

screen. The cDNA encodes a member of the ethylene response transcription factor (ERF) family. ERF proteins can act as transcriptional activators or repressors, and this particular ERF is most closely related to the former. This is consistent with a role in activation of *AL1629* promoter. ERFs are ethylene-inducible DNA binding proteins that regulate responses to abiotic and biotic stresses, and have been described in tomato, rice, *N. tabacum* and *Arabidopsis*. Of relevance here, is that ethylene can increase either resistance or susceptibility to pathogens. Transgenic plants expressing tobacco (NtERF5) or tomato (Pti4 and Pti5) *erf* genes show enhanced resistance to *Tobacco mosaic virus* or *Pseudomonas syringae* respectively.

Temporal analysis of scab on four passion fruit varieties on brasilian cerrado

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The scab (*Cladosporium* spp.) is a harmful disease of the passion fruit vine (*Passiflora edulis* f. *flavicarpa*). It can occur in all aerial parts of the plant, including leaves, little branches, flowers and fruits. It is a typical young tissue illness causing clorosis in the leaves and can lead to the defoliation of the plant. The aim of this study was to evaluate the progress of the scabon the Gigante Amarelo, Sol do Cerrado, Ouro Vermelho e Rubi varieties of passion fruit on brasilian cerrado. Were installed four experimental plots containing three plants of each variety, spaced 2.0 × 4.0 meters in cordon training system. From the flowering were performed 15 evaluations for the incidence and severity of the scab on the leaves of one branch of each plant, with an interval of seven days between them. Were calculated the area under disease incidence (AUIPC) and under disease severity (AUSPC) progress curve, and was set a model for disease progress over the time. The varieties showed no significant differences between the AUIPC and AUSPC. The monomolecular model fitted better to the incidence and severity progress curves for all varieties and showed the highest coefficients of determination and lower residual mean squares.

Grapevines infected with powdery mildew emit specific volatile organic compounds that can be utilized for pathogen detection

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Plants are known to respond to attack by pathogens with emission of specific volatile organic compounds (VOC). These chemical signals may potentially serve as indicators of the presence of disease, thereby aiding in pathogen detection via biological or electrochemical sensing. Powdery mildew (PM), *Erysiphe necator* Schwein, of grapevines, *Vitis vinifera* L., is a ubiquitous pathogen infecting a valuable crop. In this pathosystem, early detection is difficult but can reduce necessary fungicide applications, proving economically and environmentally valuable. Rooted cuttings were inoculated with PM conidia via spore suspension and compared to uninfected cohorts in terms of emitted VOC over the course of three weeks. Each plant was completely sealed within a plastic tube, randomized with respect to treatment, and grown under PM-favorable environmental conditions. Headspace from within each tube was regularly sampled using polydimethylsiloxane-coated stir bars coupled with gas chromatography and time-of-flight mass spectrometry. Detected VOC differed between infected and uninfected plants, between sampling periods, and between infection / sampling period interaction levels. This description of temporal differences in VOC emission in response to pathogen infection may aid future in situ detection attempts. Specifically, it may be possible to detect indicator VOC in lieu of physical signs of the pathogen at early stages of infection.

The cryptic dimension of host-pathogen interactions: Physiological impacts of *Fusarium circinatum* infection on symptomless *Pinus radiata*

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Fusarium circinatum causes pitch canker, a damaging disease of pines worldwide. A typical symptom of the disease is shoot dieback, but the pathogen can also reside in soil and infect seedlings, which may die or remain symptomless. Nursery-grown trees that sustain latent infections provide a vehicle for pathogen dissemination and are prone to failure after out-planting. For these reasons, it is of interest to better understand the nature of the relationship between the pathogen and the host during the symptomless stage. To this end, we established symptomless infections in *Pinus radiata* seedlings in order to assess: 1) the impact on plant growth and root system structure, 2) the extent of root rot, 3) the frequency of pathogen isolation, 4) pathogen biomass based on Q-PCR, 5) longevity of the symptomless stage, and 6) impacts on plant response to water stress. Infected seedlings could remain symptomless for over one year. Infected (but symptomless) plants grew 30% faster than control seedlings, roots showed no evidence of rot but overall root system morphology was distinctly altered, with greater mycorrhizal root branching than control seedlings. Under drought conditions, infected plants wilted more rapidly than non-inoculated seedlings, suggesting a predisposition to water stress. This may help to explain a greater mortality risk for transplants that are cryptically infected by *F. circinatum* and suggests greater complexity of pathogen impacts in native forests.

Effect of *Puccinia emaculata* infection on ethanol production potential of *Panicum virgatum*

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Rust (*Puccinia emaculata*) infections have been reported for switchgrass (*Panicum virgatum*), a model biofuel crop thought to have few disease problems. Infection may reduce biomass and ethanol yield. This study examined the effect of varying degrees of rust infection on switchgrass ethanol yield. Naturally infected leaves from field-grown Alamo and Kanlow in Knoxville, TN were visually categorized as low (LD), medium (MD), or high (HD) disease based on degree of chlorosis, necrosis, and sporulation. Rust was isolated from select leaves to confirm infection. Vegetative (V) tillers were used for LD and reproductive (R) tillers for MD and HD. Leaves from V and R tillers of a disease-free, greenhouse-grown Alamo clone were used as controls. Leaves were dried, ground to 0.063 mm, acid/heat pretreated, and subjected to simultaneous saccharification and fermentation with *Saccharomyces cerevisiae* D_5A with two runs per material set. HPLC was used to assess ethanol yield. Ethanol yield differed significantly among disease levels within cultivars and between V and R stages. In run 1, V produced 19% less ethanol than R. MD had 33 and 36% less ethanol, and HD had 54 and 57% less ethanol than LD in Alamo and Kanlow respectively. In run 2, R produced 16% less ethanol than V. MD had 13 and 48% less ethanol, and HD had 26 and 60% less ethanol than LD in Alamo and Kanlow respectively. Ethanol yield loss in rust infected switchgrass may contribute to reduced economic value.

The mixed infections of peach trees by *Pseudomonas syringae* pathovars in Mazandaran Province, Iran

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Numerous samplings were made from peach trees suffering from old and chronic canker disease. Different suspensions were prepared from leaf spot, shot hole, shoot die back and canker symptoms. All sample suspensions were cultured on both KB and NA media. A complex of bacterial isolates with distinct morphological characteristics were collected during a long run isolation procedures. A total of 30 isolates were selected for further studies. Based on physiological and biochemical characteristics, RRIC–PCR patterns and pathogenicity tests on indicator plants and host seedlings, the most prevalent bacterium was identified as *Pseudomonas syringae* pv. *syringae*. Three of the isolates were found different from other isolates. These were further characterized. According to biolog GN2 kit and persicomycine test they were tentatively identified as *Pseudomonas syringae* pv. *persicae*. The incidence of the latter is new record for Iran.

Identification and characterization of fungal endophytes from a Greek tall fescue collection

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The Epichloae (*Epichlöe* and *Neotyphodium* species) are agriculturally important fungal symbionts that associate with cool season grasses. This association is known to confer several benefits to the plant host, including drought resistance and decreased herbivory due to secondary metabolites produced by the fungal partner. A tall fescue collection from Greece was evaluated for the presence of Epichloae to identify and characterize novel endophyte strains. Of the 88 lines investigated, 38 lines were infected with

Neotyphodium species. Pure cultures of each endophyte were obtained from infected tillers. Individual isolates were classified according to morphological characteristics, including growth rate, conidia morphology, and colony appearance. Isolates were also subjected to phylogenetic analyses of the *tefA* and *tubB* genes and the ITS region. The alkaloid potential of each isolate was determined using PCR. Results indicate that the Greek isolates are likely to produce some ergot alkaloids and/or indole-diterpenes *in planta*. Characterization of these secondary metabolism clusters will result in a greater insight and understanding into the evolution of the Epichloae.

Detection and distribution of Root-lesion nematodes (Pratylenchidae) on fruit trees in Northeast regions of Iran

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Root-lesion nematodes are considered as economically important nematodes which cause severe damage to fruit trees. They are always responsible for causing necrotic lesions on the roots. During April-October of 2009 to 2011, a total of 60 soil and root samples of different pome and stone fruits were collected from several gardens in Northeast regions of Iran. Nematodes were extracted from subsamples of 5 g of roots and 250 g of soil with a modification of the sugar centrifugal flotation method. For Identification, nematodes were transferred to pure glycerin and mounted on slides. Five species belonging to three genera from Pratylenchidae were identified based on morphological and morphometric characters. *Pratylenchoides ritteri* was isolated from Mulberry, Walnut, Fig, Apricot, Apple, Nectarine, Orange, Peach, Olive and Hazelnut trees, *Pratylenchus neglectus* isolated from Walnut, Quince, Plum, Apple and Olive trees, *Pratylenchus thornei* isolated from Walnut, Quince, Plum, Cherry, Orange, Apple, Persimmon, Nectarine, Hazelnut, Walnut, Fig and Olive trees, *Pratylenchus vulnus* isolated from Walnut and Fig trees, *Zygotylenchus guevarai* isolated from Quince and Fig trees. The most prevalent species were *Pratylenchoides ritteri* and *Pratylenchus thornei* with high population density through visited regions. Infected plants had roots with dark lesions, a symptom typical of the attacks by these nematodes. To our knowledge, this is the first report of lesion nematodes infecting fruit trees plants in Northeast of Iran.

A species-specific primer for detecting *Botryosphaeria dothidea*

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Botryosphaeria dothidea can attack a wide range of hosts. It causes apple ring rot, a severe disease in China. The objective of this study is to develop a fast method for detection and quantification of *B. dothidea*. A pair of primers (ipF and ipR) was designed base on the ITS region of rDNA and a PCR product of 347bp was amplified from DNA extracted from mycelia of *B. dothidea*, but not from that of *Colletotrichum gloeosporioides*, *Alternaria alternata*, *Marssonina mali*, *Monilinia fructigena*, *Venturia inaequalis*, *Botrytis cinerea* or *Valsa* sp., the commonly present species in apple orchards, or *Botryosphaeria ribis* the closely related species. As low as 1pg DNA was detected using conventional PCR assay. The PCR assay with this species-specific primer was capable of detecting the pathogen in infected apple fruit tissues. Subsequently, a more sensitive SYBR Green real-time PCR system was developed. The detection limit of the real-time PCR system was estimated as 100fg DNA, which is more efficient and accurate than the conventional PCR. A linear relationship between number of spores counted with compound microscope and the corresponding number of spores determined with the real-PCR assay was obtained and as low as 20 spores can be detected. This method may be used in quantifying spores in spore suspensions collected in the field for the purpose of disease monitoring.

Evidence of a low rate of seed transmission of Citrus tatter leaf virus in citrus

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Citrus tatter leaf virus (CTLV) (*Apple stem grooving virus*) is mechanically-transmitted in citrus, causing bud-union crease in trees budded on trifoliate orange and its hybrids, but is symptomless in most scions. Seed transmission of a strain of CTLV has been reported in *Lilium longiflora* and *Chenopodium quinoa*. In order to test whether CTLV is seed transmitted in citrus, seed was collected from four adjacent CTLV-infected citrus trees of different species, namely Clementine mandarin, Meyer lemon, Eureka lemon and Meiwa kumquat. The resulting 355 seedlings and the four parent trees were tested for CTLV presence by RT-PCR using three primer sets. The four parents and two of the 136 Eureka lemon seedlings, were found to be CTLV positive. This is the first report of CTLV seed transmission in citrus. Cloning and sequencing of the coat protein gene amplified using primer set TL1 showed that the sequence from the seedlings had an 89.7% homology with the parent tree, but 100% homology with the Meyer lemon and Meiwa kumquat trees, suggesting a filtering effect during transmission through the Eureka lemon parent as reported elsewhere.

Effect of temperature on bacterial leaf spot of *Phalaenopsis*, caused by *Acidovorax cattleyae*

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Bacterial leaf spot of orchid, caused by *Acidovorax cattleyae*, is common in south Florida nurseries. Little is known about the epidemiology of the disease. The effect of temperature on disease development was investigated using growth chamber studies. *Phalaenopsis* sp. orchids were wound inoculated with a cell suspension (10^9 spores/ml) of an isolate of *A. cattleyae*, then placed in humidity chambers at 15, 20, 25, 30, and 35°C with a 12-hour light/dark cycle. There were six inoculated plant replications and two non-inoculated controls at each temperature. Symptoms appeared after 14 days of incubation. Every 7 days, the number of lesions and the percent disease severity was measured for the top three leaves of each plant. At 28 days after inoculation, inoculated plants at 25, 30, and 35°C had significantly more average lesions per leaf than those incubated at 15°C, which developed bacterial spot symptoms. There was an increase in disease severity at 30 and 35°C compared to lower temperatures. There was a high incidence of naturally infected bacterial soft rot at the higher temperatures. From this data, we conclude that increased temperature increases the severity of bacterial leaf spot of orchid.

Microscopic observation of the interaction between the soybean sudden death syndrome pathogen and soybean cyst nematode, in soybean roots

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Fusarium virguliforme, sudden death syndrome of soybeans (SDS), and *Heterodera glycines*, the soybean cyst nematode (SCN), impair soybean production in the U.S. Infection by SCN affects severity of SDS symptoms, but the mechanisms behind this interaction are not well understood. Our objectives were to select an optimal inoculation method with both pathogens and to determine if SCN penetration sites serve as entry points for *F. virguliforme*. Three methods were compared on an SCN- and SDS-susceptible cultivar; infestation of sand-soil mix with SCN females and inoculations of roots with SCN eggs in pouches and in sterile sand. Roots were exposed to SCN from 2 to 20 days and sampled and stained every other day to assess SCN infection. A subset of plants was then inoculated with *F. virguliforme* and incubated at 27°C for 10 days. Infestation of sand-soil mix with SCN females resulted in more penetrated juveniles per unit root length at each sampling time. The most juveniles were observed in roots incubated with SCN for 8–10 days. In initial samples, *F. virguliforme* was found in proximity to SCN juveniles as well as in their absence. Further work is needed to clarify if *F. virguliforme* colonization is more abundant around SCN infection sites. SDS foliar symptoms were observed in plants inoculated with *F. virguliforme* after 14–20 days of SCN exposure, but not in the absence of SCN. This suggests that SCN facilitates penetration of *F. virguliforme* into the root vascular tissue.

Anthracnose disease of *Capsicum* spp.

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Anthracnose disease of *Capsicum annuum* (chili) is caused by a complex of *Colletotrichum* species with *C. truncatum*, *C. acutatum*, *C. gloeosporioides*, being the most severe pathogens. Elucidation of the disease cycle for *C. truncatum* indicated that seed infection and quiescent leaf infection were important sources of inoculum thus necessitating more efficient use of integrated disease management practices to prevent fruit infection. Taxonomy of the *Colletotrichum* spp was validated using three fungal gene sequences (ITS rDNA, partial β-tubulin; translation elongation factor 1-alpha) and species-specific microsatellite markers (STMS). Pathogenicity analysis of *C. truncatum/C. capsici* isolates collected from various hosts in Australia identified the existence of *formae specialis* subgroups that were host specific to soybean and custard apple. Differential reactions on mature green and ripe chili fruit of 10 genotypes of cultivated *Capsicum* spp identified five, 11 and

three pathotypes of *C. truncatum*, *C. gloeosporioides* and *C. acutatum* respectively. This will have profound effect on chili breeding programs where novel sources of resistance genes from related species are being incorporated into commercial *C. annuum* varieties. Putative PR genes have been identified through transcriptional analysis from a virulent pathotype of *C. truncatum* and an *Agrobacterium*-mediated fungal transformation system developed for assessing function of these genes.

High throughput screens reveal *Salmonella* behaviors required for persistence in tomatoes

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The outbreaks of vegetable-associated gastroenteritis suggest that enteric pathogens multiply and persist in plants for extended periods of time, eventually infecting people. The pathways which control how enterics colonize plants, are still poorly understood. To better understand interactions of *Salmonella enterica* sv. Typhimurium with tomatoes, a collection of deletion mutants and a *gfp*-tagged *Salmonella* promoter library were screened inside fruits. Dozens of constructs that were differentially regulated in tomato relative to *in vitro* growth were identified. The expression of these promoters was tested *in planta* using recombinase-based *in vivo* expression technology and fitness of the corresponding mutants was tested. Gene expression in *Salmonella* was affected by tomato genotype and maturity. For example, a *fadH* promoter was upregulated in immature tomatoes in response to linoleic acid. The regulation of *cysB* was activated in the fruit of cv. Hawaii 7997 (resistant to *Ralstonia solanacearum*) more strongly than in the varieties that are more susceptible to *Salmonella* proliferation. *Salmonella* motility and animal virulence genes (*hilA*, *flhDC*, *fliF* and those encoded on the pSLT virulence plasmid) did not contribute significantly to fitness inside tomatoes. Thus *Salmonella* relies on a distinct set of metabolic and regulatory genes, which are differentially regulated *in planta* in response to host genotype and fruit maturity.

Response of pepper (*Capsicum annuum*) genotypes to co-infection by *Phytophthora capsici* and *Meloidogyne incognita*

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Phytophthora capsici (PC), the causal agent of Phytophthora blight, and *Meloidogyne incognita* (MI), the southern root-knot nematode, are both important pathogens of pepper (*Capsicum annuum* L.) in the U.S. We studied the responses of five pepper genotypes with differing resistances to PC and MI to co-infection by both pathogens, with and without mandipropamid and oxamyl, in greenhouse tests. Pepper genotypes were CM-334, a serrano-type pepper (resistant to MI and PC), and 4 bell peppers: Charleston Belle (MI resistant); R4 (moderately high resistance to PC); Aristotle (moderately resistant to PC); and Jupiter (PC susceptible). CM-334 and Charleston Belle exhibited high resistance to MI (root galling range = 0% and 1%, respectively). Jupiter, Aristotle, and R4 were susceptible to MI (root galling range: 45% to 68%). Oxamyl treatment was effective in controlling MI in the bell genotypes (root galling range: 0% to 2%). CM-334 was resistant to PC with 7% root necrosis and 12% crown necrosis. All bell peppers were susceptible to PC (root necrosis range: 30% to 33% and crown necrosis range: 50% to 65%). Bell peppers treated with mandipropamid had significantly less (P = 0.0165) crown necrosis than non-treated plants (23% vs. 58%). Inoculation with MI did not affect Phytophthora blight symptom development in any genotypes. The results in this study indicate that MI does not have a significant effect on predisposing PC-resistant or susceptible pepper genotypes to Phytophthora blight.

Pre- and post-anthesis activity of fenbuconazole and triforine against blueberry flower infection by *Monilinia vaccinii-corymbosi*

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Monilinia vaccinii-corymbosi infects open blueberry flowers via stigma and style, causing mummy berry disease. Management of flower infection relies on multiple fungicide applications based on bloom progression. Since bloom can be highly protracted, however, flowers that open between applications remain prone to infection because the stigmatic surface is not exposed to active ingredient – unless there is systemic movement in the pistil and/or residual activity in the ovary. In greenhouse experiments with fungicide applications at defined flower stages and conidial inoculations conducted 1 day after anthesis, the systemic fungicides fenbuconazole and triforine provided excellent protection to newly opened flowers sprayed at anthesis, but differed in their pre-anthesis activity in unopened flowers. Fenbuconazole suppressed infection in unopened flowers treated 2.5 days pre-anthesis, whereas triforine provided substantial protection to earlier flower stages up to 7 days pre-anthesis. In a separate experiment to quantify post-infection activity, fenbuconazole and triforine significantly suppressed infection when applied within 2 and 6 days after anthesis, respectively. Thus, the total window of activity of fenbuconazole is only 4.5 days, whereas that of triforine is up to 13 days. To better manage flower infection by *M. vaccinii-corymbosi*, active ingredients such as triforine with superior systemic and/or residual activity in flowers need to be identified.

IR-4 Project fungicide registration on specialty crops update

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In 2010 and early 2011, the IR-4 Project obtained new uses of 5 fungicides on many specialty crops with a total of 147 new fungicide uses being registered through U.S. EPA. New fungicide registrations on food crops included cyazofamid, difenoconazole, flutolanil, fluazinam, and mancozeb. Azoxystrobin, fludioxonil, and difenoconazole were submitted for post-harvest use on potato to control silver scurf and Fusarium dry rot. IR-4 is developing data to verify that bacterial spot of pepper is controlled with low rates of acibenzolar without yield loss. Cyazofamid was submitted for use on basil downy mildew. Switch (cyprodinil + fludioxonil) was submitted for anthracnose control on pepper and spinach. Acibenzolar was submitted for use on strawberries to control angular leafspot. Metconazole was submitted for use on potatoes to control early blight. Mancozeb was labeled on additonal tropical fruit, ginseng, and the entire cucurbit crop group. Cyazofamid was labeled on brassica crops for downy mildew and club root control as well as downy mildew control on turnip greens, spinach, and hops, and white rust of spinach. Fluazinam was labeled on onions and lettuce for control of a number of diseases. Flutolanil was labeled for use on brassica crops for control of witestem caused by *Rhizoctonia* spp. Two developing crops, dragon-fruit (Pitaya) and wasabi, will soon obtain registrations of a number of fungicides:L chlorothalonil, azoxystrobin and Switch on dragon-fruit, and azoxystrobin on wasabi.

Tradeoffs between host adaptation and vector transmission of Soybean Dwarf Virus

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New emergent viruses must surmount critical limitations for transmission, infection, and replication in order to expand their host range. To evaluate the risk of indigenous clover strains of Soybean Dwarf Virus (SbDV) as an emerging plant pathogen threatening soybean production in the United States, the clover isolate SbDV-MD6 was serially transmitted from clover to soybean and pea by aphid vectors, *Nearctaphis bakeri* and *Acrythosipon pisum*, respectively. Genomic RNA sequence analysis of SbDV-MD6 following passages on soybean and pea identified 11 non-synonymous consistent mutations in soybean, and 4 mutations in pea. The d_N/d_S analysis indicated positive selection pressure on MD6 in soybean, but not in pea. Significantly increasing virus titers with each sequential transmission support this analysis. In the soybean line, virus titer in the third passage was approximately 100 times more than the first passage. However, transmission efficiency on soybean decreased with each passage from 53% to 0%, until the virus was no longer aphid transmissible. The level of intra-host genetic diversity of virus populations in a single infected plant increased from 0.08% on average to 0.11% after reaching equilibrium in the new host. Results indicated that the clover strain of SbDV-MD6 adapted readily to soybean by improved replication and/or movement, but selection for host adaptation created tradeoff factors decreasing host-to-host transmissibility by aphid vectors.

Study on molecular targets of hydrogen peroxide in fungal pathogen mitochondria under oxidative stress

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We mainly investigate the mechansitic process at the proteomic level in Penicillium expansum, by identifying the molecular targets of H2O2 that is frequently used as a response of the host cells. Then, we performed mitochondrial sub-proteomic analysis to seek the molecular targets of H2O2. A set of mitochondrial proteins were identified, including respiratory chain complexes I and III, F1F0 ATP synthase, and mitochondrial phosphate carrier protein. The functions of selected proteins were further investigated to determine their effects on the H2O2-induced cell death. Through fluorescent

co-localization and the use of specific inhibitor, we provide evidences that complex III of the mitochondrial respiratory chain contributes to ROS generation in mitochondria under H2O2 stress. The undesirable accumulation of ROS caused oxidative damage of mitochondrial proteins and led to the collapse of mitochondrial membrane potential. Additionally, we prove that ATP synthase was involved in the response of fungal pathogen to oxidative stress, because inhibition of ATP synthase by oligomycin decreased cell survival. The results suggest that mitochondrial impairment due to functional alteration of specific proteins is associated with cell death of fungal pathogen caused by H2O2. The identification of mitochondrial targets can provide a basis for future development of novel antifungal agents.

Effect of environmental conditions and lesion age on sporulation of *Phytophthora ramorum* on California bay, rhododendron, and camellia
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Phytophthora ramorum is the causal agent of the disease known as Sudden Oak Death (SOD). The objective of our research was to determine the environmental conditions and lesion age favorable for *Phytophthora ramorum* sporulation under field conditions. For two years, new camellia, rhododendron, California bay (Umbellaria californica) nursery stock were seasonally inoculated (every 3 months) on foliage. They were covered overhead to prevent rainfall from falling on the plants, but otherwise the plants were completely open to the natural environment. Consistent leaf wetness periods were produced with overhead misting systems and controlling sensors to simulate rainfall, fog, dew, or other conditions that might be supportive of sporulation in an irrigated nursery or landscape. For each season, these wetness conditions began when leaf lesions were 3, 6 and 9 weeks old and, at each of these time points, the wetness conditions were maintained for 8 days. Sporulation was evaluated by washing leaf lesions before the wet period began and at 1, 2, 4 and 8 days during the wet period. Leaf wetness and temperature were measured near the plants. For rhododendron, a Poisson regression model demonstrated that sporulation decreased with increasing lesion age. Sporulation increased with increasing consecutive hours of leaf wetness up to about 48 hours. Sporulation decreased with higher maximum temperatures. Sporulation often occurred when leaves were coated with naturally forming dew.

Characterization of *Pythium nunn* newly recorded in Japan on antagonistic activity against *P. ultimum* and *P. aphanidermatum*
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Pythium nunn is a potential biocontrol agent first recorded from a grassland soil in Colorado, U.S.A. This species, which has never been recorded as a plant pathogen, efficiently suppresses several soilborne diseases. *P. nunn* was recently first recorded in Japan and was characterized by its antagonistic activity against *P. ultimum* var. *ultimum* and *P. aphanidermatum*. Three *P. nunn* isolates were obtained from soils in Nagano, Osaka and Fukuoka prefectures. The morphology of all isolates corresponded with those of the original description of *P. nunn*. The rDNA-ITS sequences of the three isolates were identical to each other and had a high similarity with the sequences of the type strain of *P. nunn*. The *P. nunn* isolates were mycoparasitic toward *P. ultimum* var. *ultimum* and *P. aphanidermatum*. They suppressed damping off of cucumber caused by *P. ultimum* var. *ultimum* and damping off of creeping bentgrass caused by *P. aphanidermatum* at an early growth stage of the plants.

Effect of barley chromosome addition to wheat on the preference and performance of the migratory locust *Locusta migratoria* (Orthoptera: Acrididae)
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The migratory locust *Locusta migratoria* exhibits density-dependent phase polyphenism and is potentially one of the most destructive agricultural pests worldwide. The locust feeds on various Poaceae such as wheat, but does not eat barley. Identification of the barley genes inhibiting feeding by the locust is useful for developing resistant crops. Using six barley chromosome disomic addition lines of wheat (2H-7H), we investigate the effects of barley chromosome addition to wheat on the preference and performance of *L. migratoria*. The locomotor activities of hatchlings given seedlings of wheat and 2H-7H were not significantly different one another, but lower than those of hatchlings given barley. Feeding preference by locust hatchlings was investigated by choice tests in which hatchlings were given wheat and one of the chromosome addition lines for 24 hours. The mean amount of wheat consumed by hatchlings was significantly larger than that of 2H, 5H, and 6H consumed. Growth performance of the hatchlings fed with wheat, barley, or 2H-7H was assessed by examining their survival rate and developmental period. The duration of the first instar for hatchlings given 2H or barley was significantly prolonged than that for those given wheat or other addition lines. These results suggest that the barley genes involved in the inhibition of feeding by locusts exist on barley chromosomes 2, 5, and 6, and those causing slow development of the locust on barley chromosome 2.

From bacteriosis of the fall webworm *Hyphantria cunea* to development of bio-insecticide based on *Bacillus thuringiensis* in Kazakhstan
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The fall webworm (*Hyphantria cunea* Drury) is a quarantine pest for Kazakhstan. Its mass invasion of tree plantations was discovered in 2003 in southeast Kazakhstan. In 2004 its nests with dead larvae were found on a maple tree (*Acer negundo* L.) with sign of bacterial infection. Septicemia was caused by crystal-forming bacteria *Bacillus thuringiensis var. kurstaki*. Strain 2127-3k with high production of parasporal endotoxin-crystals was derived from the R-form of *B. thuringiensis*. The mortality of the larvae after infection with *B. thuringiensis* strain 2127-3k was 3.8 times higher than with strain Z-52 of the Russian commercial bio-insecticide "Lepidocide" used as a control. In 2009, based on *B. thuringiensis* strain 2127-3k, the first local bio-insecticide "Ak Kobelek" was registered in Kazakhstan against a broad range of Lepidopterous pests.

Host specificity of *Cochliobolus* sp., a new pathogen of warm-season turfgrasses
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A new foliar disease was initially observed on bermudagrass (*Cynodon dactylon*) and zoysiagrass (*Zoysia japonica*) putting greens and fairways at golf courses in the Houston, Texas area in 2007. Disease symptoms on individual leaves exhibited prominent, black, elliptical lesions along the leaf margins. Symptoms on the closely mowed turfgrass appeared as dark, brownish-black spots 5-cm in diameter. Isolates of *Cochliobolus* sp. were consistently recovered from field samples and Koch's postulates were conducted to confirm pathogenicity. A greenhouse study was conducted to determine the host range of these isolates. Surface disinfested seed of bermudagrass, zoysiagrass, centipedegrass (*Eremochloa ophiuroides*), and seashore paspalum (*Paspalum vaginatum*) were sown into *Cochliobolus*-infested soil contained in 5-cm pots. St. Augustinegrass (*Stanotaphrum secundatum*), propagated from stolons, was also tested as a possible host. Disease severity was greatest in zoysiagrass which appeared scorched or desiccated. Leaf symptoms consisted of elliptical lesions with gray, necrotic centers and black margins. Bermudgrass and St. Augustinegrass developed foliar lesions but severity was low. Centipedegrass and seashore paspalum only had a reduction in seedling establishment. *Cochliobolus* sp. was re-isolated from all hosts except seashore paspalum. The fungus did not cause root discoloration in any of the hosts indicating that it is only pathogenic on foliar tissues of select warm-season turfgrasses.

Efficacy of spring fenarimol applications for spring dead spot control in a Tifway bermudagrass fairway in Mississippi
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Spring dead spot, caused by *Ophiosphaerella korrae*, is an annual disease of bermudagrasses that undergo dormancy. A study was conducted on a golf course with a Tifway bermudagrass fairway in West Point, MS to evaluate the effectiveness of spring fenarimol fungicide treatments and fertilizer for controlling this disease. Treatments served as the main plot factor and included fenarimol (spring, fall, spring followed by fall), propiconazole, myclobutanil, and azoxystrobin (all fall), and a check. Fertilizer nitrogen source (sub-plot factor) was applied as an organic or inorganic (ammonium sulfate) 12N-2P-12K at 0.5 N kg per 93 m^2. Spring dead spot severity and percent disease were visually assessed in April, 2009 and 2010. Turfgrass quality was rated monthly and spring green-up was recorded in March of both years. The fenarimol, spring only treatment, was equally effective, based on SDS severity, as fall treatments of fenarimol, propiconazole, myclobutanil, and azoxystrobin. The nitrogen source did not influence spring dead spot control. Turfgrass quality and spring green-up were similar for all treatments.

An advantage of a spring fungicide application is that only areas where the disease is active and exhibiting SDS symptoms are treated. This allows the superintendent to make sight-specific fungicide applications on fairways that may in turn be more cost effective as well as provide an acceptable level of spring dead spot control.

Micro-biota associated with wild and cultivated strawberry and their potential use as biological control agents for strawberry black root rot

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Black Root Rot (BRR) is a disease complex on strawberry conferred by one or more organisms including Pythium, Fusarium, Rhizoctonia spp. and several species of nematodes. Eighty percent of strawberry acreage is pre-plant fumigated with methyl bromide. The pending elimination of MB use concerns strawberry growers and has stimulated the search and evaluation of ecologically-based strategies for the management of BRR. Project objectives are to survey, isolate and identify microorganisms present in the rhizosphere and endosphere of wild (Fragaria virginiana) and cultivated strawberry (F. x ananassa). Microbiological and molecular techniques were used to determine the microbiota present in roots, crowns and rhizosperic soil of 5 different populations of strawberry (2 nurseries, 2 wild and 1 organic system). The diversity of pathogenic fungi as well as bacteria and fungi populations differed in each plant source. The average CFU gr-1 dry soil of Pythium was 288 on cultivated strawberry soil, versus 2268 on wild strawberry's. For Rhizoctonia, 6281 on cultivated versus 11602 on wild. For general fungi, 37543 on cultivated and 172810 on wild strawberry soil. Despite of this, no disease symptoms were found on wild strawberries. Over 150 isolates have been obtained including potential beneficial Trichoderma spp. and Paecilomyces lilacinus. Further evaluations will be made to determine the role of this and other beneficials and their use in an integrated disease management system for strawberry BRR management.

Clonal and sexual dispersal of *Armillaria mellea* in an ornamental landscape

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High densities of planted hosts and frequent irrigation have contributed to severe Armillaria root disease in Golden Gate Park, San Francisco, CA. Our objective was to assess the relative contribution of vegetative growth and basidiospore dispersal to the colonization of the park by *Armillaria mellea*. We investigated the genetic structure of *A. mellea* at a fine spatial scale using microsatellite data. Ninety-five unique multilocus genotypes were identified among 166 isolates. Only 28 genotypes (29%) were shared by two or more isolates (clones). The largest two clones, resulting from vegetative growth of one genotype, measured 216 m and 322 m. Spatial autocorrelograms of kinship coefficients, with and without clones, converged at an average distance of 130 m, indicating that this distance constitutes the linear spatial dimension above which clonality does not affect the genetic structure of the population. Up to 100 m, genetic similarity between pairs of isolates decreased linearly with an increase in spatial distance. Beyond 100 m, a random spatial distribution of genotypes was observed, consistent with an establishment from sexual spores from distant sources. The absence of multilocus linkage disequilibrium and the high proportions of genotypes detected only once suggest that most infections in the park resulted from basidiospores. However, 29% of genotypes infected multiple trees as a result of subterranean, vegetative growth.

Use of *Datura stramonium* and *Nicotiana benthamiana* to study Acibenzolar-S-Methyl-induced SAR against Iris yellow spot virus (genus *Tospovirus*)

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Acibenzolar-S-Methyl (ASM) is a functional analog of salicylic acid (SA) that activates plant's systemic acquired resistance (SAR) response against a wide variety of pathogens including bacteria, fungi and viruses. ASM has been shown to induce SAR against *Tomato spotted wilt virus* (TSWV) in *Nicotiana tabacum* and other hosts. Iris yellow spot virus (IYSV) is an economically important tospovirus of onion. We are using *Datura stramonium* and tobacco *Nicotiana benthamiana* as model plants to develop a set of descriptors to study the effect of SAR inducers on IYSV-host interactions. ASM- and buffer-treated *D. Stramonium* and *N. benthamiana* plants were mechanically inoculated with IYSV. Symptom development and virus levels were monitored. After 10–14 days post inoculation, leaves were tested for IYSV by ELISA. Significant reduction in virus levels in the ASM-treated plants was noticed. The ELISA results were confirmed by RT-PCR. The level of SAR response was assessed by measuring the lesion sizes on the systemically infected leaves of the ASM-treated plants. ASM-treated plants showed reduced viral symptoms compared to buffer-treated plants. Our results suggest that both *N. benthamiana* and *D. stramonium* can be used efficiently to study the effect of SAR inducers on IYSV. These model plant systems also facilitate studies to quantify the levels of various IYSV genes during the ASM-induced plant defense response.

Adapting synthetic gene circuits for plant-based detection of pathogen indicators: A test case

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Recently the principles of synthetic biology were applied to the development of transgenic detector plants that visibly de-green or turn white in the presence of specific chemical compounds. The strategy required three components: a receptor that relays a signal in the presence of a chemical input, a response-relay protein that carries the signal to the nucleus, and genes activated in response to the signal (i.e., encoding a de-greening response gene circuit or luciferase reporters). We are adapting this strategy to develop the first generation of plants that detect and respond to small molecules secreted by bacterial plant pathogens. *Xylella fastidiosa*, a devastating quarantine pathogen of citrus and grape, secretes a small fatty acid diffusible signal factor (Xf-DSF). We designed a synthetic receptor that triggers the response relay circuit in the presence of Xf-DSF. In a bacterial reporter system, the synthetic DSF receptor elicited a measurable response to nanomolar concentrations of a synthetic Xf-DSF analogue, as well as to crude extracts of *X. fastidiosa* culture supernatants. The receptor is more responsive to fatty acid signals made by *X. fastidiosa* than those of *Xanthomonas* species. *Arabidopsis* lines expressing the receptor exhibited an increase in reporter gene activity in response to infiltration with DSF. These efforts represent the first steps toward the application of plant synthetic biology to detection of pathogens.

Effect of Huanglongbing on the structure and functional diversity of microbial communities associated with citrus rhizosphere

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The diversity and stability of the rhizosphere bacterial community heavily influence plant productivity and ecosystem sustainability. The goal of the study was to understand the influence of 'Candidatus Liberibacter asiaticus' (known to cause Huanglongbing, HLB) on the structure and functional potential of microbial communities associated with citrus rhizosphere. The results of clone library sequencing and quantitative real-time PCR revealed that 'Ca. L asiaticus' infection re-structured the native microbial community of citrus rhizosphere. Geochip 3.0 used to determine the effect of 'Ca. L. asiaticus' infection on the functional diversity of rhizophere microbial communities showed that HLB disease has significant effects on various functional guilds of bacteria. Many genes involved in key ecological processes such as nitrogen cycling, carbon fixation, phosphorus utilization were significantly greater in healthy as compared to HLB diseased citrus rhizosphere. HLB infection also caused shifts in the carbon utilization patterns of rhizosphere microbial community. Overall our study provides evidence that change in plant physiology mediated by 'Ca. L. asiaticus' infection could elicit shifts in the composition and functional potential of rhizosphere microbial communities. Our results indicate that plant diseases not only affect plant productivity but also cause disturbances in ecosystem equilibrium.

Effect of pre-sowing soil incorporated treatments on *Alternaria radicina* in carrot *Daucus carota*

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Alternaria radicina, a seed- and soil-borne pathogen of carrot can cause problems for carrot seed producers. As the pathogen is present in New Zealand soils, the pre-sowing soil incorporation of potential control products

was investigated in a glasshouse study. Four fungicides (difenoconozole, difenoconozole + chlorothalonil, iprodine and pyraclostrobin), three fumigants (formaldehyde, dichloropropene + chloropicrin, and metam sodium) and two biocontrol products (*Trichoderma atroviride* and *T. harzianum*) were incorporated at recommended label rates into soil which contained 250 CFUs/g of the pathogen. Two carrot varieties were sown 15 days later and emergence recorded at 15 days after sowing. *A. radicina* soil population density was determined at 0, 4, 16 and 32 weeks after application using a soil dilution plating method. Carrot emergence did not differ between the varieties, and emergence for all fungicide treatments and formaldehyde did not differ from that of the uninoculated control. *T. atroviridae* significantly increased emergence over the inoculated control. All chemical treatments had reduced the *A. radicina* soil population to between 10 and 100 CFUs/g by 4WAA, and by 32WAA they were between 50 and 150 CFUs/g depending on the treatment. The biocontrol products took 16 weeks to significantly reduce the pathogen population (to ca. 180 CFUs/g) and by 32 weeks had reduced it to ca. 150 CFUs/g. Whether this reduction would have continued is worthy of further investigation, as is field validation.

Calosphaeria canker of sweet cherry in California

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California is the second largest sweet cherry producer in the United States, with annual revenues up to $200 million. Canker diseases and associated branch dieback are responsible for extensive damage throughout California's sweet cherry orchards, reducing annual yields and tree longevity. Recent surveys and isolation work identified *Calosphaeria pulchella*, *Eutypa lata* and *Leucostoma persoonii* (Syn: *Cytospora leucostoma*) as the main fungi associated with branch dieback of sweet cherry in California. The most prevalent pathogen, *C. pulchella*, was isolated from nearly all of the surveyed orchards. Pathogenicity studies showed that *C. pulchella* operates as a primary pathogen of sweet cherry, infecting healthy tissue through wounds and subsequently causing cankers in the wood. Spore trapping studies conducted in two orchards near Davis and Linden showed that rain as well as sprinkler irrigation water were important factors for aerial dissemination of the ascospores of *C. pulchella*, which constitute the main inoculum. Plant stress provoked by excessive irrigation and extensive pruning are suspected to constitute additional factors that contribute to the expression of the disease. Pruning of cherry trees during summer when inoculum availability is low combined with irrigation practices which do not wet trees should diminish the risk of pruning wound infections with *C. pulchella*.

Diversity and population dynamics of *Xanthomonas axonopodis* pv. *manihotis* in Colombia from 2008 to 2010

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Phytopathology 101:S179

*Xanthomonas axonopodi*s pv manihotis (*Xam*) is the causal agent of cassava bacterial blight and the most important bacterial problem in this crop. In Colombia, processes of pathogen migration were detected between regions in the 90's with a concomitant high diversity index. With the purpose of characterizing the current population structure of the pathogen, sampling collections were carried out from September of 2008 until November 2010. Bacterial isolates were characterized using AFLPs then clustering analysis allowed to identify geographical distribution patterns. Additionally, genes coding for the Type Three Effectors (T3E) were sequenced to establish their degree of variability and to assess the presence and nature of selection exerted by the host. The results confirmed a prominent diversity of the pathogen on the Colombian Caribbean coast and haplotype migration through time. On the other hand, a low variation was detected on the T3E genes, possibly indicating their importance in pathogenesis. However, additional T3E genes are been sequenced in order to confirm these observations. This study shows the current condition of populations of Xam in Colombia and it will contribute to the generation of measurements to manage bacterial blight.

Survey and screening of classical biological control agents for Japanese knotweed (*Fallopia japonica*)

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Phytopathology 101:S179

Japanese knotweed (*Fallopia japonica*, Polygonaceae) is a serious invasive weed in the UK, North America and large parts of Europe where there is an urgent need for classical biological control (CBC) strategy. Surveys have confirmed the absence of any significant natural enemy pressure in the UK, and the presence of an extensive guild of specialized natural enemies in the native range of Japan. Among these, although a psyllid, *Aphalara itadori*, has been approved ahead for release in the UK, the plant pathogens still remain as potential CBC agents for use against *F. japonica*. The results of preliminary surveys showed that three fungal diseases of two rusts and a *Mycosphaerella* leaf spot were predominantly common and widespread in the fields. These rusts were identified as *Puccinia polygoni-amphibii* var. *tovariae* and *Aecidium polygoni-cuspidati*, and confirmed their severe infection to *F. japonica* both in the field and laboratory studies. However, it suggested that they were heteroecious rusts pathogenic to the non-target species, indicating to be eliminated from the prior agents of CBC ones. On the other hand, a leaf spot fungus morphologically identified as *Mycosphaerella polygoni-cuspidati* caused severe damaging disease of *F. japonica*. Additionally, endophytic fungi associated with *M. polygoni-cuspidati* promoted the disease severity. In conclusion, developments of new strategy for integrated CBC of invasive weeds are suggested.

Effect of fungicide programs on white rot of garlic in central California

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Phytopathology 101:S179

Sclerotia of *Sclerotium cepivorum*, the fungus that causes white rot in onion and garlic, are capable of survival in the soil for decades. In Central California production areas, once the disease is detected, the field is no longer used for production of allium crops, and at least 5,900 ha are currently infested. Adoption of drip irrigation in these crops presents an opportunity for application of pest control materials. In this study conducted in a naturally infested field in western Fresno County California, fungicide programs including applications in the planting furrow and injected into the drip irrigation system were evaluated from 2008 to 2010. Treatments consisted of 2 to 4 applications through the drip irrigation system. Materials applied first were either tebuconizole or fludioxonil, rotated with the other followed by 1 or 2 applications of boscalid. In subplots, the same three materials were tested as applied immediately before planting into the trench. As compared to untreated controls, the at-planting applications reduced disease levels by an average of 30, 86 and 42 percent, in 2008, 2009 and 2010, respectively. There were no differences detected between the 3 fungicides tested. Drip irrigation applications yielded no benefit as compared to the untreated control. Potential of 3 fungicides in managing white rot when applied at planting were documented in central California, but drip applications were consistently ineffective.

Projected distribution and severity of clubroot of canola in the Canadian prairies

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Clubroot of canola, caused by *Plasmodiophora brassicae* Woronin, was first reported in the St. Albert region of Alberta, Canada in 2003 and has subsequently appeared over a broad area around Edmonton, AB. The simulation program CLIMEX® was used to model potential distribution and severity of clubroot of canola in the Canadian prairie region under: 1) current climate using long-term climate normal data (LCND); 2) incremental temperature and precipitation scenarios; and 3) an irrigation scenario. Initial projections of clubroot based on LCND were consistent with observations on cruciferous vegetables in the lower mainland of British Columbia and central Canada, and canola in Alberta. The model suggested clubroot could affect canola over a wide area of the prairies, especially wetter regions. Incremental temperature increases of 1 to 3°C resulted in an expansion of the area potentially affected by clubroot. A scenario of 120% of normal rainfall during the growing season resulted in greater projected clubroot distribution and severity, compared to incremental temperature increases. Incremental decreases in rainfall resulted in substantial reductions in the projected distribution and severity of clubroot. Irrigation may compensate for dryer conditions in southern Alberta, resulting in the development of clubroot. A proactive approach to extension and research is recommended for projected 'at risk areas' to limit the potential impact of clubroot on dryland and irrigated canola.

The impact of fungicide and herbicide timing on barley leaf disease severity, weed management and crop productivity

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Field trials were conducted in 2010 to determine the efficacy of tank mixing herbicides and fungicides on leaf disease and weed management, and barley grain yield. Combinations of the herbicide Axial® (pinoxaden) and the fungicide Tilt® (propiconazole) were applied to malting barley cv. AC Metcalfe at the 2-3 leaf stage, 5-6 leaf stage, or the flag leaf stage at three experimental sites (Lacombe, AB, and Melfort and Scott, SK). Prior to seeding of the barley, the plot area was cross-seeded with tame oat as a model weed. Penultimate leaf samples were collected for assessment of leaf disease severity, while weed biomass was also assessed. Plots were harvested and grain yield and kernel quality assessed. Penultimate leaf disease severity was significantly higher for the 2-3 or 5-6 leaf stage no fungicide, herbicide only treatments and the combination herbicide and half rate fungicide treatments at the 2-3 or 5-6 leaf stage compared to all other treatments. Yield and thousand kernel weight tended to be highest for those treatments where the fungicide treatment included a flag leaf stage application. Model weed biomass was very low and generally not influenced by the treatments due to effective herbicide applications at each of the sites. Preliminary results suggest that for malt barley, fungicide applications should include a flag leaf stage timing to ensure protection of upper canopy leaves, thus contributing to enhanced yield and grain filling. The experiment will continue for two more years.

Prevalence and aggressiveness of *Alternaria solani* and *A. alternata* on potato in the Columbia Basin of the Pacific Northwest

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Phytopathology 101:S180

Early blight and brown spot on potato are caused by different species of *Alternaria*. *A. solani*, a well-documented pathogen on potato and the cause of early blight, forms tan-colored lesions that have large concentric rings. Lesions of brown spot, caused by *A. alternata*, tend to be smaller and darker in color, but can be numerous on a leaf. *A. alternata* was not described as a pathogen on potato until the 1980s. In 2009, 214 isolates of *Alternaria* were isolated from leaves with lesions from 22 potato fields in the Columbia Basin. In 2010, 163 isolates of *Alternaria* were collected from 20 fields. The average frequency of isolation for *A. solani* vs. *A. alternata* between 6/09 and 8/09 was 9% and 1%, respectively, but after 8/25/09 it was 15% vs. 71%, respectively. In 2010, *A. solani* was isolated less frequently (18%) than *A. alternata* (75%) throughout the season except on 7/3/10. Pathogenicity and aggressiveness assays using 34 isolates of both species collected in 2009 were performed on detached Russet Norkotah leaves. All 17 isolates of *A. solani* (100%) resulted in lesions whereas 53% (9 of 17) isolates of *A. alternata* caused lesions. Early blight lesions enlarged more rapidly than brown spot lesions, indicating greater aggressiveness on potato by *A. solani*. The differences in prevalence and aggressiveness of these two pathogens will likely impact the timing of their management in the region.

The use of arthrospore formulation of antagonistic *Streptomyces* for the control of diseases caused by *Phytophthora* species

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Phytopathology 101:S180

The diseases caused by *Phytophthora* spp. have been long time serious problem for the cultivation of various important crops worldwide. For the disease control, antagonistic *Streptomyces* strains with superior competence of sporulation in submerged culture were isolated. An intellectual proprietary pilot scale (up to 750L) fermentation technology which yield well suspended arthrospore formulation at more than 10^{10} cfu/mL was established. The effectiveness of the attempted biofungicide on disease control was demonstrated on late blight of tomato caused by *Phytophthora infestans* as well as foot rot and gummosis of citrus caused by *P. palmivora*. The success of disease control depends greatly upon mycoparasitism of the biocontrol agent. For field grown tomato spray treated with the biocontrol agent, the mycelia and zoosporangia of *P. infestans* on the existing lesions were parasitized and killed; and the expansion and spread of the lesion was inhibited. Likewise, for field grown citrus shown declined growth and gummosis symptoms typical of *Phytophthora* infection, a coverage treatment with cellulosic nonwoven tape pre-soaked with the arthrospore formulation resulted in healing up of the infected tissue and restored growth vigor. Accumulated evidence indicated clearly great potential for the use of antagonistic *Streptomyces* as microbial biofungicide for the control of diseases caused by *Phytophthora* species.

Distribution, pathogenicity, and molecular analysis of *Puccinia psidii* in Hawaii

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Ohia rust caused by *Puccinia psidii* was first discovered on the island of Oahu in 2005 and is now found infecting Myrtacea on all of the major Hawaiian Islands. The genus of susceptible hosts include five *Syzygium*, five *Eugeniea* including the highly endangered *Eugenia koolauensis* (nioi), four *Metrosideros*, and one each from genus *Callistemon*, *Chamelaucium*, *Melaleuca*, *Myrciaria*, *Myrtus*, *Pimenta*, and *Rhodomyrtus*. In pathogenicity tests, *M. polymorpha*, *E. koolauensis*, *S. jambos*, *S. samarangense*, and *P. dioica*, all react to *Puccunia psidii* spores from *S. jambos* in the same severe manner. Plants are nearly killed with many urediniospores. On a few species, visual variation can be attributed to the host. This is seen for *S. paniculatum* and *R. tomentosa*. Molecular analysis of urediniospores collected from various hosts indicate that of the 15 *P. psidii* genotypes, the rust found in Hawaii is a single strain and is similar to one found in Florida and from an intercept of infected myrtle from California.

Dissemination, incidence and severity of *Leifsonia xyli* subsp. *xyli* in sugarcane of Sao Paulo state, Brazil

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Sugarcane is the third most important cash crop in Brazil with 8.03 million hectares and production of 624.99 million ton. Despite its importance, research on sugarcane pathology stands far behind. Little information is available on ratoon stunting disease (RSD), caused by *Leifsonia xyli* subsp *xyli* (Lxx), the most important disease of sugarcane worldwide. Therefore, the objective of the present work was to examine the dissemination, incidence and severity of Lxx among sugarcane varieties in 2009 and 2010. Sap of 100 stalks from each field was sent by mills to the laboratory for a routine RSD analyses which allowed examining the incidence. The presence of Lxx was checked by the "dot blot immunoassay" which identified different bacterial populations by the blue dots of different gradations which permitted examining severity. The present work analyzed 187 fields from 35 varieties in 2009 and found Lxx disseminated in 23% of fields in 21 varieties whereas in 2010 from 166 fields and 33 varieties dissemination was 26.5% in 20 varieties. In total 30 out of 49 varieties showed presence of Lxx. Incidence of Lxx in three of the most planted varieties (44.9%) varied from 1 to 72% in RB867515, from 1 to 25% in SP81-3250 and from 1 to 56% in RB855453. Some fields of these varieties showed severity enough to indicate susceptibility of main Brazilian varieties to RSD.

The status of grapevine trunk diseases in British Columbia

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Grapevines (*Vitis vinifera* L.) is one of British Columbia's emerging crops with vineyards becoming established in different regions including, the southern interior, southwestern region, and pacific islands. Nowadays, British Columbia wine industry comprises over 5,000 ha representing an economic impact that exceeds $45 million. Grapevine trunk diseases have recently become a major concern among growers. However, the importance that grapevine trunk diseases have on grapevine health in British Columbia has not yet been evaluated. Therefore, field surveys were conducted throughout the main grape-growing regions and diseased samples showing characteristic dieback symptoms including, central vascular necrosis, dark streaking of the wood, light-brown wood discoloration, and perennial cankers were collected from both young and mature vines in British Columbia. Morphological characteristics along with combined multi-allelic DNA sequence analyses from the rDNA (ITS1-5.8S-ITS2), β-tubulin and EF1-α genes allowed us to identify several fungal species in the families Botryosphaeriaceae, Calosphaeriaceae, Diatrypaceaeae, Nectriaceae, Valsaceae, as well as species in the genera Cadophora, *Phaeomoniella*, *Truncatella*, and *Pestalotiopsis* associated with the different vascular symptoms. This study reveals for first time the presence of grapevine trunk diseases in British Columbia including, black foot disease, Botryosphaeria canker, Eutypa dieback, esca, and young vine decline.

Production of both carboxy-coterminal coat protein forms of *Lolium latent virus* is required for efficient systemic movement

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The *Lolium latent virus* (LoLV, *Lolavirus*, *Alphaflexiviridae*) genome is encapsidated by equimolar amounts of carboxy-coterminal coat protein (CP) variants of apparent MW 33 and 28 kDa. The CP ORF contains two 5′-proximal AUGs, encoding Met 1 and Met 49, respectively promoting translation of the 33 kDa and 28 kDa CP variants. The 33 kDa CP N-terminal domain includes a 42 aa sequence encoding a putative chloroplast Transit Peptide (cTP) with a predicted cleavage site upstream of AUG2. Ablation of AUG1 in an infectious clone yielded mutant LoLV-K1, which was able to replicate in inoculated leaves of *Nicotiana benthamiana*, but not spread systemically. Mutation of AUG2 to UUG yielded mutant LoLV-K2, which was able to infect plants systemically. LoLV-K1 revertants that regained expression of a CP form of >28 kDa (by restoration of wild-type AUG1; by mutation to AUG at another site; or by mutation to an upstream CUG alternate initiation codon) were able to infect plants systemically. Substitution of four amino acids at the predicted cTP cleavage site combined with AUG2>CCC yielded mutant LoLV-C4, in which systemic infection was significantly delayed and symptoms altered. The N-terminal cTP sequence is crucial for efficient cell-to-cell and systemic movement, as well as homologous CP interactions and particle formation, but is not required for virus replication. Lack of production of the 28 kDa CP by either internal initiation or proteolytic cleavage limits systemic infection.

***Aspergillus* section *Flavi* populations in cornfields of Jalisco and its potential for aflatoxin contamination in maize**

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Aflatoxin monitoring is the second key to dimensioning the contamination problem and suggest strategies to prevent the extent of contamination. The objectives of this research were to isolate toxigenic populations of *Aspergillus* section *Flavi* from soil and grain samples from seventeen municipalities of Jalisco, and determine the distribution of aflatoxin contamination. Some *A. flavus* and *A. parasiticus* populations were obtained from at least all municipalities of Jalisco. The highest frequency was recorded for *A. flavus*. The greater *Aspergillus* populations was associate to North Coast and South of Jalisco. For type of *A. flavus* strain, S-type (small sclerotia < 400 µm in diameter) population was only found in Puerto Vallarta. An important percentage (36%) of *Aspergillus* was described as unnamed taxon. In vitro not all isolates were capable to produce aspergillic acid on AFPA medium. Results with the rapid test of AflaCheck® by VICAM, shown only four municipalities were aflatoxin positive (Tequila, Puerto Vallarta, Atotonilco El Alto and La Barca) for the limit of 20 ppb of more. This study demonstrates that main Jalisco corn producer regions has potential populations of toxigenic *Aspergillus*. To our knowledge, this is the first report of *Aspergillus* section *Flavi* populations (including *A. flavus* S, T or L morphotypes) in Mexico and its relationships with aflatoxin contamination. Monitoring and biological control studies are in process.

Managing gladiolus rust in Mexico with fungicides

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Uromyces transversalis, the causal agent of gladiolus rust, is a quarantine-significant fungus in the United States. When gladiolus rust is found, quarantine and eradication procedures are implemented including destruction of diseased plants and application of fungicides to minimize inoculum spread. However, efficacy data on registered fungicides for this disease are lacking. In Mexico, *U. transversalis* was first observed in 2004 in Morelos, State of Mexico, and Puebla and in Michoacán in 2005. Since then, it has been recorded in Guerrero, Oaxaca, Veracruz, and Chiapas. In 2010, two trials were conducted in commercial gladiolus fields in Mexico—in Cuautla, Morelos and Atlixco, Puebla. Four applications of 11 fungicides (azoxystrobin, boscalid+pyraclostrobin, epoxiconazole, fluoxastrobin, kresoxim-methyl, myclobutanil, oxycarboxin, propiconazole, pyraclostrobin, tebuconazole, and trifloxystrobin) were made every 14 days. Disease severity in each plot was recorded each week for 7 weeks after the first treatment (WAT). All fungicides significantly reduced disease severity in Cuautla, Morelos; the 3 triazoles exhibited the greatest reduction. In Atlixco, Puebla, the triazoles and trifloxystrobin significantly reduced disease starting 3 WAT; other fungicides reduced disease starting at 6 WAT. Timely applications of fungicides should reduce rust severity on field-grown gladiolus in Mexico, a leading source of cut gladiolus flowers, and, therefore, help reduce the movement of *U. transversalis* into the United States.

Comparative analysis of the host response of citrus leaf, stem and root tissues to infection by *Candidatus* Liberibacter asiaticus

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Candidatus Liberibacter asiaticus (Las) is known to cause citrus Huanglongbing (HLB) disease. Host response of leaf, stem and root tissues of Valencia sweet orange (*Citrus sinesis*) to Las infection was investigated using Affymetrix microarray. Using a 2 fold change and $p < 0.05$ as cut-off values, a total of 1008, 580 and 58 transcripts were up-regulated in leaves, stems and roots, respectively, while 1109, 350 and 58 were down-regulated, respectively. The following metabolic pathways were altered: transport, amino acid, hormone, lipid, secondary, carbohydrate, signaling, transcription and cell wall biogenesis. PR genes were mostly up-regulated in leaves and stems but repressed in roots. JA genes were up-regulated in stems, down-regulated in roots but showed both patterns in leaves. Leucine-rich repeat and DUF26 receptor-like kinases were the two receptor groups altered in all tissues. Genes encoding major intrinsic proteins, zinc and Fe(II) transporters were regulated in leaves and stems but unaffected in roots. Genes encoding ADP-glucose phosphorylase, starch synthase, and starch branching enzyme were up-regulated in stems and leaves but repressed or unaffected in roots while amylase genes showed both patterns in leaves and stems. Sucrose transporter 4 was up-regulated in leaves, sucrose synthase was repressed in stems but invertases showed both patterns in the two tissues. The implication of the differences in the response of leaves, stems, and roots to Las infection will be discussed to their distinct functions.

Detection of the begomovirus *Clerodendrum* golden mosaic China virus in *Salvia splendens* cv. Dancing Flame

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'Dancing Flame' is a popular salvia (*Salvia splendens*) cultivar with red flowers, described as having variegated foliage. After careful observations of plants at a local nursery in Baton Rouge, LA; we noticed that the variegated foliage resembled symptoms caused by plant viruses. The symptoms included bright yellow mosaic, yellow vein, and leaf distortion. Suspecting that a virus infection may be involved, we conducted graft and mechanical inoculations, dsRNA analyses and ELISA tests using antisera for several common plant viruses. Graft inoculations to four healthy *S. splendens* cultivars reproduced the symptoms observed in 'Dancing Flame'. ELISA testing and dsRNA analyses were negative. Suspecting an infection by a begomovirus, total DNA was extracted from a selected symptomatic 'Dancing Flame' plant and used as template for the rolling circle amplification (RCA) method. Two putative viral DNAs were obtained, cloned, and sequenced. Sequence comparisons of the 2.78 (DNA-A) and 2.73 kbp (DNA-B) molecules resulted in 99 and 98% identity with the corresponding genomic DNAs of *Clerodendrum* golden mosaic China virus (ClGMCNV) isolate YX1. The virus was detected by PCR using begomovirus-specific primers in all *S. splendens* plants showing variegated foliage but not in non-variegated plants. ClGMCNV was also detected in graft inoculated plants. These results strongly suggest that ClGMCNV is associated with the variegated foliage of 'Dancing Flame'.

Does weed management for sweet corn differ with planting date?

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Sweet corn in the mid-Atlantic region of the U.S. is often planted over a two-month period to provide a consistent supply for either processing or fresh market. This requires a weed management program that is robust enough to control weeds over a range of environmental conditions. A field trial was conducted in 2009 and 2010 in Delaware on coastal plains soils. 'Overland' hybrid was planted over five planting dates, spaced 10-days apart. In 2009, planting began May 10 and ended June 20 and in 2010 dates were May 1 to June 10. Five weed control strategies were implemented, three soil-applied programs (s-metolachlor + atrazine [Bicep] at full and reduced rates; full rate of s-metolachlor + atrazine + mesotrione [Lumax]) and two postemergence programs (s-metolachlor followed by carfentrazone + bentazon [Aim+Basagran]; and s-metolachlor followed by topramezone + atrazine [Impact]). Visual weed

control was collected by species. Smooth pigweed, common lambsquarters, common ragweed, and morningglory species were present both years and large crabgrass was present in 2009. Across all species, Impact was consistently the most effective treatment. Lumax was the most effective soil-applied program, but was not as consistent as Impact. For instance, morning-glory control ranged from 58 to 82% for Lumax, while Impact ranged from 74 to 86% control. There were no trends that planting date influenced weed control or that weed emergence differed by planting date.

Did *Phytophthora ramorum* already invade Italian forests? A possible answer by mass sequence approach
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Since the first record of *P. ramorum* in nurseries stocks in Italy, Italian forests were largely inspected for *Phytophthora* by means of classical baiting methods followed by morphological and molecular identification. Results never reported the presence of *P. ramorum.* Recent introduction of mass sequence techniques in biodiversity studies provide the opportunity to analyse in one step, and high sensitivity, Phytophthoras population in forest soils. However outcomes of mass sequence must be treated with caution due to the risk of false positives, and need to be confirmed with species specific PCR and/or biological detection. Pyrosequencing analysis of beech and chestnut soils has been carried out in different sites in Italy to evaluate the diversity of Phytophthoras community. Sequence data analysed with dedicated database identified a range of Phytophthoras including species known to be common in forest soils in Italy. Some new species resulted to be present: among these, *P. ramorum* was detected both in beech and chestnut soils. To confirm the detection, DNA's utilised for pyrosequencing was amplified with species specific primers sets for *P. ramorum* and the amplicons sequenced. Sequences matched with *P. ramorum* on database. Next step will be to bait the soils in order to obtain *P. ramorum* living cultures. Cryptic presence of *P. ramorum* in forest would represents an improvement of knowledge on epidemiology and invasion mechanisms of this species in Mediterranean climate.

Testing bait sprays and male annihilation traps for area-wide management of the invasive fruit fly *Bactrocera invadens* in Senegal, West Africa
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Bactrocera invadens is a recently introduced invasive tephritid fruit fly. It has become the most economically important tephritid on mango since its recent arrival and expansion across Africa. A large-scale field trial was carried out in the main commercial mango production region of Senegal at a time when fruit fly pressure was high. Male annihilation using pheromone traps was compared to male annihilation plus weekly applications of a protein bait that attracts females. 30ha parcels of each treatment were replicated three times. *B. invadens* populations dropped six-fold in the parcels receiving only male annihilation. The addition of the protein bait sprays resulted in a further six-fold decrease in the target population after the first two weeks, progressing to an 11-fold difference from the effect of male annihilation alone. These results show that even relatively small management areas can overcome outside population influx.

Characterization of QoI resistant isolates in *Alternaria alternata* causing Alternaria brown spot in citrus
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Alternaria brown spot (ABS) is one of the most important foliar fungal diseases in tangerine hybrids. ABS affects leaves, twigs and young fruit, reducing yield and quality of the fruit. Q_oI fungicides have been widely used for ABS control and have been the most effective products for ABS control registered for Florida citrus until recently. The site-specific mode of action of the Q_oI fungicides, blockage of electron transfer in the mitochondria at the cytochrome bc1 complex, increases the risk of resistance. Therefore, resistance monitoring is essential for successful resistance management programs. Media amended with logarithmically-diluted fungicide and resazurin-based microtiter assays were optimized for determining the EC_{50}. Twelve representative isolates showed highly correlated EC_{50} values for both methodologies. Isolates were more sensitive to pyraclostrobin than azoxystrobin. The EC_{50} values for sensitive isolates were less than 0.019 µg/ml and greater than 1.73 µg/ml of pyraclostrobin for resistant isolates. Values for azoxystrobin ranged from less than 0.607 µg/ml for sensitive to greater than 10 µg/ml for resistant isolates. Partial sequence of the cytochrome bc1 gene of resistant isolates revealed the expected G143A point mutation as compared with the susceptible isolates. The presence of the point mutation correlated with the higher EC_{50} values of resistant isolates and explains observations of Q_oI fungicide failure for ABS in Florida citrus orchards.

Development of encapsulation methods for CO_2 attractants and plant extracts as plant protection products
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Because of the demand for eco-friendly plant protection products there is a high interest in the development of formulation methods for natural substances such as plant extracts and attractants. The systematic development of formulation methods like encapsulation is essential to improve stabilisation, release and handling. Attractants based on CO_2 have the potential to control the Western Corn Rootworm, *Diabrotica virgifera*. Because of the larvae's orientation towards CO_2-emitters CO_2 beads can lure the larvae away from the roots. Artificial CO_2 sources were successfully encapsulated in hydrogel beads. Experiments have shown a significant CO_2 emission over two weeks. Furthermore, lipophilic CO_2 plant extracts were encapsulated in hydrogel beads, and their antifungal potential was tested against the phytopathogenic fungi *Phytophthora infestans*, *Rhizoctonia solani* AG1-IB and *Phoma lingam*. The extracts were encapsulated in 2% Ca-alginate beads (2.8 mm diameter). For *Origanum vulgare* (oregano) leaf extract (1.8 µg) and *Thymus vulgaris* (thyme) leaf extract (2.4 µL) agar diffusion tests showed considerable inhibitory effects. For *Allium sativum* (garlic) bulb extract (2.4 µg) only *P. lingam* was significantly inhibited. Further investigations will include the physico-chemical characterization of encapsulated plant extracts and the development of prolonged CO_2-emitting capsules as well as attract and kill beads.

Current status of legume viruses in the Pacific Northwestern U.S.A.
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A survey was conducted to determine the prevalence of *Bean leafroll virus* (BLRV) on alfalfa and *Pea enation mosaic virus* (PEMV) on pea in commercial fields in Washington (WA) and Idaho (ID), U.S.A. Pea and alfalfa samples, randomly collected from commercial fields in Nez Perce (ID) and Whitman (WA) counties in June and August 2010, were tested for the presence of BLRV and PEMV by antigen-coated plate (ACP)-ELISA. Antisera raised against the recombinant coat proteins (CP) of BLRV and PEMV were used. PEMV was found in one field out of 27 sampled in June, 2010 and in 20 out of the same 27 fields sampled in August, 2010. None of the samples tested positive for BLRV. PEMV consists of PEMV-1 (genus *Enamovirus*) and PEMV-2 (genus *Umbravirus*). The genomic sequences of PEMV-1 and PEMV-2 from selected samples obtained from this survey in ID and WA were characterized. Sequence comparisons and phylogenetic analysis of PEMV-1 and PEMV-2 sequences from ID and WA showed more than 95% sequence identity with isolates from Germany, UK and U.S.A.

Inverse responses of two major genes against bacterial blight of rice at different temperature regimes
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Transcriptome analysis using customized microarray platform was done to evaluate global gene expression patterns in IRBB4, a bacterial blight resistant line carrying *Xa4* in comparison with the susceptible IR24 at early stages of disease development. Differential gene expression including PR genes peaked

at 72 hrs post-inoculation in both genotypes, but bacterial population counts from whole leaves did not differ significantly. There were 152 differentially expressed genes (DEGs) between the resistant and susceptible genotypes. Lesion lengths at 35/31°C (day/night) were significantly higher than lesion lengths at 29/21°C both in phytotron and screenhouse conditions. No significant difference in whole leaf population counts was observed between temperature regimes. Segment plating after the visible end of the blight lesions showed that bacterial counts across segments were not significantly different at warm temperature but significantly different among leaf segments at low temperature, suggesting host regulation of pathogen spread and symptom development but not pathogen multiplication. IRBB7, another resistant line carrying *Xa7* shows increased effectiveness at high temperature regime. There were no differences in whole leaf counts but segment plating shows more efficient regulation of bacterial spread at high than at low temperatures. We are examining gene expression levels of IRBB4 DEGs in IRBB7 to find clues on their inverse responses at high and low temperatures.

Genome-enabled primer design to distinguish geographic origin of *Xanthomonas oryzae* pvs. *oryzicola* and *oryzae*
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Xanthomonas oryzae pathovars *oryzae* (Xoo) and *oryzicola* (Xoc) are the causal agents of bacterial leaf blight and bacterial leaf streak, respectively. While study of *X. oryzae* has focused predominantly on strains collected from Asia, diverse strains of X. oryzae have been isolated from infected rice in North and South America and Africa. Recently we used genomic sequence to design primers that differentiate *X. oryzae* strains by pathovar. In this study, we have analyzed draft genomic sequence of African Xoo and Xoc strains to develop specific diagnostic primers that distinguish Xoo and Xoc based on geographic origin. Primers were designed based on 40 predicted ORFs specific to 3 African Xoo strains, and 15 ORFs specific to African Xoc strains. The same strategy was used to design primers specific to Asian strains of Xoo and Xoc. Primers were validated on an extensive collection of DNA from *X. oryzae* and other bacteria collected worldwide. This study demonstrates how genomic data can be used to expedite design of tests that distinguish closely-related pathogens by geographical origin. These tests will serve as a valuable resource in the development of testing programs to rapidly identify and characterize *X. oryzae* in rice fields and seed lots. As sequencing costs continue to drop, genome-enabled ultraspecific diagnostics may become important epidemiological and regulatory tools for a variety of pathogens.

Effect of the localization of *Acidovorax citrulli* in watermelon seeds on pathogen detection
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Recently, we reported that in naturally infested watermelon seeds, *Acidovorax citrulli* may be located under the seed coat (when infested via the fruit pericarp) or in endosperm tissues (when infested through the pistil of female flowers). However, the effect of the location *A. citrulli* in seeds on pathogen detection has not been investigated. Hence, the goal of this study was to determine the effect of *A. citrulli* location in seeds on the ability to extract the pathogen by washing or crushing methods. Fifty infested watermelon seedlots were generated by inoculating female watermelon stigmas or by swabbing the pericarps of fruit ovaries with *A. citrulli* cell suspensions (~ 10^8 CFU mL^{-1}). Samples (~ 5 g of seed/lot) from pericarp- and pistil-infested lots were crushed or washed for 60 minutes and genomic DNA was purified from the seed extract and subjected to real-time PCR. Samples (40-80 seeds/lot) from each lot were also tested by a modified seedling grow-out assay. For pericarp-infested lots, *A. citrulli* was detected in 86% of the seed wash samples as compared to 26% for seed from pistil-infested lots. In contrast, when seed samples were crushed, *A. citrulli* was detected in 100% of the pericarp- and pistil-infested lots. BFB seedling transmission was observed for 100% of the seedlots from both infestation type, when planted under conditions of 28°C and 80% R.H. *A. citrulli* from seeds infested by pistil invasion, and that crushing is necessary for accurate pathogen detection.

Nutritional cues and ambient pH modulate the *in vitro* activity of a polygalacturonase isozyme produced by *Penicillium expansum*
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Penicillium expansum is an economically important pathogen of apple and pear fruit that causes blue mold in storage. This fungus produces polygalacturonase (PG) isozymes in decayed fruit which macerate host tissue and may be regulated by nutritional cues and ambient pH. Inhibition of this virulence factor along with understanding its underlying regulatory mechanism(s) could aid in disease control methods. Therefore, we investigated the roles of carbon and nitrogen nutrition and ambient pH, on *P. expansum* PG activity in vitro. The greatest PG activity was detected when the fungus was grown on a medium with apple pectin and ammonia as the sole carbon and nitrogen sources at pH 4. *P. expansum* PG activity was also affected by the form of galacturonic acid and the degree of pectin methylesterification when utilized as a sole carbon source. After 7 days of fungal growth in culture, the pH of the apple pectin-ammonia medium increased from 4 to 6, total soluble polyuronides decreased, and ammonia levels remained unchanged. A single PG isozyme with a pI of ~7.9 was produced *in vitro* which was different from those produced by *P. expansum* in decayed apple or pear fruit. Our results indicate that carbon, nitrogen, and pH modulate PG activity but do not affect the production of the single, prominent PG isozyme in culture.

Suppressiveness to *Phytophthora infestans* infection in potato tubers by Andean soils from three provinces of Ecuador
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Phytophthora infestans affects potato production worldwide through foliar and tuber damage. Tuber blight is almost nonexistent in the Ecuadorian Andes, despite the high incidence of foliage infection and tuber susceptibility. This study aimed to understand abiotic and biotic components of Ecuadorian soils that contribute to tuber blight suppression. Soils were collected from three sites where potatoes were grown. Suppressiveness to tuber blight was evaluated after exposure of sporangia to non-treated and heat-treated soils. Infection in whole tuber and tuber slices was significantly reduced after exposure to non-treated soils when compared to heat-treated soils. In addition, tuber infection was reduced with longer exposure of sporangia to soil. Contact to soil over time resulted in a decrease of germinated sporangia, as well as an increase in lysed/latent sporangia. Differences between heat-treated and non-treated soils suggest microbial populations contribute to tuber blight suppression. However, suppression occurred also in heat-treated soils and infection levels varied between sites. These results suggest that physical-chemical characteristics are important as well in suppression. Understanding natural tuber blight suppression will contribute to improve management practices to reduce tuber infection in high incidence areas.

***Alternanthera Mosaic Virus* identified in clock vine in Florida**
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Phytopathology 101:S183

Clock vine (*Thunbergia laurifolia*) is a new host for *Alternanthera Mosaic Virus*. A sample of clock vine from a commercial greenhouse in Florida was observed to have virus-like symptoms including mottling, mosaic patterns, ringspots, and chlorosis. Approximately 10% of 50 plants were affected, including the mother plant used for vegetative propagation. ELISA tests using *Papaya Mosaic Potexvirus* antisera and microscopic observation of spindle-shaped inclusion bodies indicated the plant was infected with a potexvirus. Reverse transcription polymerase chain reaction (RT-PCR) using Miglino's potex-4 and potex-5 primers produced an expected amplicon of 280bp. The PCR product was sequenced and compared to sequences in NCBI GenBank, resulting in a 97% identity match with *Alternanthera Mosaic Virus* (AltMV) GenBank accession DQ393785. AltMV has been previously reported in Australia, Europe, North America and South America in ornamentals such as *Crossandra* spp., *Phlox stolonifera*, *Portulaca grandiflora*, and *Scutellaria* spp.

The role of silicon transport in improving plant disease resistance
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Phytopathology 101:S183

Silicon (Si) is not considered as an essential element for plant growth yet its uptake is beneficial in alleviating abiotic and biotic stresses. These positive effects are variable since accumulation differs among plant species. This differential accumulation would be attributable to the presence of specific

genes involved in Si uptake. These genes have first been recently described in rice with homologs reported in maize and barley. The objective of this project was to investigate, identify and characterize the presence of Si-transport genes in wheat, a species known to accumulate Si, and to determine their functionality and localization. Our results have allowed the identification and the cloning of a putative Si-transport gene presenting high homology (>80%) with the Si-influx protein in rice known as *Lsi1*. Transient expressions of the wheat *Lsi1* Si transporter (*TaLsi1*) coupled with GFP in *Nicotiana benthamiana* indicated that this protein was localized across the plasma membrane, a feature typical of other members of the *Lsi1* family. The Si transport activity of *TaLsi1* was confirmed in a heterologous system, *Xenopus laevis* oocytes, and its efficiency at transporting Si was comparable to that of the rice *Lsi1*. The discovery of these transporters provides a unique opportunity to understand and optimize the uptake of Si in a strategy to control plant diseases.

Incidence and prevalence of fungal pathogens on switchgrass seed produced in the U.S.A.

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Switchgrass (*Panicum virgatum* L.) production is increasing in acreage due to current initiatives for commercial biofuel production in the United States and worldwide. Due to a lack of seed certification programs for switchgrass, seedborne plant pathogens have likely been shipped along with the seeds to switchgrass producers. The aim of this study was to identify prevalent seedborne fungal pathogens of switchgrass from commercially available seed produced in the U.S. Seven cultivars, including 'Alamo', 'Blackwell', 'Cave-in-Rock', and 'Kanlow', from 12 sources were tested. A randomly-selected subsample of seed from each 454-g lot was surface-sterilized in 1% NaOCl for 1 min, rinsed three times with sterile water, and dried on sterile filter paper. Three hundred surface-sterilized seed per lot were plated on potato dextrose agar (PDA) amended with 100 mg/L chloramphenicol and incubated at 22°C. Seed were evaluated daily for development of fungal colonies. Emergent colonies were transferred to fresh PDA plates for identification. Rates of fungal infection among the 31 sampled seed lots ranged from 0 to 85.6%. The most prevalent pathogens isolated included *Bipolaris sorokiniana*, *Bipolaris oryzae*, *Alternaria alternata*, and *Fusarium graminearum*. Managing seedborne pathogens could increase stand establishment and crop yields, while decreasing the likelihood of a seedborne epidemic.

Wheat streak mosaic virus outbreak in North Dakota 2010

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North Dakota ranks second in the nation in total wheat production and is the largest producer of pasta wheat (durum), hard red spring wheat, and barley. Wheat streak mosaic (wsm) virus is a devastating disease of wheat and can also infect barley. In 2010, producers suffered extensive losses from this disease because of "a perfect storm" of conditions. Once the wsm virus is established in a field the crop must be destroyed or risk having the virus spread to neighboring fields. A key means of prevention is to "break the green bridge" between the wheat curl mite that spreads the disease and its host by destroying wheat volunteers from previous growing seasons with herbicides or tillage two weeks prior to planting the next wheat crop. In response to calls from producers, consultants, and crop insurance adjusters, on site visits were made to infected wheat fields. In addition to making visual assessments based on disease symptoms, ELISA testing confirmed the virus in 71 samples. As a result of the timely and effective response of NDSU extension, many of these producers were able to replant infected wheat fields with another non-host crop rather than suffer poor wheat yields and risk spread of the disease to adjacent fields. Best management practices to combat this disease were conveyed to producers with on farm visits, newspaper, TV, and radio interviews, a user friendly management brochure, weekly updates in the NDSU Crop and Pest Report and numerous presentations.

Bacillus subtilis, strain QST 713: Soil applications for disease control, crop yield and quality ehancement

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Phytopathology 101:S184

Bacillus subtilis, QST 713, is a soil borne strain of plant growth promoting rhizobacteria. It is unique from other strains of B. subtilis in its production of anti-fungal and anti-bacterial products. These properties have previously been employed for the control of foliage plant pathogens under the trademark Serenade®. More recently, research has exhibited the advantages of soil applications of QST 713 in terms of disease suppression and beneficial plant effects leading to a product extension, Serenade® Soil. Soil applications, whether applied via seed treatment or drench, result in more vigorous plants as measured by topgrowth and rootmass. In the presence of soil borne pathogens QST 713 soil applications suppress disease with resultant increases in plant vigor, improved yields and, in some instances, quality. The aforementioned properties are the result of a protective biofilm on the roots of plants and disease suppression from an array of lipopeptides and other biochemicals known to suppress plant pathogens. Finally, plant modulating chemicals are also produced that may contribute to plant vigor and disease suppression. Practical implications of these new findings are discussed in regards to solanaceous crops, fruiting vegetables and cucurbits.

Two new broad-spectrum fungicides for use on pome fruits, stone fruits, fruiting vegetables and potatoes

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Merivon™ and Priaxor™ are two new broad-spectrum fungicides under development in the United States by BASF Corporation for control of key fungal diseases of pome fruits, stone fruits, fruiting vegetables and potatoes. Merivon is a premix fungicide containing two active ingredients, fluxapyroxad and pyraclostrobin in a 1:1 ratio and is currently being used for research on pome fruit and stone fruit diseases. Research in university and private cooperator trials has indicated Merivon is highly effective at controlling diseases such as scab (Venturia inaequalis) and powdery mildew (Podosphaera leucotricha) of apple; blossom blight and brown rot of peach (Monilinia spp.) and powdery mildew of cherry (Podosphaera clandestine) in the rate range of 146 – 250 g ai/ha. Priaxor is a premix fungicide containing a 2:1 ratio of pyraclostrobin to fluxapyroxad and is currently being used for research on fruiting vegetables and potato diseases. Research has indicated Priaxor is highly effective at controlling early blight (Alternaria solani) in both tomato and potato as well as powdery mildew (Leveillula taurica) and black mold (Alternaria alternata) in tomato and black dot (Colletotrichum coccodes) in potato in the rate range of 146 – 300 g ai/ha. Trial results from 2009 and 2010 will be presented. EPA registration is expected in 2012.

Grape hosts infested with glassy-winged sharpshooters produce volatile compounds which may attract egg parasitoids

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Glassy-winged sharpshooter (GWSS), *Homalodisca vitripennis* (Germar), is an important vector of Xylella fastidiosa Wells, which causes Pierce's disease in grapes. Current management strategies in GWSS infested areas include mass release of the egg parasitoid *Gonatocerus ashmeadi* Girault and related species. However, little is known about egg parasitoid host finding behavior. Thus, volatile emissions from non-infested grapes and grapes infested with GWSS egg masses were compared using gas chromatography and mass spectrometry. Three compounds, tentatively identified as beta-ocimene, (1, 8)-cineole, and beta-farnesene, were emitted in greater levels from grape hosts infested with GWSS egg masses than non-infested plants. Parasitoid attraction to synthetic versions of these compounds will be evaluated. If confirmed to be attractants for the egg parasitoids, these compounds could be deployed as lures to monitor egg parasitoid populations in a locale. These compounds also could be screened in grape breeding programs aimed at reducing GWSS populations, because infested grapes producing more of these compounds will improve attractiveness to the parasitoids that prey on GWSS eggs.

Zebra chip disease is associated with increases in pathogenesis-related protein activity and host defense-associated secondary metabolites in tubers

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Zebra chip disease, putatively caused by the bacterium 'Candidatus Liberibacter solanacearum', is an emerging problem for potato growers throughout North America. However, little is known about the physiological changes that occur in diseased plants beyond the eponymous zebra chip symptom. One physiological change that occurs in diseased plants, presumably as a response to pathogen infection, is increased production of pathogenesis-related (PR) proteins including beta-glucanases, exo-chitanases, peroxidases, and polyphenol oxidases. Increased production of phenolic compounds also occurs. The levels of these proteins and phenolics were compared between healthy and zebra-chip diseased tubers. PR protein levels were quantified based on relative enzymatic activity, whereas phenolic compounds were extracted in methanol and analyzed by high-performance liquid chromatography. Diseased tubers, compared with non-diseased tubers, had twice the levels of beta-glucanase, exo-chitanase, and polyphenol oxidase;

and eight times the levels of peroxidase. Protein concentrations were also positively correlated with disease assessment ratings. Phenolic levels were much greater in diseased tubers, especially levels of chlorogenic acid derivatives and precursors. Zebra chip diseased tubers could exhibit increased browning when cut or fried because of these observed increases in enzyme levels and phenolic content.

Xylella fastidiosa infection of grapevines affects host secondary metabolite and defense-related protein levels within xylem

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Phytopathology 101:S185

Pierce's disease of grapevine is a serious threat to grape production and is caused by the xylem-dwelling bacterial pathogen *Xylella fastidiosa*. Microscopy studies have documented morphological changes to grapevine xylem due to infection by *X. fastidiosa*. Comparatively, less is known about the biochemical interactions between *X. fastidiosa* and grapevine. In this study, phenolic content of xylem sap collected from non-inoculated and *X. fastidiosa*-inoculated grapevine was assessed using high-performance liquid chromatography. In addition, peroxidase, polyphenol oxidase, exo-chitanase, and beta-glucanase levels of non-inoculated and *X. fastidiosa*-inoculated grapevine were compared using enzymatic activity assays. Greater levels of four phenolics were observed in infected grapevine compared to uninfected grapevine. While beta-glucanase levels were reduced in infected grapevine compared to uninfected grapevine, effects of infection on other proteins was unclear. A better understanding of the role of phenolic compounds in grapevine defense against infection may aid in the development of novel management strategies. Likewise, documented shifts in compound levels could be used to develop detection methods for Pierce's disease that are host-based as opposed to pathogen-based.

Alteration of host gene silencing during root-knot nematode infection

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Root-knot nematodes (RKN, *Meloidogyne* spp.) are an agronomically important pathogen that are capable of establishing intimate feeding sites (giant cells) within the roots of a variety of plant hosts. Elucidating the mechanisms that allow RKN to establish and maintain these giant cells will be crucial in improving our ability to manage the economic damage they cause to crops world-wide. Much of the molecular underpinnings of this interaction however, remain unclear. We utilized functional genomic tools in order to gain more insight into what is necessary for a successful RKN infection. Trends observed in the transcriptome of laser-captured giant cells in *Arabidopsis thaliana* roots (microarray data obtained from 14 and 21 days post infection) suggest that the RKN infection process may be influencing mechanisms in host gene silencing. A subset of genes altered in their expression during nematode-infestation are found to be up-regulated in Arabidopsis plants over-expressing the suppressor of gene silencing, Hc-Pro. Furthermore, genes normally down-regulated by trans-acting small RNAs are also up-regulated during the nematode infection process. Gene silencing is known to play an important part in plant defense responses to other pathogens, yet little is known with regards to how this pathway is involved during RKN infection. These results may help elucidate the role of gene silencing during the RKN infection process.

Biological control of invasive common ragweed, _Ambrosia artemisiifolia_ L. with beneficial insect herbivores in China

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Phytopathology 101:S185

Common ragweed, *Ambrosia artemisiifolia* L. is unintentionally introduced into China in the 1930s. Since there are no effective biological control agents, it has rapidly spread to 21 provinces in China. Biological control of common ragweed has been conducted since the1980s in China, since several insect herbivores. *Epiblema strenuana* and *Ophraella communa* were considered as two biological control agents of common ragweed in China. In recent decade years, many studies were focused on host specificity, biology, climatic adaptation, mass rearing and application of *E. strenuana* and *O. communa*. The results indicated that both *E. strenuana* and *O. communa* were safe and available. They reveal a higher fecundity under optimum temperatures, and are well adapted to subtropical climatic conditions. This suggests significant potential for using *E. strenuan* and *O. communa* to suppress common ragweed because most of areas invaded by common ragweed belong to subtropics in China. Based on the biological and ecological studies, mass rearing of *E. strenuana* and *O. communa* have been achieved in China. Since the spatial niches between *O. communa* and *E. strenuana* are significantly heterogenous, combined control strategy of common ragweed with the two insect species were recommended and used in many areas invaded by common ragweed in China. Significantly, the two insect species can overwinter successfully, thus they can sustainable-suppress the population of common ragweed in the field.

Oviposition or host-feeding: Host handling strategy in the whitefly parasitoids _Eretmocerus hayati_ and _Encarsia sophia_

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Phytopathology 101:S185

Host feeding provides nutrients that allow parasitoids to mature eggs. Thus, when handling an accepted host, the parasitoid chooses between current reproduction through oviposition and future reproduction through host feeding. Two whitefly parasitoid species, *Eretmocerus hayati* and *Encarsia sophia* were examined to determine if host density and host instar can affect their host handling decisions. In a single-instar no-choice experiment, the whitefly host, *Bemisia tabaci*, was introduced to *Er. hayati* and *En. sophia* females at densities of 5, 10, 20, 30, 40, 50, 60, 70 and 80 second or third instar nymphs per 3.5 cm^2, respectively. Similarly, in a mixed-instar choice experiment, the whitefly host was introduced at densities of 20, 40, 60, 80 mixed-instar nymphs per 3.5 cm^2. It was found that with the increase of host density, more hosts were killed by females with host feeding and parasitism. The number of host parasitized by *Er. hayati* peaked at the density of 40 nymphs per 3.5 cm^2. Meanwhile, there was a sharp increase in the number of host fed, indicating that the oviposition was prior to host feeding in *Er. hayati*. In the mixed-instar choice experiment, when the host density was low, *Er. hayati* was found to oviposit under the optimal host instars and feed on alternative host instars. However, with the increase of host density, *Er. hayati* was found to oviposit and feed both on the optimal host instars. In contrast, *En. sophia* females showed different host handling strategy that host-feeding was prior to oviposition.

Gut bacterial communities in the Bactrocera dorsalis and their luring activities on host

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In this paper, The 16S rDNA cloned libraries from the intestinal tract of lab-reared, lab sterileugar-reared and field-collected populations of B. dorsalis were compared. Phylogenetic analysis of 16S rDNA revealed that Gammaproteobacteria was dominant in the all samples (73.0%–98.3%). Actinobacteria and Firmicutes were judged to be major components of a given library since they constituted 10% or more of the total clones of such library. The Flavobacteria, Deltaproteobacteria, Bacteroidetes, and Alphaproteo-bacteria were observed in small proportions in various libraries. LIBSHUFF analysis showed that the bacterial communities of B. dorsalis from the three populations were significantly different from each other ($P < 0.0085$). Those results indicated that the intestinal tract of B. dorsalis adult contains a diverse bacterial community, and different environmental conditions and food supply could influence the diversity of the harbored bacterial communities and increase community variations. The whole beer, filtered and autoclaved super-natants of fermentation cultures of the cultured gut bacteria had attractiveness to B. dorsalis adults to some extent in laboratory bioassays. Autoclaved supernatants were significantly more attractive than the whole beer or filtered supernatants. Six isolates, which autoclaved supernatants were most attractive among all cultured bacteria, were selected to attract to B. dorsalis adults in field. Results showed that LRC38 was more attractive than others.

Food and microhabitat preferences of _Mononchus_: A preliminary investigation

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Phytopathology 101:S185

Predatory nematodes are known to be potential nematode biocontrol agents, but they feed on nematodes opportunistically which means they also consume free-living nematodes and other microorgansims. Objectives of this project were to 1) determine the efficacy of a commonly occurring predatory nematode, *Mononchus*, to prey on plant-parasitic nematodes, and 2) to explore microhabitats favorable for *Mononchus* reproduction. DNA was extracted from individual *Mononchus* isolated from fields infested with burrowing (*Radopholus similis*), or root-knot (*Meloidogyne* spp.) nematodes. To determine if *Mononchus* habitually feed on plant-parasitic nematodes, PCR was conducted on the Mononchus DNA using primers specific for *R. similis* and *Meloidogyne*. The percentage of samples that tested PCR positive was evaluated to establish feeding preferences of Mononchus in cultivated soils.

To examine favorable environments for *Mononchus*, 5 artificial microhabitats were inoculated with 6 *Mononchus* each. These microhabitats included: 1) 10 root-knot nematodes suspended in water in a 0.5-ml watch glass, 2) 10 g soil (frozen to free indigenous nematodes) inoculated with 40 root-knot nematode juveniles, 3) 3 g of vermicompost media, 4) water agar with a flamed carrot disc, co-cultured with Rhabditidae, and 5) 10 g soil amended with 1% (w/w) of dried sunn hemp (*Crotalaria juncea*) powder. *Mononchus* were extracted from these microhabitats 3 months after inoculation and counted.

Genome-wide identification of genes regulated by RcsB and RcsC in *Erwinia amylovora*

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Phytopathology 101:S186

The exopolysaccharide amylovoran is one of the major virulence factors in *Erwinia amylovora*, causal agent of fire blight of apples and pears. We have previously demonstrated that the RcsBCD phosphorelay system is essential for virulence by controlling amylovoran biosynthesis. We have also found that the hybrid sensor kinase RcsC differentially regulates amylovoran production *in vitro* and *in vivo*. To further understand how the Rcs system affects *E. amylovora* virulence, we performed genome-wide microarray analyses to determine the regulons of RcsB and RcsC in liquid medium and on immature pear fruit. Our array analyses identified many novel genes differentially regulated by RcsBC. Consistent with our previous findings, we confirmed that, while RcsB acted as a positive regulator in both conditions, RcsC positively controlled amylovoran biosynthetic gene expression *in vivo*, but negatively *in vitro*. Other virulence traits such as type III secretion, regulatory, and levansucrase genes were also regulated by the RcsBCD system. In addition, a hidden Markov model (HMM) was used to predict candidate RcsB binding sites in the intergenic regions of the *E. amylovora* genome. Predicted target genes were compared with RcsBC-regulated genes identified in the microarray assay. Based on our findings, a working model has been proposed to elucidate how the Rcs phosphorelay system regulates virulence gene expression in *E. amylovora*.

SSR markers closely linked with a major QTL on chromosome 12 associated with resistance to phylotype I strains of *Ralstonia solanacearum* in tomato

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Phytopathology 101:S186

Bacterial wilt caused by phylotype I strains of *Ralstonia solanacearum* is a major disease of tomato in Southeast Asia. Tomato variety 'Hawaii 7996' has been shown to have durable resistance against the pathogen. Previous studies have associated the resistance with a major quantitative trait locus (QTL) on chromosome 12. This study constructed a new linkage map with good genome coverage. Simple sequence repeats developed from bacterial artificial chromosome sequences of tomato were the main marker types used. Attempts were made to saturate the reported QTL regions. A 188-recombinant inbred line (RIL) population derived from 'Hawaii 7996' (H) and 'West Virginia 700' (W) was used for resistance assays. The population was evaluated at seedling stage against phylotype I strains Pss4 and Pss186 isolated from Taiwan, and strain TM151 from the Philippines. Three field evaluation trials were conducted in Indonesia, Taiwan, and Thailand. QTL analyses identified the presence of a major QTL located on a 2.5-cM interval of chromosome 12 controlling 9.0 – 37.4% of total resistance variation over trials. The "H" group of the RIL had 22.5 – 50.5% less wilted plants compared with the "W" groups over trials. This QTL should be the same as *Bwr-12* reported previously, which contributes greatly to the durable resistance in 'Hawaii 7996' against phylotype I strains. Potential for marker-assisted selection using markers linked to *Bwr-12* identified in this study will be discussed.

Resistance selection and risk assessment of fenpropathrin against *Panonychus citri* (Acari: Tetranychidae)

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Phytopathology 101:S186

The citrus red mite, *Panonychus citri* McGregor (Acari: Tetranychidae), is an important pest mite in the world that devastates citrus trees and thus severely affects yield and quality of fruit. This mite is difficult to manage because of their ability to rapidly develop resistance to various acaricides. Increasing levels of resistance to the most commonly used acaricides have caused multiple treatments, including overdoses, raising serious environmental and human health concerns. In this study, a population of the citrus red mite was selected for resistance to fenpropathrin in laboratory, and the resistance increased 29.92-folds after 16 times of selection. Based on the estimation, the realized heritability of fenpropathrin resistance was 0.1544. Theoretically, it required 16 or 8 generations to develop a 10-fold increase in resistance for fenpropathrin under 50–90% selective pressure. Field populations would be expected to require more generations to obtain the same resistance levels. Bioassay showed that this strain had high cross-resistance to pyridaben, dicofol, and azocyclotin, suggested that fenpropathrin had high resistance risk to control *P. citri.* In addition, the roles of some enzymes including CarE, AChE, GST, and MFO in fenpropathrin resistance were also elucidated. The results of this study provided some important information for the resistance management of the citrus red mite.

Comparative studies of acetylcholinesterase purified from various field populations of *Bactrocera dorsalis* (Diptera: Tephritidae)

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Phytopathology 101:S186

Acetylcholinesterace (AChE) is known to be the major target for organophosphate and carbamate insecticides and biomolecular changes to AChE seem to be an important mechanism for insecticide resistance. As one of the most economically important fruit fly pests, *Bactrocera dorsalis* has the potential to invade new areas and to adapt to severe insecticides pressure. In this study, AChE from three field populations of *B. dorsalis* was purified by affinity chromatography and subsequently characterized by its Michaelis-Menten kinetics to determine if detectable changes to AChE have occurred. Bioassays revealed that the potential resistance threat of the fly in Guangdong (GD) was greater than either Hainan (HN) or Yunnan (YN). Compared to the other two populations, the YN population possessed the highest specific activity of purified AChE. Kinetic analyses indicated that the purified AChE from YN population expressed both a significantly higher affinity and a higher catalytic activity to acetylthiocholine iodide than GD and HN populations. *In vitro* studies of AChE suggest five test inhibitors all possess strong inhibitory effects with eserine having the strongest inhibitory effect against purified AChE. According to bimolecular rate constants, the purified AChE from GD population was least sensitive to all inhibitors among the populations. The current results attribute to guide pesticide application and control practices for the oriental fruit fly among the three locations.

Mapping soybean QTL conferring resistance to *Phytophthora sojae* through different phenotypic methods and assessment of their contribution to yield

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Phytopathology 101:S186

Partial resistance to *Phytophthora sojae* in soybean is expressed as reduced infection efficiency, smaller root lesions and reduction in oospore production. This type of resistance is conferred by several quantitative trait loci (QTL). In several host-pathosystems, individual QTL have been reported to be effective towards specific pathogen isolates or influenced by environmental conditions. In addition, the contribution of QTL towards yield is an important factor for selecting QTL candidates for resistance breeding. In this study, QTL in the 'Conrad x Sloan' $F_{4:6}$ population were mapped against three P. sojae isolates using two greenhouse phenotyping assays. Soybean QTL with smaller effects, especially those which originated from the susceptible parent, were not consistently detected among isolates or between phenotyping assays. Of the ten QTL mapped, four QTL on Chrs. 18 and 19 from Conrad had the largest effects and were detected with all three isolates and both phenotyping assays. The RILs with resistant alleles from these four QTL had significantly higher yield than RILs with susceptible alleles. These results indicate the important role of these four QTL in conferring partial resistance to *P. sojae* populations as well as their contribution towards overall yield.

Comparison of genes underlying two QTL conferring partial resistance to *Phytophthora sojae* from resistant and susceptible soybean genotypes

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Partial resistance in soybean is mediated by quantitative trait loci (QTL) which confer broad-spectrum durable resistance to *Phytophthora sojae*. Two

QTL on Chr. 19 in resistant cultivar Conrad were identified. Approximately 160 genes from these regions were amplified with long-range PCR, including 1.2 kb upstream and 400 bp downstream regions, from this resistant cultivar and the susceptible cultivar Sloan. Products ranged from 2-8 kb and were sequenced using Illumina GA. Reads were assembled against the sequenced soybean genome, Williams 82. A total of 1025 single nucleotide polymorphisms (SNPs) were identified from the amplicons between Conrad and Sloan. In comparison to both Sloan and Williams82, Conrad had 304 SNPs in 54 genes, and there were 11 genes in which Conrad sequence variation was unique. Twenty-nine SNPs were selected and verified by designing SNP markers using PCR Amplification of Multiple Specific Alleles (PAMSA) technique. This variation in sequence among these key genes may contribute to the difference in gene functions or changes in expression levels in response to pathogen infection. Expression patterns of 20 genes in these regions in response to inoculation with *P. sojae* will also be discussed. This study provides additional SNP markers for fine mapping and marker-assisted resistance breeding for this trait.

Research on the elimination of CyMV and ORSV from *Phalaenopsis amabilis*

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The production and quality of orchid were influenced by *Cymbidium mosaic virus* (CyMV) and *Odontoglossum ring spot virus* (ORSV) in China. It was necessary to set up the technical system to get the virus-free orchid plant. *Phalaenopsis amabilis* was cultured in vitro firstly. Its formula of culture medium and culture procedure was optimized. The infected plants with CyMV and ORSV were tested by the bioassay and DAS-ELISA, and the tissue culture system of the infected plants was set up separately. the vigor plants were treated with the standard procedures of chemotherapy. The proliferation medium supplemented with a filter-sterilized solution of ribavirin 5, 10, 20, 30 mg/l respectively. 30 days later, Meristems (0.5–1.0 mm diameter) of plantlet were cut and cultured on shoot tip medium. Regenerated plants were tested by ELISA again. Frequencies of virus-free plantlets produced by ribavirin 20 mg/l treatment (85% for CyMV and 64% for ORSV) were higher than those by ribavirin 5 mg/l treatment (35% for CyMV and 0% for ORSV) and ribavirin 10 mg/l treatment (58% for CyMV and 0% for ORSV), similar to those by ribavirin 30 mg/l treatment (90% for CyMV and 71% for ORSV). Survival from ribavirin 20 mg/l treatment (83.6%) was higher than those from ribavirin 30 mg/l treatment (36.8%). When treated with 30 mg/l of ribavirin, the meristem became transparent and brown. The results above suggested that the chemotherapy of ribavirin was effective and could be used in the production of healthy orchid in future.

Temperature effects on appressorial formation of *Colletotrichum cereale* on two turfgrass hosts

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Turfgrass anthracnose, caused by Colletotrichum cereale, is a devastating disease on annual bluegrass (AB) and creeping bentgrass (CRB). Intensified putting green management has increased both incidence and severity of the disease worldwide, yet the biology of the pathosystem remains unclear. An experiment was designed to investigate the effects of temperature on C. cereale appressorial formation on two different hosts. Detatched three-week-old leaves of either AB or CRB were inoculated with a conidial suspension ($2.5*10^6$ conidia/ml) of C. cereale. Inoculated leaves were incubated in dark growth chambers set at one of the following temperatures: 12 C, 18 C, 22 C, 26 C, 30 C and 34 C with high relative humidity (>95%). Twenty-five arbitrarily selected conidia were examined microscopically for appressorial production at 6, 12, 24, 48 and 72 hours after inoculation. Appressoria were observed in each temperature treatment, but developed most rapidly between 18 C and 26 C. At 30 C and 34 C, apressorial development occurred, although development was significantly hindered when compared to the other temperature treatments These results suggested that C. cereale may infect AB and CRB well prior to the onset of symptoms.

QTL analysis for transgressive resistance to root-knot nematode in a cotton RIL population derived from interspecific susceptible parents (*Gossypium* spp.)

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The root-knot nematode (RKN, *Meloidogyne incognita*) is a major parasite of cotton, causing significant yield losses in most production areas. A genetic standard recombinant inbred population of 138 lines developed from a cross between Upland cotton TM-1 (*Gossypium hirsutum* L.) and Sea island cotton Pima 3-79 (*G. barbadense* L.), both susceptible to RKN, was used to identify responses to RKN in two greenhouse experiments. Compared to both parents, 50.7% and 51.4% of lines showed less galling index and lower nematode egg production, respectively. Highly resistant lines were identified in the RIL population. Four quantitative trait loci (QTLs) accounting for 7.2 to 13% of the phenotypic variance (R^2) in galling index, and two QTLs accounting for 6.7% and 8.4% egg production variance were identified based on interval mapping (LOD score ≥2 and Kruskal-Wallis (KW) analysis ($P \leq 0.005$). These QTLs were located on chr3, 4 and 17 for galling index and chr14 and chr23 for egg production. In addition, 15 putative QTL accounting for 3.8% to 5.8% ($2>LOD\geq1$ and $P \leq 0.05$) of phenotypic variance in galling index, and 12 QTLs accounting for 3.2% to 5.2% in egg production were identified. In lines with combinations from both parents of 2 to 5 QTL with positive alleles, dramatic reductions of > 50% in both root galling and egg production were recorded. These epistatic effects in progeny derived from susceptible parents indicate that pyramiding these QTLs present a new level of nematode resistance in cotton.

Nematode community analysis for soil ecosystem health prediction

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Nematodes are good indicators of the structure and function of the soil ecosystem, and provide a reference for soil health conditions. However, performing nematode community analysis is laborious and technically challenging. The overall goal of this project is to develop a molecular tool that might replace conventional nematode community analysis. The first step of this approach is to identify four soil ecosystems in Hawaii with distinct soil health conditions, and to verify the reliability of conventional nematode community analysis. Four ecosystems examined including a forest site (dominated by *Eucalyptus* spp.) at Lyon Arboretum, Manoa, a low maintained collard green (*Brassica oleracea*) field in an organic site at Waimanalo, a pineapple (Ananas comosus) field previously planted with sunn hemp (*Crotalaira juncea*) at Whitmore, and a beach site dominated by naupaka (*Scaevola taccada*) bushes at Hanauma Bay. Four replicated subsamples were collected from each site. Nematodes were extracted by elutriation, counted to genus and assayed by nematode community analysis. Among the nematode community indices, richness, total abundance, percentfungivores, percent omnivores, enrichment index and channel index segregated ($P < 0.05$) the four ecosystems as anticipated. These indices would provide a standard to verify future molecular tool for nematode community analysis.

***Pcg2*, a novel pathogenicity gene in *Magnaporthe oryzae* encodes a transcription factor that activates and represses expression of distinct genes**

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APSES proteins are a class of transcription regulators specific to fungi. In this study, *PCG2* encoding an APSES transcription factor was isolated by T-DNA mutagenesis and was functionally characterized in *Magnaporthe oryzae*. The Δ*pcg2* mutant show defects in hyphal growth, conidiogenesis, and reduction in virulence. Pcg2 is a protein localized in nuclei with two transcription activation domains, and could bind both the MCB-box and the SCB-box. Microarray analysis revealed that expression of 188 genes with either or both of the two boxes were activated or suppressed over two-folds in the Δ*pcg2* mutant, indicating that Pcg2 is a transcription repressor and activator. Pcg2 has two forms in mycelia, the full-length $Pcg2^{715}$ and the truncated $Pcg2^{530}$. The full-length $Pcg2^{715}$ was confirmed to function as a transcription activator and the truncated $MoPcg2^{530}$ as a transcription repressor. *MoBTB1*, a novel gene with one MCB-box in its promoter region is activated in the Δ*pcg2* mutant, and its constitutive expression resulted in slower hyphal growth of transformants. *MoUNQ1*, a novel specific gene to *M. oryzae* with one MCB-box in its promoter region is suppressed in the Δ*pcg2* mutant and deleting this gene resulted in both slower hyphal growth and reduction in virulence. Our study is significant not only for identifying a novel pathogenicity gene but also for providing new insight into the mechanisms on how a protein functions as a transcription activator and repressor.

Identification and characterization of *Pectobacterium* species causing potato blackleg disease in North China

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Thirteen pectolytic strains were isolated from diseased plants, sampled from 7 different potato fields located in Inner-Mongolia, GanSu and HeBei provinces, and confirmed on CVP agar plate. By using the strain *P. atroseptica* SCRI 1043 as positive control and based on the test of pathogenicity, ELISA, physiological and biochemical characteristic detection, 16S rDNA sequence analysis and specific PCR, it revealed that all strains were pathogenic to potato tubers and seedlings on three potato varieties after inoculation in greenhouse, and lead to similar symptoms as SCRI 1043. All of the nearly complete 16S rRNA gene sequences of the strains were determined to be 1490 bp, and sequence identity from 12 out of 13 strains range from 99%–100%, compared to Pa SCRI 1043. Only one strain (DL07) from Inner-Mongolia showed 97%–99% identity to the species of *P. carotovora* in GenBank. Twelve of 13 strains were ELISA positive tested by Pa monocolnal antibody (Agdia) except DL07. The physiological and biochemical characteristic and specific PCR tests revealed the similar results as ELISA. In PCR assay, the DNA of DL07 can be amplified by *P. carotovora* species-specific primers and yielded the expected amplicon, but not by published Pa specific primers. In conclusion, 12 strains can be identified as *Pectobacterium atroseptica*, and DL07 was considered as atypical *P. carotovora*. All of them were the pathogen and responsible for the occurrence of potato blackleg diseases in North China.

Grosmannia clavigera, a mountain pine beetle associated pathogen, has efficient ABC transporters for excreting monoterpenes or their derivatives

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Western North American pine forests are being devastated by the mountain pine beetle and its associated fungi. *Grosmannia clavigera*, one of the most pathogenic fungi in this epidemic ecosystem, survives the toxic terpene/phenolic defense chemicals produced by host pines. Further, *G. clavigera* can kill pine trees when inoculated without the beetle. With the availability of the genome and transcriptome sequences of *G. clavigera*, we identified fungal genes that are important in the host-pathogen interaction. The fungus responded to application of terpenes by regulating enzymes (e.g. CYP450s) and membrane proteins like ABC transporters. Here, we describe the ABC transporters in the *G clavigera* genome, and report their transcription profiles under different terpene treatments using RNA-seq data. We functionally characterized one PDR transporter (GcABC1), which is highly induced (>100 fold) by monoterpenes. Both gene knockout and a yeast heterologous expression demonstrated this gene confers tolerance to monoterpenes. While the *G. clavigera* wild type grows with monoterpenes as a single carbon source, the mutant (GcABC1) cannot survive under these conditions, despite having other mechanisms to detoxify or utilize monoterpenes. We conclude that GcABC1 is a potential monoterpene efflux transporter that plays a critical role in removing toxic monoterpenes or their modified products from the fungal cell, allowing the fungus to grow in the presence of pine defense chemicals.

A common scab resistant potato cultivar is not explained by pathogen growth in soil or window of infectivity

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Potato cultivars differ in resistance to common scab (CS); this might be due to differential growth of the pathogen at the plant root, or a shorter susceptibility window in resistant potatoes. Resistant (Superior) and susceptible (Chippewa) potato cultivars were inoculated by watering with a *Streptomyces* spore suspension at 10-fold differences in density over 5 orders of magnitude at planting or at biweekly intervals for 12 weeks in pot experiments conducted in a greenhouse. Tubers initiated 6 weeks after planting. CS severity was recorded on tubers 16 weeks after planting. The experiment was performed twice. Disease pressure was higher in the second experiment, but CS disease and *Streptomyces* growth patterns were similar. Quantitative PCR data from soil DNA extracted at 2 week intervals showed similar growth and timing of *Streptomyces* clinging to soil of both susceptible and resistant plants, while disease incidence and severity were very different. No clear window of susceptibility could be recognized in either cultivar, although disease was greatest when plants were initially treated at medium-high inoculum densities 2 to 4 weeks after planting. CS severity was lower at the highest inoculum density in both resistant and susceptible plants. The results showed that CS resistance was not explained through an inhibition of pathogen growth in soil on the root surface or by a shorter window of pathogen susceptibility in the resistant cultivar.

Development of an *in vitro* bioassay to screen *Prunus* spp. for resistance to *Armillaria ostoyae*

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Armillaria root rot is an important fungal disease of stone fruit trees within the genus Prunus. In Michigan, Armillaria ostoyae has been identified as the most prevalent species infecting tart cherry. There are no effective chemical controls available to the cherry industry, thus resistant rootstocks must be developed to reduce the decline and loss of tart cherry orchards due to Armillaria. An in vitro bioassay was developed to screen Prunus spp. for resistance to A. ostoyae. Seventeen Prunus spp. were evaluated for resistance to six strains of A. ostoyae. Branch segments (3cm) from two year-old wood were placed adjacent to the leading edge of a 14 day-old A. ostoyae culture grown on yeast malt peptone glucose medium. The plates were incubated for 10 days at 25°C and then evaluated for penetration of the fungus into the host wood. The branch segments were cut longitudinally, and the periderm and cambium layers were peeled back to assess the extent and location of penetration by the fungal mycelial fans. The average range of mycelial fan penetration by the six A. ostoyae strains among the Prunus spp. was 9–18 mm, with the exception of P. maackii which exhibited an average penetration of 3.72 mm. Based on these results, P. maackii exhibited the highest level of resistance to A. ostoyae. Our data corroborate with previous research findings from in planta screening, and thus shows promise as a quick and reliable technique to screen Prunus spp. breeding stock.

Construction of plasmid based expression vectors for the production of recombinant proteins in *Xylella fastidiosa*

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Recombinant production of certain *Xylella fastidiosa* (Xf) proteins has proven difficult in commercially available *E. coli* and *P. pastoris* expression systems. Xf polygalacturonase (PG) is one of these proteins. Xf possesses a single PG gene and it was shown that if the gene encoding Xf PG was disrupted, the resulting PG-mutant was completely non-pathogenic in grapevines. Thus, identifying peptides or proteins that could inhibit the activity of Xf PG may provide a viable means for protecting grapevines from Pierce's Disease. To identify such PG inhibitors, it is necessary to produce adequate quantities of enzymatically active Xf PG. In order to express active Xf PG, we constructed a plasmid-based Xf protein expression system. These expression plasmids are based on the pBBR1MCS and pPROBE broad host range cloning vectors and use the constitutive nptII promoter to drive protein expression. Green fluorescent protein (GFP) reporter constructs made using this system stably expressed GFP in Xf under antibiotic selection. We are currently screening our Xf PG constructs to determine if they will express biologically active Xf PG.

House fly regurgitation spots may be a source of *E. coli* O157:H7 contamination of leafy greens

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Filth flies are known mechanical vectors of human enteric bacteria in hospital and restaurant settings. However, the role of flies in the movement of human pathogens to pre-harvest food plants is largely unknown. The fate of an attenuated strain of *E. coli* O157:H7 acquired by the house fly, *Musca domestica*, from contaminated manure and deposited on spinach via regurgitation spots was studied by molecular methods and scanning electron microscopy. Regurgitation spots were excised from spinach plants at 0, 4, 8, and 12 days after deposition by flies. Retention of bacteria on fly body parts was studied by relative quantitative PCR analysis of the *eae*gene. It revealed that *E. coli* numbers in the regurgitation spots increased from day 1 to day 4, then dropped to levels comparable to the negative control spots. The same *E. coli* strain when acquired by flies from LB ampicillin plates did not increase in number, suggesting that manure-acquired *E. coli* O157:H7 was more capable of replication on the spinach surface than plate-acquired bacteria. Scanning electron micrographs of regurgitation spots from flies that acquired from contaminated manure show bacteria-like organisms embedded in a dense matrix of regurgitated manure. These results suggest that filth flies may pose a risk for contamination of leafy greens with *E. coli* O157:H7.

Influence of genetic background of bacterial blight resistance gene *Xa7* on population and movement of *Xanthomonas oryzae* pv. *oryzae*

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Xanthomonas oryzae pv. *oryzae* (*Xoo*) is the causal agent of rice bacterial blight, a destructive rice disease worldwide. Although the *Xa7* gene has not been cloned, the gene is a potential source of broad and durable resistance to the disease. In this study, the function of resistance gene *Xa7* to *Xoo* T7174 (Japanese race IA) and the effects of genetic background of *Xa7* on bacterial growth and movement were analyzed. Plants with *Xa7* in different genetic background, susceptible cultivar *indica* IR24 and *japonica* T65, were inoculated with *Xoo* T7174 using the leaf clipping method. No significant difference in lesion length of the plants harbored *Xa7* in *japonica* (T65-*Xa7*) and *indica* (IR24-*Xa7*) background was found. However, *Xoo* T7174 population in T65-*Xa7* was higher than that of IR24-*Xa7*, suggesting that genetic background may play a role in resistance meditated by *Xa7*. To examine the effect of heterozygous (*Xa7xa7*) and homozygous (*Xa7Xa7*) on the disease resistance, bacterial population dynamics throughout the inoculated leaves with *Xoo* T7174 wild type and *Xoo* T7174 expressing green fluorescent protein (GFP) were investigated by cutting the top 15 cm of each leaf below the point of inoculation into three 5-cm sections. The results showed that bacterial movement in *Xa7* genotypes were not significantly different. In conclusion, bacterial population but not movement was influenced by the genetic background of *Xa7*.

Detection of sour skin of onion, caused by *Burkholderia cepacia*, using zNose technology

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In Georgia, sour skin of onion (*Allium cepa*), caused by *Burkholderia cepacia*, is responsible for the losses of onions in storage units that have a capacity of 40,000 bushels. Removing and re-grading onions can be an effective management strategy if the disease is detected early. zNose technology was explored as a new approach for the rapid detection of sour skin in stored onions. The zNose is a portable gas chromatograph used to rapidly identify volatile compounds in head space and can produce a volatile gas profile for the air in the storage room. Research was conducted in the spring of 2010 using Peruvian sweet onions and zNose technology to evaluate the differences between healthy and diseased onions. Surface disinfested onion bulbs were inoculated using a sterile toothpick contaminated with a 24 hour culture of *B. cepacia* which was inserted ~1cm into the shoulder of the onion bulb. Inoculated onions were incubated in sealed 2L glass jars for 72 hours, data were collected at 48 and 72 hours post inoculation. Also, onion bulbs infected with 10 different strains of *B. cepacia* were evaluated to determine if variation in volatile profiles was strain related. Results showed that the zNose can quantitatively differentiate between healthy onions and onions infected with *B. cepacia* after 3 days of incubation. There were no qualitative differences among volatile profiles produced by the 10 strains of *B. cepacia*.

A novel M RNA reassortant of *Groundnut ringspot virus* and *Tomato chlorotic spot virus* infecting vegetables in Florida

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Groundnut ringspot virus (GRSV) was recently identified using serology and nucleocapsid gene sequence from tomato plants with severe tospovirus symptoms in south Florida, which extends the geographic range of this virus from South America and South Africa to now include North America. Full genome sequence analysis demonstrated that the Florida GRSV isolate was actually an M RNA reassortant with the S and L RNA segments coming from GRSV but with the M RNA segment coming from *Tomato chlorotic spot virus* (TCSV), a related but genetically distinct tospovirus species described in South America. This is the first report of a natural reassortant (i.e. $L_GM_TS_G$) between two tospovirus species. Regions of each of the three genomic RNA segments were sequenced to confirm that the $L_GM_TS_G$ genotype was present in tomato samples collected in five south Florida counties starting in December 2009. The $L_GM_TS_G$ genotype was also detected in pepper and tomatillo with typical tospovirus symptoms in December 2010. Western flower thrips (*Frankliniella occidentalis*) transmitted the $L_GM_TS_G$ genotype and other thrips species are currently being investigated for their ability to transmit this virus. Neither parental genotype (GRSV or TCSV) nor alternate reassorted genotypes have been detected in any samples. These results suggest that $L_GM_TS_G$ was introduced to the U.S. in its current form and that reassortment between distinct tospovirus species may be more frequent than previously thought.

Evaluation of leaf blight-resistant plant introductions of *Brassica juncea* and *Brassica rapa* and elucidation of inheritance of resistance

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Brassica leafy greens (*Brassica juncea* and *Brassica rapa*) represent one of the most economically important vegetable crop groups in the southeastern United States. In the last 10 years, numerous occurrences of a leaf blight disease on these leafy vegetables have been reported in several states. One of the pathogens responsible for this blight is *Pseudomonas cannabina* pv. *alisalensis* (Pca). Two *B. rapa* (G30710 and G30499) and two *B. juncea* (PI418956 and G30988) plant introductions (PI) with moderate to high levels of resistance to this pathogen in greenhouse studies were tested for field resistance in comparison to eight commercial cultivars of *B. rapa*, *B. juncea* and *B. oleracea*, which include turnip greens, mustard greens, collard and kale. The two *B. juncea* PI and one of the *B. rapa* PI (G30499) were found to have significantly less disease than all tested cultivars except Southern Curled Giant mustard (*B. juncea*) and Blue Knight kale (*B. oleracea*). Inheritance of resistance studies performed with populations derived from the resistant G30988 and two susceptible, rapid-cycling PI indicate that the resistance is probably multigenic.

Making foliar fungicide applications to corn consistently profitable in Illinois

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Foliar-applied fungicide use on corn has increased recently in the North Central U.S. In part, this is due to increased marketing and promotion of fungicides to corn growers. In some cases, fungicides are being applied based on the potential of increasing yields without considering foliar disease risks or field scouting. To determine the effect of fungicides on corn yields in Illinois, trials were conducted at multiple sites across the state in 2008–2010. Products evaluated included quinone outside inhibitor (QoI) fungicides, demethylation inhibitor (DMI) fungicides, and QoI-DMI mixtures. Fungicides were applied between tassel emergence and silking, and disease severity was measured (% ear leaf area affected) approximately 21–30 days after application. In total, fugnicides were evaluated in 21 different environments, which were divided into three different categories based on final disease severity levels on the non-treated controls (<10%, 10–15%, and >15%). Fungicide applications were considered profitable if the yield response was at least 600 kg/ha. The mean yield response was 6, 419, and 949 kg/ha in low, medium, and high disease intensity environments, respectively. A yield response of at least 600 kg/ha was achieved 14%, 17%, and 75% of the time in low, medium, and high disease intensity environments, respectively. These results indicate that disease should be considered in order to achieve consistent profitable yield responses with foliar fungicides.

Identification of yeasts associated with grape sour rot in the north of China

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Sour rot becomes a very serious problem for grape growers in main grape producing area of China recently. It attacks grape berries and has a damaging effect on wine quality. The objective of this study was to identify yeast species associated with grape sour rot. The twenty-six strains of yeast were isolated by the tissue culture technique from the samples (diseased fruits and pupae of Drosophila spp.), which were taken from four different vineyards in the north of China. To determine the pathogenicity of the isolated yeast strains, artificial inoculations were carried out in laboratory. Yeast identification was done by classical and molecular methods. On the basis of 26S rDNA D1/D2 domain sequence analysis, morphological and physiological characteristics, the yeast isolates were identified as follows: Pichia fluxuum, P. membranaefaciens, P. novegensis, Schizosaccharomyces pombe, Issatchenkia orientalis, I. terricola, I. scutulata, Arthroascus javanensis, Cyptococcus magnus and Hanseniaspora uvarum. Supported by the earmarked fund for Modern Agro-industry Technology Research System (nycytx-30-bc-03).

Distribution and sequence analysis of the rDNA-ITS region of cereal cyst nematodes from different locations in China

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An extensive survey on cereal cyst nematode (CCN) occurring in Jiangsu, Anhui, Shandong and Henan Province was carried out. The results indicated that CCN was detected in all 30 samples and the cyst density range was 1-80/100 g soil. The rDNA-ITS regions of the 30 CNN populations were amplifiled by PCR and the sequences were analyzed. The similarities of nucleotide sequences of 30 isolates are 97.7% to 100%. The phylogenetic tree of 30 CCN populations and other related species of Heterodera spp. reported in GenBank was constructed based on its rDNA-ITS sequences. The 30 CCN populations and the published CCN populations from China (AY148382, EU106175), Australia (AY148395) and Russia (AY148351) were clustered in the same group, showing high homology level in evolution. Characters of cereal cyst nematode (CCN) populations occurred on wheat in Jiangsu and other provinces were identified with molecular methods and might provide theoretical basis for control of the CNN disease.

Development of PCR assay using simple sequence repeat primers for detection of '*Candidatus* Liberibacter solanacearum'

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Genetic variations of "*Candidatus* Liberibacter solanacearum (Lso)", the bacterium associated with the zebra complex of potato have been detected and differentiated based on simple sequence repeat (SSR) genotyping markers and sequencing of the amplicons of 16S-ISR-23S rRNA gene. More recently, 3 haplotypes have been reported with two present in North America, one in New Zealand and a third haplotype recovered from carrot in Finland. Current Lso detection assays are based on the 16S rRNA or 23S-ISR rRNA gene, since limited genetic variation exists in these regions among isolates, differentiating Lso strains has to be relied on sequencing the PCR amplicons which is expensive and time-consuming. In this study, a PCR assay was developed for both Lso strain detection and genotyping using one of our SSR primer pairs. The low detection limit of the PCR assay was approximately 100 copies of the target templates per reaction. This assay is more sensitive than previously published PCR assays using 16S, ISR or 23S rRNA gene and is able to distinguish two types of Lso by comparing PCR products on an agarose gel. This PCR assay has been validated using fresh or archived plant and psyllid samples associated with zebra complex disease obtained from potato commercial fields in the U.S.A. and Mexico. Both potato and psyllid samples were shown to have either type 1, type 2 or type 1 plus type 2 Lso.

MCW-2 for management of root-knot nematode on carrot, tomato and cucurbits

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Five RCB field trials with 5 replicates were conducted to evaluate the effectiveness ($p = 0.05$) compared to an untreated control (UC) of MCW-2 for management of root-knot nematode (RKN), *Meloidogyne javanica*, on carrot, tomato, squash, cucumber, and cantaloupe. Treatments in all trials were MCW-2 at 2, 3, 4, 6, and 8 kg ai/ha, oxamyl at 4.7 l/ha, metam sodium (MS) at 589 l/ha, 1,3-dichloropropene (1,3-D) at 84 l/ha and UC. 1,3-D was injected 14-days preplant. MS, MCW-2 and oxamyl were applied 7-days preplant followed by rototilling and sprinkler irrigation. Evaluations were conducted at harvest. The 3 and 8 kg rates of MCW-2 had a higher percent of marketable carrots. All MCW-2 rates and 1,3-D had fewer RKN. On tomatoes, 4 kg MCW-2 had a greater weight of fruit plus foliage. MS had a greater weight of fruit plus foliage and a greater weight of fruit. 3, 4, and 8 kg MCW-2 and 1,3-D had a lower root gall rating (RG). 4 and 8 kg MCW-2 and 1,3-D had fewer RKN. On cucumbers, 4 and 8 kg MCW-2 had a greater number, weight and size of fruit. MCW-2 at 2, and 4 kg had a lower RG. All treatments had fewer RKN. On squash, fruit size was greater for 4 and 8 kg MCW-2. MCW-2 at 2, and 4 kg had a lower RG. All treatments had fewer RKN. On cantaloupe, all treatments except 3 kg MCW-2 and oxamyl had a greater fruit weight. MCW-2 at 4 kg and MS had a larger number of fruit. MCW-2 at 3 kg had larger fruit. At 2 and 8 kg MCW-2 had a lower RG. All treatments had fewer RKN.

Screening newly released Northwest Potato Variety Development Program cultivars for resistance to Pythium leak

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Pythium leak caused by *Pythium ultimum* has been a major storage disease problem in the Pacific Northwest (PNW) potato production areas for many years, but in recent years reported losses have increased. In storage, *Pythium* infected tubers rot quickly and create wet areas which enhances tuber breakdown, leading to severe losses. Current measures for controlling Pythium leak include fungicide applications of mefenoxam to foliage. Recently, mefenoxam resistant strains of *P. ultimum* have been found in PNW potato production areas. One potentially effective approach for controlling storage rots is through the use of resistant cultivars. In recent years, the Northwest Potato Variety Development Program (NPVDP) has developed new cultivars with higher disease resistance than the standards, e.g. Russet Burbank and Russet Norkotah. The objective of this study was to screen newly released NPVDP cultivars against Pythium leak to see if there are any differences in resistance to the disease compared to the standards. Tubers from each cultivar (Classic Russet, Clearwater Russet, Alpine Russet, Russet Norkotah, Premier Russet, Gem Russet, Alturas Russet, and Ranger Russet) were inoculated with P. *ultimum* by immersion in an inoculum suspension for 36 h. Tubers were then stored at 15°C for 2 weeks before being rated for disease incidence and severity. Results showed that several of the new cultivars had less than 5% disease in storage.

Control of potato early blight tuber rot using post-harvest fungicide treatments

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Early blight of potato caused by *Alternaria solani* is a common foliar disease found in most U.S. potato growing areas. Although primarily recognized as a foliar pathogen, *A. solani* can cause tuber lesions in certain cultivars. Tuber symptoms of early blight include circular to irregular lesions that are slightly sunken and often surrounded by a raised border. The underlying tissues are leathery to corky in texture and usually dark black with a yellow border. Lesions reduce the quality and marketability of fresh market tubers and present a challenge to potato processors as tuber lesions often require additional peeling to remove the darkened lesions and underlying tissues. The cultivar Western Russet is highly susceptible to both foliar and tuber early blight and growers have struggled to control the disease on tubers after placing potatoes in storage. The objective of this study was to screen a range of pre- and post-harvest fungicides for the control of tuber early blight. Naturally infected tubers were artificially bruised by tumbling and then treated with a range of fungicides before being placed in storage at 15°C for 3 months. Results showed that effective control of tuber early blight with registered post-harvest fungicides is possible. Tubers treated with phosphorous acid had significantly fewer lesions and smaller lesion diameters than the untreated control. These results and the results of the other products tested are presented and discussed.

Potato virus Y resistance from Ry_{adg} and Ry_{sto} genes: Practical application in a potato breeding program

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In the Aberdeen Potato Breeding Program two different sets of clones and cultivars were tested for Potato Virus Y (PVY) R-gene markers. The first set of eighteen breeding clones/cultivars were selected based on *Solanum tuberosum* ssp. *andigena*, S. *stoloniferum* background, or known PVY resistance. R-genes in these species are reported to confer resistance against all PVY strains (extreme resistance). These clones/cultivars have been utilized in the breeding program as parents in a PVY resistance crossing block. A second set of clones derived from other parents with evidence of PVY resistance were selected based on acceptable agronomic characteristics. Both sets of were tested with SCAR marker RYSC3 for Ry_{adg} and an SSR marker STM0003 for Ry_{sto}. All entries were further characterized by their PVY reactions in the field or by grafting in the greenhouse. Strategies to hybridize marker-confirmed PVY resistant parents with superior agronomic parents should result in higher frequencies of selected clones with extreme PVY resistance and desirable agronomic traits. Confirming PVY markers after initial agronomic selection would eliminate the need for greenhouse and field bioassays to determine resistance to individual PVY strains. Results show initial selection of ten russet types for processing or fresh pack and one for specialty. Of these eleven selections, three have markers for Ry_{adg} and Ry_{sto} genes, while the remainders have a marker for either one of the genes.

Variation in copy number, expression, and sequence of *Avr1a*/*avr1a* among populations of the oomycete plant pathogen, *Phytophthora sojae*

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Phytophthora sojae has reemerged as a prominent pathogen in some areas of the Midwest due to the pathogen's ability to adapt to many of the resistant (Rps) genes, deployed in soybean cultivars. Recent research identified several *Avr* genes namely, *Avr1a*, *Avr3a* and *Avr3c*, and showed they belong to the RXLR family of effectors. Several mechanisms by which these effectors may contribute to changes in virulence in pathogen populations were proposed including the copy number variation of avirulence genes, differential regulation of the transcription of the genes and changes in amino acid composition of the proteins. However, this research only evaluated a few standard isolates. We compared the *Avr1a* locus across field isolates of *P. sojae* from Iowa and Ohio to discern which mechanism(s) maybe more critical at the population level. Preliminary data from both Iowa and Ohio suggest that the variation in copy number in the putative *Avr1a* gene may not be a major contributor to the variation in the avirulence/virulence response towards Rps1a.

Sporulation potential of *Phytophthora kernoviae* compared to *P. syringae* and *P. cactorum* on selected hosts

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Phytophthora kernoviae (Pk) is presently only found in the United Kingdom and New Zealand. There is concern that serious ecological damage could occur if it should enter the U.S. However, very little is known about the biology of this species. Oospore and sporangia production of Pk was compared on *Rhododendron*, *Magnolia tripetala*, *Liriodendron tulipifera*, and *Kalmia latifolia* with two other species, *P. syringae* (Ps) and *P. cactorum* (Pc). Leaf disks of each host were inoculated with Pk, Ps, or Pc zoospores and set at 20 C in the dark. After 1 wk, the formed sporangia or oospores were counted. On *Rhododendron* and *M. tripetala*, Pc produced more than five times as many oospores as either Pk or Ps. On *L. tulipifera*, all species produced approximately the same amount, while on *K. latifolia*, Pk and Ps oospore production was almost nonexistent. Production of sporangia was different, resulting in significantly higher numbers for Pk on all hosts, except for Pc on *Rhododendron*, which was similar. These results show that secondary inoculum production of *P. kernoviae* via sporangia and oospores is more or similar to that of species already present in the U.S., and that the host plays a significant role.

Management of *Phytophthora ramorum*-infested nursery soil with *Trichoderma asperellum*

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Disinfestation of *Phytophthora ramorum*-infested nursery sites is hindered by the inability to mitigate soil, a potential factor in recurrent infections. Current APHIS-PPQ protocols for disinfection include steam sterilization, soil fumigation, or the application of cement over infested soil. However, we present a more feasible alternative for soil mitigation that is being investigated. Recent laboratory studies showed that *Trichoderma asperellum* (TA) can reduce soil populations of *P. ramorum* to non-detectable levels within 2 wk. To demonstrate the effectiveness of TA in a nursery setting, fall and spring field trials were carried out at the National Ornamentals Research Site at Dominican University of California. There, forty microplots were inserted into natural field soil in a raised bed and infested with *P. ramorum* chlamydospores 1 wk prior to treatments. Five treatments, including a non-treated control, a chemical, two commercially-available biological control products, and an experimental *T. asperellum* isolate (TA1) were applied separately to soil within a given plot in a randomized split-plot design. Soil samples were collected over time and assayed to monitor *P. ramorum* and *Trichoderma* spp. populations. After 2 wk, *P. ramorum* populations declined in the plots treated with TA1, while TA1 populations increased over time. These preliminary results suggest that *T. asperellum* is a promising alternative for managing *P. ramorum* populations in nursery soils.

Comparison of culture based and culture independent methods for identifying *Rhizoctonia solani* AG2.1 and 3 inhabiting infected plant material of potato

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Rhizoctonia solani causes stem and stolon canker on potato plants, as well as black scurf on potato tubers. AG2.1 is prevalent as mycelium and AG3 is less prevalent and survives as melanised sclerotia in potato field soil. Historically, AG3 is the most dominant anastomosis group isolated from potato plants but AG2.1 may not be easily isolated and therefore not implicated in causing stem and stolon symptoms. Cultivation-based analysis captures only isolates that are readily culturable or fast growing strains while cultivation-independent PCR based analysis allows the detection of non culturable or slow growing strains providing a more complete picture of the infecting strains. AG2.1 and AG3 were inoculated into soil in combination at various rates and one cv, Coliban minituber was planted per pot with 5 replicate pots, and grown in the glasshouse. Emergence was assessed 4 weeks after planting. Stolon pruning and stem canker lesions were assessed 4 times at 5, 8, 11 and 14 weeks after planting. Infected potato plant parts were divided into two with half being used for isolation of a culture and the other half being used directly for DNA isolation. qPCR analysis of cultures isolated from infected tissue found that AG3 is the most common strain. Direct qPCR of infected plant material found that AG3 is the most common strain but the prevalence of AG2.1 increased using this technique. This research reveals that AG2.1 may be implicated in disease of potato.

The nature of the relationship between Soybean Cyst Nematode population densities and soil pH

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A consistent positive relationship exists between soil pH and soybean cyst nematode (SCN), Heterodera glycines, egg population densities in the field. The basis of this relationship is not understood. Soil pH may directly or indirectly affect SCN or the host status of soybean, and the relationship could involve other soil factors closely related to soil pH. To determine how quickly the relationship develops, greenhouse experiments were conducted using three different soils with low (5.6), medium (6.1), and high (7.5) pH. Soils were infested with 4,000 SCN eggs per 100 cm^3 soil. After 30 days (time for one SCN generation), there were two and a half to five times more SCN females and eggs on a susceptible and a resistant soybean variety in the high pH soil than the medium and low pH soils, which were not significantly different. There were no differences in the numbers of eggs per female among the three soils or the two soybean varieties. Results were similar after 60 days. The results indicate that soil pH-SCN population density relationship develops quickly, which may imply that a direct effect is occurring. The experiment is currently being repeated and new experiments are underway in which SCN-infested soil with a pH of 5.5 was amended with three different rates of lime to increase the soil pH. SCN population densities and soil pH will be assessed at 30 and 60 days on resistant and susceptible soybeans grown in the soils receiving the different rates of lime.

Revisiting flag leaf-based foliar fungicide application thresholds for Stagonospora nodorum blotch management in soft red winter wheat

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Field trials were established in Illinois, Indiana, Ohio, and Wisconsin during the 2009/10 season to evaluate associations among disease severity at different crop growth stages and leaf positions and grain yield in an effort to establish fungicide decision thresholds. A randomized complete block design was used with two soft red winter wheat cultivars, one susceptible and one resistant to Stagonospora nodorum blotch (SNB), serving as whole plot. Plots were inoculated at either Feekes 6 or 9 (sub-plot) using four inoculum densities (sub-sub-plot): an uninoculated check, 125,000, 250,000 and 500,000 conidia/ml. SNB severity was assessed at weekly intervals at three positions in the wheat canopy: on the flag leaf and the two leaves below the flag. In general, SNB increased over time and decreased from the lower to upper canopy. Based on preliminary results from linear mixed model covariance analyses, disease severity throughout the canopy and at various growth stages had significant ($P < 0.05$) negative linear relationships with yield. Lower canopy disease severity had a positive linear relationship with upper canopy severity in many cases. This suggested that disease severity on lower leaves in the canopy could potentially be used to make early foliar fungicide application decisions to minimize the risk of severe damage to the flag leaf. Preliminary disease-yield models and fungicide decision thresholds based on these models will be discussed.

Efficacy of pre-flag leaf emergence foliar fungicide application for Stagonospora nodorum blotch management in soft red winter wheat

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The objective of this study was to evaluate claims that foliar fungicide applications prior to flag leaf emergence provide yield and economic benefits and adequate foliar disease control in soft red winter wheat. Field trials were established in Illinois, Indiana, Ohio and Wisconsin during the 2009/10 season. Two cultivars, one susceptible and one resistant to Stagonospora nodorum blotch (SNB), were planted in a randomized complete block design, with cultivar as whole-plot, fungicide treatment as sub-plot, and inoculation-timing as sub-sub-plot. There were nine foliar fungicide treatments: separate full-rate applications of Headline and Prosaro at Feekes 5, 8, and 10; double half-rate applications of each fungicide at Feekes 5 and 8; and an untreated check. Cultivar was statistically significant ($P < 0.05$) for SNB severity in IL and OH and for yield in IL. Fungicide treatment had significant effects on SNB severity in IL, IN and WI, significant effects on yield in IL and OH, and marginal significant effect on yield in WI ($P = 0.069$). The effect of inoculation was not significant for SNB or yield at any location. Overall, the resistant cultivar had lower disease severity and higher yields than the susceptible cultivar. Fungicide applications at Feekes 8 or 10 resulted in lower SNB severity and greater yields than the check. In general, applications at Feekes 5 were not significantly different from the untreated check in terms of SNB severity and yield.

Development of an electronic-nose technology for the rapid detection and discrimination of subterranean termites within wood in service

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The application of effective IPM controls for subterranean termites and fungal decays of wood in service requires the detection and identification of these pests within the hidden walls and floor boards of buildings. Our objective was to developed methods for the detection and identification of four subterranean termite species, including *Coptotermes formosanus*, *Reticulitermes flavipes*, *R. hageni*, and *R. virginicus*, using an electronic-nose technology based on headspace volatiles released from termite bodies. Analyses were conducted with an Aromascan A32S electronic-nose instrument fitted with a 32-sensor array and 15 volts across sensor paths. Headspace volatiles from 25-50 individuals were collected in a 500 ml sampling bottle and run for 120 s at 4% RH and 25 C. Three-dimensional principal component analysis (PCA) differentiated the four termite species with the first two principal components accounting for more than 98% of the sample variability ($P < 0.01$). Unique electronic aroma signature patterns (EASPs) were produced from the sensor array output that effectively distinguished between termite species based on specific combinations of volatile metabolites released from their bodies. The electronic-nose methods developed here provide a novel non-invasive (nondestructive) means for the detection and identification of relatively small infestations of termites present in wood in service by sampling termite volatiles released into the void spaces in the internal walls and floors of buildings.

Effectiveness of early-season fungicide programs for the control of *Sclerotinia homoeocarpa*, the causal agent of dollar spot

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Dollar spot, caused by *Sclerotinia homoeocarpa*, is the most important turfgrass disease in the United States with respect to fungicide expenditures. Single early-season applications delay symptom development, but do not provide season long control of dollar spot. This field study compares the efficacy of a conventional dollar spot fungicide program to early-season programs. This study was conducted at the O.J. Noer Turfgrass Facility and at Milwaukee Country Club in Wisconsin. Conventional applications started June 1 and were applied every 14 days using full label rates of propiconazole and chlorothalonil. This program was compared to several early-season treatments applied May 1, followed up with applications of a tank mixture of propiconazole and chlorothalonil at either ¾ rates every 21 days or full label rates applied every 28 days. Treatments were arranged in a randomized complete block design with four replications with individual plots measuring 2.8 m^2. Disease severity was rated visually by counting individual dollar spot foci every two weeks. The 21-day early-season program suppressed disease development, but not to acceptable levels (<5% disease severity). The 28-day early-season program provided excellent suppression that was comparable to the conventional program. One fungicide application could be eliminated by using a 28-day early-season program instead of a 14-day conventional program, resulting in reduced expenditures and environmental inputs.

Effects of temperature on growth and aggressiveness of *Sclerotinia homoeocarpa*

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Dollar spot, caused by *Sclerotinia homoeocarpa*, is an important disease of most turfgrass species worldwide. *S. homoeocarpa* was described almost a century ago by F.T. Bennett, however the basic biology and epidemiology of the pathosystem is still unclear. Four isolates of *S. homoeocarpa* from Wisconsin and six isolates from Oklahoma were grown on native soil and USGA greens-grade sand. WI isolates were grown with and without creeping bentgrass (CRB) debris and incubated at temperatures ranging from 11 to 34C. OK isolates were grown with CRB debris only at temperatures of 15, 20, 25, 30, and 35C. Radial growth of mycelia was recorded at 24, 48, 72, and 96 hours past infestation. Growth for all isolates was most rapid between 20 and 30C. WI isolates grew best on native silt loam with CRB debris, while the effect of soil treatment was not significant for the OK isolates. Growth was limited and sporadic at temperatures below 15C. To assess aggressiveness, CRB plants were inoculated with 3 WI isolates and 6 OK isolates and placed in separate growth chambers set at 10, 14, 20, 25, 30 or 34C. Disease severity was visually assessed every 24 hours for four days. Disease developed in each temperature treatment, yet was most severe at 14 and 20C for all isolates. These data indicate a temperature window for dollar spot development, but also demonstrate that another environmental parameter such as relative humidity is likely more important for dollar spot development.

Differential effects of host plants on accumulation, competition and transmission of curtoviruses from single and mixed infections

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Curly top disease, caused by viruses in the genus, *Curtovirus*, has impacted U.S. agriculture for over a century; however, over that period the viruses responsible for the disease have changed. The two most abundant curtovirus species today, *Beet severe curly top virus* (BSCTV) and *Beet mild curly top virus* (BMCTV) have not always been the dominant forms, and in some areas of the southwestern US new curtovirus species have been identified. To identify factors that drive the emergence of new species, as well as determine what factors cause a variant to become dominant, studies were undertaken to examine virus accumulation, competition and transmission among common weed and crop curtovirus hosts. Single and mixed infections of BSCTV and BMCTV were established in several weed and crop hosts, to determine efficiency of accumulation in each host plant species individually, as well as which virus dominates during mixed infections using TaqMan probes. Results indicated differential accumulation of each virus depending on host plant, and shifts in accumulation patterns during mixed infection. Transmission studies demonstrated variation in transmission efficiency of each virus among host plants. Evaluation of the relationship between source plant virus concentration and transmission efficiency is ongoing. Results add to the knowledge of factors driving emergence and dominance among curtoviruses and contributes to overall knowledge of curtovirus ecology and epidemiology.

Endophytic colonization and induced resistance by *Pseudomonas aeruginosa* strain UPMP3

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Colonization patterns of oil palm (*Elaies guineensis*) roots by a plant growth-promoting bacterium, *Pseudomonas aeruginosa* strain UPMP3, were studied in axenic conditions. UPMP3, both wild-type and tagged with *gusA* and *gfp* genes were used for enumeration and visualization of colonized root tissues of 3-month-old seedlings. Epiphytic and endophytic bacteria colonization was enumerated using dilution plating assays. Colonization patterns were visualized using fluorescent microscopy. The expression of two pathogenesis-related genes, chitinase and β-1,3 glucanase, in oil palm roots colonized with UPMP3 was monitored using RT-PCR for 28 days. There was significant increase in the rates of epiphytic and endophytic colonization by UPMP3. The bacteria entered the roots via joints at secondary adventitious roots adjacent to the elongation zone and first detected at the epidermal cells, then in cortex cells and finally at the surface of vascular tissues within seven days. Chitinase and β-1, 3 glucanase were differentially expressed in oil palm roots where the highest expression for both genes occurred at 5 days after inoculation of the bacteria. These phenomena showed that the bacteria cells are capable of establishing themselves in oil palm roots and triggering some defense mechanisms of the host.

Genetic diversity of *Xanthomonas oryzae* pv. *oryzicola* from West Africa

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Bacterial Leaf Streak (BLS) caused by *Xanthomonas oryzae* pv. *oryzicola* (*Xoc*) was first reported in Africa in the 1980s. Following the recent expansion of rice cultivation in West Africa, a substantial reemergence of BLS was observed in Burkina Faso and Mali. *Xoc* strains were isolated from cultivated and wild rice varieties showing BLS symptoms. Samples were collected at various sites in three different regions of Burkina Faso and Mali, respectively. A collection of 58 *Xoc* strains was evaluated for virulence on rice varieties. African *Xoc* strains showed high variation in lesion length on susceptible cultivars. A set of strains was further characterized using a MultiLocus Sequence Analysis using seven housekeeping genes. Dendrograms generated for the data sets obtained from MLSA clearly separated different groups among African *Xoc*. RFLP analysis was performed using the TALE *avrXa7* as a probe, resulting in the identification of six haplotypes. PCR-based analyses of two conserved TTSS (*avrRxo1* and *xopW*) also differentiated the strains into distinct groups. *avrRxo1* was detected in only 30% of African *Xoc* strains. Functionality of *avrRxo1* was confirmed by leaf infiltration on rice Kitaake *Rxo1* lines. Sequence analysis of *xopW* revealed three distinct groups among Asian and African *Xoc* strains. Together, our results demonstrate that African *Xoc* strains, while differentiable from the Asian strains, are highly diverse and rapidly evolving.

The use of natural plant volatile compounds for the control of potato blemish disease pathogens

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Many naturally occurring plant volatiles are known for their anti-fungal properties. However, they have limited use because they diffuse rapidly after coming in contact with air. In an initial study, acetaldehyde and 2E-hexenal were chosen as prototype volatiles in order to investigate the use of volatiles for control of blemish pathogens in fresh-pack potato packaging. Pure cultures of the three main potato blemish pathogens, *Colletotrichum coccodes*, *Helminthosporium solani*, and *Pectobacterium atrosepticum* were used in the study. Pathogen cultures were exposed to the pure volatiles in sealed jars for 7 days at 23°C. Results showed that 2E-hexenal was the more effective of the two volatiles with 2.5 µL providing complete inhibition of growth for all three pathogens. In the current study, experiments were repeated using inoculated tubers instead of pathogen cultures. The potatoes were inoculated by means of spore or bacterial suspension onto sterile filter paper that was placed onto a single point of wounding on the tuber surface. Pure volatile organic compounds were injected using a syringe into the headspace of sealed jars through an airtight valve. The headspace of the jars was sampled daily using SPME, and the volatile concentration measured by GC/MS. Fungal and bacterial growth were measured daily. Results are presented and discussed in relation to the future use of these volatile compounds in active packaging systems for the control of potato blemish diseases.

Influence of nickel on severity of pecan scab

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Pecan scab, caused by Fusicladium effusum, is a major factor limiting profitability of pecan (Carya illinoinensis) in humid environments. The effect of nickel (Ni) on the severity of pecan scab was examined in both field and lab studies in 2005 to 2010. Application of Ni sprays to foliage in tree canopies appeared to reduce subsequent scab severity. Host genotype influenced efficacy – those most resistant to scab ('Desirable') were most responsive to Ni treatment and those most susceptible ('Wichita' and 'Apache') were least responsive to Ni treatment. Addition of Ni to fungicide treatments delivered by air-blast sprayers to commercial orchards reduced the severity of scab disease on fruit by 6–52%, depending on cultivar. Ni augmented fungicide sprays on 'Desirable' also increased fruit weight and kernel filling. Ni was toxic to the fungus both in vitro and in vivo, but was not as efficacious as triphenyltin hydroxide, a standard fungicide used in commercial orchards. These studies establish that Ni can provide some protection against pecan scab when used alone at high concentration or when combined at lower concentration with conventional pecan fungicides. Protection appears to be both indirect via enhancement of host resistance, and direct via toxicity to the scab fungus.

Evaluating the use of solid-phase microextraction to detect aflatoxin-producing isolates of the fungus *Aspergillus flavus*

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Aflatoxins produced by *Aspergillus flavus* accumulate in maize during pre- and postharvest, and the detection of aflatoxin-producing isolates of the fungus is important to ensure food safety. To date, most methods used for detection are destructive. This study describes a potential non-destructive method utilizing volatile organic compounds (VOC's). The objective of this project is to rapidly detect *A. flavus* in maize. Laboratory studies were initially conducted to examine the possibility of utilizing this type of method. Headspace solid-phase microextraction (HS-SPME) and gas-chromatography/mass spectrometry (GC-MS) were used in these studies. Replicated experiments were performed in the laboratory employing an aflatoxin-producing isolate *A. flavus*, an atoxigenic isolate, or a media control for comparison. A volatile profile was assembled by GC-MS for all samples measured. A series of optimization experiments were performed including four types of SPME fibers (65-micrometer PDMS/DVB, 50/30-micometer DVB/CAR/PDMS, 85-micrometer CAR/PDMS, and 85-micrometer PA) and five SPME fiber exposure times (30 minutes, 1 hour, 1.5 hours, 2.0 hours, 2.5 hours, 3.0 hours). Our data suggest that a 65-micrometer PDMS/DVB SPME fiber and a 30 minute exposure time was the optimum sample preparation technique. Future research will investigate the potential for examining and detecting VOC's in whole plant systems infested with *A. flavus* in greenhouse-grown maize utilizing a portable Mini GC.

Evaluating resistance to *Aspergillus flavus* in maize genotypes using stem inoculations

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Few researchers have studied the systemic infection of *Aspergillus flavus* in maize. Our data suggest that fungal densities found within inoculated maize seedlings may be correlated with *A. flavus* kernel infection. These methods may be comparable to ear inoculations currently being used by the USDA. Genotypes susceptible (S) and resistant (R) to *A. flavus* were inoculated by inserting a toothpick colonized with either an aflatoxin-producing isolate or an atoxigenic isolate into the stem. A sterile toothpick was used for comparison. Necrotic lesions observed in the S genotype inoculated with the toxigenic isolate were significantly larger than lesions observed in both R and S plants inoculated with the atoxigenic isolate and the control ($P < 0.0001$). Traditional isolations and quantitative polymerase chain reaction data detected the pathogen in tissue outside the visible stem lesion in both genotypes. *A. flavus* genomic DNA was detected 1.0 to 1.5 cm further from the edge of the necrotic region in the S genotype compared to 0.25 to 1.0 cm in the R genotype. These results indicate that genotypes resistant to kernel infection may have a mechanism that can limit fungal growth within the stem. Our data also suggest that the fungus is able to spread much farther within the stem tissues in susceptible genotypes compared to resistant genotypes.

Management of Sclerotinia blight of peanut in Texas: An integrated approach

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Phytopathology 101:S193

Sclerotinia blight, caused by the soilborne fungus *Sclerotinia minor*, is an important disease of peanut (*Arachis hypogaea*) in parts of Texas. The effects of fungicides, application timing and method, as well as partially resistant cultivars were evaluated from 2007–2010 in central and west Texas. Preventative applications of boscalid and fluazinam increased yields by 1814 and 1727 kg/ha, respectively over the non-treated control. Overall, preventative fungicide applications provided superior disease control and higher yields than applications that were made following the onset of disease. Banded applications of fungicides improved yields by 147 kg/ha when compared to broadcast applications; however, differences in efficacy among application methods were more variable. Dramatic differences in disease incidence and yield have been observed between the cultivars evaluated. Use of the partially resistant cultivar, TamrunOL02 resulted in a 51% reduction in disease incidence and a 931 kg/ha increase in yield when compared to the commercial standard Flavorrunner 458. Despite the increased level of resistance in Tamrun OL07, significant yield responses are observed when fungicides are applied.

Effect of temperature on potato psyllid reproduction and Liberibacter titer level in tubers

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"*Candidatus* Liberibacter *solancearum*", vectored by the potato psyllid (*Bactericera cockerelli*), is associated with zebra chip (ZC), a potato disease which imparts dark colorations to fried chips rendering them unmarketable. As a newly emerging disease, little or no information is available on factors which influence ZC epidemiology. To determine the effect of temperature on psyllid reproduction and Liberibacter titer, growth chamber studies were conducted at 4 constant temperatures levels (15, 21, 27, and 32C). Potatoes were grown in caged pots (4 pots per cage) and after bloom infested with 30 bacterialiferous psyllids per cage. Plants were similarly set up in uninfested cages to serve as controls. Development of new adult psyllids was monitored periodically using yellow sticky traps. At the end tubers were harvested and tested for Liberibacter titer levels using qPCR. Development of new adults was delayed by 10 days in the 21C chamber and the number of new adults was substantially lower compared to those in the 27C chamber. No new adults were observed in the 15C and 32C chambers. Tubers from all psyllid-infested plants tested positive for Liberibacter but tubers from the 15C chamber had the lowest titer compared to those from the other temperature chambers. The results suggest that cool temperatures slow psyllid reproduction and the buildup of Liberibacter titer in tubers. This finding represents a significant addition to our understanding of the epidemiology of ZC in relation to temperature.

Fungi in Botryosphaeriaceae causing stem blight in the Southeast and latent infection in southern highbush blueberry propagative material

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Stem blight of southern highbush blueberries (SHB) in Florida is caused by a species complex in Botryosphaeriaceae (Bot) that includes *Botryosphaeria dothidea*, *Lasiodiplodia theobromae*, and *Neofusicoccum ribis*. In 2010, 365 stem blight samples were collected from SHB and rabbiteye cultivars from 28 sites in the southeastern United States (AL, FL, GA, NC, and SC); 86% of the samples were initially identified as Bot species. Sequence analysis of fungal ribosomal DNA (rDNA) was used for phylogenetics and to design PCR restriction fragment length polymorphism (PCR-RFLP) assays to discriminate among Bot species. *Neofusicoccum ribis* and *L. theobromae* were identified as the two predominate species causing stem blight in the southeastern U.S.; *Botryosphaeria corticis*, *B. dothidea*, and *Diplodia seriata* were found infrequently. In an additional survey, propagative material was collected monthly from May to October and fungal isolations identified latent Bot pathogens on apparently healthy softwood cuttings (swc). Bot fungi also were isolated from lisate washed from healthy swc. Using PCR-RFLP, *L. theobromae* and *N. ribis* predominantly were identified from both isolations; pathogenicity of select isolates was confirmed. Our results suggest up to 45% of swc could have latent infections. Additional research is needed to determine the impact of these latent infections on blueberry production and pathogen distribution.

Evaluate Actigard applied through drip irrigation for suppression of Xanthomonas contamination in carrot seed

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Bacterial blight caused by *Xanthomonas hortorum* pv. *carotae* (*Xhc*), is the most important disease on carrot seed crops in the Pacific Northwest because contaminated seed is the primary inoculum for this disease in commercial carrot production. Currently, there is no effective way to control seed contamination, and seed lots commonly require hot water-treatment, which is expensive and reduces seed vigor. The objective of this study was to evaluate Actigard, a systemic resistance inducer, applied through drip tapes for suppression of *Xhc* in carrot seed crops. In two greenhouse experiments, 6-week-old carrot seedlings in 4-inch pots were drenched with 0, 5, 10, and 15 mg Actigard, and inoculated with *Xhc* suspension 1, 3, and 6 wk after. The results on dose and timing effects of Actigard drench were inconsistent. Suppression of *Xhc* was observed positively related to the Actigard dose on plants inoculated 6 wk post-drench in the first experiment and 3 wk post-drench in the second experiment. In a field trial, Actigard applied through drip tapes two to three times at 2 to 8 oz/A was compared with the commercial standard, two foliar ManKocide sprays. Similar to ManKocide, Actigard applied through drip irrigation reduced *Xhc* contamination on carrot seed although it did not control *Xhc* population on leaves and umbels. The results revealed that Actigard through drip is promising for suppression of *Xhc* on carrot seed, and more studies are needed to optimize method, timing and dose of Actigard application.

Effect of vulculic acid produced by *Nimbya alternantherae* on chloroplast function of alligatorweed

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Phytopathology 101:S194

Vulculic acid produced by *Nimbya alternantherae* can cause leaf blight of alligatorweed. We investigated the effects of the toxin on chloroplast function of alligatorweed and its mode of action. Thylakoids were isolated and treated with the toxin at concentrations of 0.042, 0.083, 0.208, 0.292 and 0.417 mmol•L^{-1}. Thylakoid suspensions were then analyzed for electron transport rates of the whole chain, of photosystem II (PS II) and of photosystem I (PS I), and for the activities of non-cyclic and cyclic photophosphorylation, and of Mg^{2+}-ATPase and Ca^{2+}-ATPase. Phosphate buffer at pH 4.1 and double distilled water were used as controls, with three replicates for each treatment. When treated at a concentration of 0.417 mmol•L^{-1}, the electron transport rates of PS II and the whole chain, the activities of non-cyclic photophosphorylation and Mg^{2+}-ATPase and Ca^{2+}-ATPase were reduced by 48.0%, 60.6%, 42.0%, 41.0% and 39.1%, respectively, compared to the phosphate buffer-treated control. The electron transport rate of PS I and the activity of cyclic photophosphorylation decreased by 16.0% and 7.5%, respectively, at the same concentration. Photosynthetic pigment content changed little after the detached leaves were treated for 48 h at 0.417 mmol•L^{-1}. These results suggested that the toxin might affect the chloroplast function of alligatorweed, and its main action might be the inhibition of electron transport in PS II and of the activity of non-cyclic photophosphorylation.

Susceptibility to nucleopolyhedrovirus and mitochondrial DNA sequence variation among different geographic populations of *Ectropis oblique* Pount

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Phytopathology 101:S194

The tea looper, *Ectropis oblique* Prout, is a recurring major tea pest that is widely distributed in South China. Ectropis oblique nucleopolyhedrovirus (EoNPV), as a commercial baculovirus insecticide, has highly toxicity to tea looper larvae. In recent years, some field observations suggested that EoNPV was failing to control tea production damage. The laboratory bioassay experiment was indicated that was a significant difference of susceptibility to EoNPV among the different geographic populations. Compared with the susceptible population, EoNPV virulence ratio of the resistant population reached up to 724.5-fold. Based on mitochondrial DNA sequence of the Cytochrome Oxidase Subunit I gene from 11 populations was analyzed, the N-J tree showed that 36 unique haplotypes diverged into 2 groups which have 5% of genetic difference. Correlated to the result of susceptibility bioassay, the group one was more susceptible with EoNPV infection than the group two.

Dissect the evolutionary process of *Potato virus Y* to overcome host resistance during single-host passages

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Resistance breaking (RB) variants of *Potato virus Y* (PVY) can emerge during single infection events of the avirulent PVY-NN in partially resistant tobaccos VAM and NC745. Both tobacco lines contains the recessive resistance gene *va*, but VAM also encodes extra resistance genes. These genetic conditions shaped differential resistance responses after plants were inoculated with PVY-NN. RB variants emerged in some NC745 plants and caused systemic infections. In contrast, RB variants were restricted to the inoculated leaves of tobacco VAM, without causing symptoms. An additional passage to another VAM plant was required for the RB variants to spread systemically. All RB variants accumulated at a higher level than PVY-NN in the common host, tobacco Burley 21 when they were individually inoculated. However, they were outcompeted by the parental PVY-NN strain in growth competition assays. Despite this interference, mixed infections in Burley 21 significantly increased the total virus concentration. The RB variants selected after passage through NC745 required further adaptation to become highly infectious in tobacco VAM, but this adaptation was accompanied by a loss of competitiveness in tobacco Burley 21. Sequencing of the viral virulent

determinant, the VPg gene, revealed that stepwise mutations were responsible for PVY to completely overcome the VAM resistance and for the changes in its fitness.

Molecular analysis of complete genomic sequences of four isolates of *Gooseberry vein banding associated virus*
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Presence of *Gooseberry vein banding associated virus* (GVBaV), a badnavirus in the family *Caulimoviridae*, is strongly correlated with gooseberry vein banding disease in *Ribes* spp. In this study, full-length genomic sequences of four GVBaV isolates from different hosts and geographic regions were determined to be 7649-7663 nucleotides. These isolates share identities of 96.4–97.3% for the complete genomic sequence, indicating low genetic diversity among them. The GVBaV genome contains three open reading frames (ORFs) on the plus-strand that potentially encode proteins of 26, 16 and 216 kDa. The size and organization of GVBaV ORFs 1-3 are similar to those of most other badnaviruses. The putative amino acid sequence of GVBaV ORF 3 contained motifs that are conserved among badnavirus proteins including aspartic protease, reverse transcriptase and ribonuclease H. The highly conserved putative plant $tRNA^{met}$-binding site is also present in the 935-bp intergenic region of GVBaV. The identities of the genomic sequences of GVBaV and other badnaviruses range from 49.1% (*Sugarcane bacilliform Mor virus*) to 51.7% (Pelargonium vein banding virus, PVBV). Phylogenetic analysis using the amino acid sequence of the ORF 3 putative protein shows that GVBaV groups most closely to *Dioscorea bacilliform virus*, PVBV and *Taro bacilliform virus*. These results confirm that GVBaV is a pararetrovirus of the genus *Badnavirus*.

Biological characterization and complete genomic sequence of *Carrot thin leaf virus*
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A cilantro isolate of *Carrot thin leaf virus* (CTLV) from diseased plants in a commercial field in California was characterized. The experimental host range of the virus included 15 plant species in the families *Apiaceae*, *Chenopodiaceae* and *Solanaceae*. Almost all infected plant species showed symptoms of local lesions, chlorosis, stunting and/or thin leaf. CTLV was transmitted to all 9 host species in the *Apiaceae* by green peach aphids. It reacted with the potyvirus group antibody in both ELISA and western blot analysis. The complete genomic sequence of CTLV was determined to be 9,491 nucleotides, excluding the 3′ poly(A) tail. The CTLV genome comprises a large open reading frame encoding a single polyprotein of 3066 amino acid residues. Its genomic organization is typical of potyviruses, and the deduced polyprotein sequence contains conserved motifs found in members of the genus Potyvirus. Comparisons with available genomic sequences of other potyviruses indicate that CTLV shares 22.4–56.4% identities with species of the existing genera and unassigned members in the family *Potyviridae* at the polyprotein sequence level. Phylogenetic analysis based on the deduced sequences of the polyprotein and individual proteins indicates that CTLV is distinct from *Carrot virus Y* (CarVY) and several CarVY-related potyviruses infecting apiaceous plants.

Radiosynthesis of tritium-labelled and the stability of novel cis-configuration nitromethylene neonicotinoids
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Phytopathology 101:S195

Recently, novel cis-configuration nitromethylene neonicotinoids were developed as insecticides in China, such as Paichongding and Cycloxaprid. The metabolism and stability of these two compounds are our focus. The radiosynthesis of [3H2]-Paichongding and [3H2]-Cycloxaprid were achieved using NaB3H4 reduction. The labelled compounds could be used as radiotracers for further study of metabolism and toxicology. In addition, the photodegradation of neonicotinoid compounds with cis-nitro configuration had been studied in distilled water. The degradation pathways of Paichong-ding were proposed according to the structures of photolytic products.

Colonization of tomato seedlings by bioluminescent *Clavibacter michiganensis* subsp. *michiganensis* under different humidity regimes
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The dissemination of *Clavibacter michiganensis* subsp. *michiganensis* (*Cmm*), the causal agent of tomato bacterial canker, is facilitated by mechanical wounds that are easily made during seedling production and crop maintenance. Little is known regarding translocation of *Cmm* in tomato seedlings through wound infection or the influence of environmental factors on *Cmm* growth as an endophyte. A virulent, stable, constitutively bioluminescent *Cmm* strain BL-Cmm 17 coupled with an *in vivo* imaging system (IVIS, Xenogen), a quantitative low-light device, allowed visualization of the *Cmm* colonization process in tomato seedlings in real-time. The dynamics of bacterial infection in seedlings through wounds were compared under low (45%) and high (83%) relative humidity. Bacteria multiplied rapidly in cotyledon petioles remaining after clip inoculation and moved in the stem towards both root and shoot. Luminescent signals were also observed in tomato seedling roots over time and root development was reduced in inoculated plants maintained under both humidity regimes. Wilting symptom development was more severe in seedlings under high humidity regimes. A strong positive correlation between light intensity with bacterial population *in planta* suggests that bioluminescent *Cmm* strains will be useful in evaluating the efficacy of bactericides and host resistance.

Identification of an RNA silencing suppressor encoded by Southern rice black-streaked dwarf virus S6
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Phytopathology 101:S195

Southern rice black-streaked dwarf virus (SRBSDV), a member of the genus *Fijivirus* within the family *Reoviridae*, is a novel virus that has been observed on rice (*Oryza sativa*) in Guangdong Province and Hainan Province recent years. In this study, we identificated an RNA silencing suppressor encoded by SRBSDV. SP6, encoded by SRBSDV segment 6, exhibited silencing suppressor activity in coinfiltration assays with the reporter green fluorescent protein (GFP) in transgenic *Nicotiana benthamiana* line 16c carrying GFP. It suppressed local and systemic silencing induced by sense RNA, but did not interfere with local and systemic silencing induced by dsRNA. SP6 can reverse the GFP silencing as well as prevent long distance spread of silencing signals which have been reported to be necessary for inducing systemic silencing in host plants. Expression of SP6 enhanced Potato virus X pathogenicity in *N. benthamiana*. Collectively, our results establish SP6 as a suppressor of RNA silencing encoded by a plant dsRNA virus and further suggest that SP6 targets an upstream step of dsRNA formation in the RNA silencing pathway.

Novel plant activator PRDA-003 for soil-borne disease
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Phytopathology 101:S195

The plant disease-resistant activator is one kind of new plant protection variety with the green-chemistry concept. Plant scientists play attention to it because of its potential comprehensive superiority to other kinds of agricultural protecting agents. The plant activators don't have notable antimicrobial activities in vitro, but they can induce their immune system in vivo to protect themselves from being invaded by plant diseases. Based on BTH, we have designed and synthesized a series of new BTH analoges. Their biological activities were also studied. At first, we studied that the novel synthesized plant cell culture elicitors induce SAR activities on pathogen in vitro. The results showed that these elicitors didn't have directive pathogens inhibition, which corresponds to the properties of plant activators. Then we studied the activators inhibiting Soil-borne disease. PRDA-003 has excellent inhibition on Cyclamen root rot disease, up to 93.2% inhibition in 25 mg/L. Also, the disease-resistant has sustainability, up to 35-day. PRDA-003 also has disease-resistant on Potato scab 58.88% in 250 mg/L.

Concentration and cultivar effects on efficacy of ACM941-CL01 biofungicide in controlling Fusarium head blight of wheat
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Phytopathology 101:S195

Fusarium head blight (FHB) is a destructive disease of wheat. This research was to examine the effect of concentration and cultivar on the efficacy of ACM941-CL01, a formulated product of *Clonostachys rosea* strain ACM941, in controlling FHB and deoxynivalenol (DON) contamination in wheat. Seven concentrations of ACM941-CL01, ranging from 104 to 108 CFU/mL, were tested for the control of FHB and significant effects observed for concentrations at or above 8×10^6 cfu/mL in the greenhouse trials or 3×10^6

cfu/mL in field trials. In the greenhouse, ACM941-CL01 reduced the area under the disease progress curve (AUDPC) by 65–83%, Fusarium damaged kernels (FDK) by 68–92%, and DON by 51–95%. Under the field conditions, the bioagent reduced FHB index by 30–46%, FDK by 31–39%, and DON by 22–33%. These effects were less but not significantly different from those of the registered fungicide Folicur® (tebuconazole) used in these trials. ACM941-CL01 and the fungicide were applied to the wheat cultivars AC Foremost, Quantum, and AC Nass, representing highly susceptible, intermediate and moderate resistance (the highest level of resistance commercially available) reactions to FHB, respectively, in field trials in 2009 and 2010. The bioagent was most effective on the moderately resistant cultivar AC Nass and least effective on the highly susceptible cultivar AC Foremost. Results of this study suggest that ACM941-CL01 is a promising biocontrol product for managing FHB on wheat.

Use of standard area diagrams to improve assessment of pecan scab on fruit

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Phytopathology 101:S196

Pecan scab (*Fusicladium effusum*) causes significant economic losses of pecan throughout the southeastern U.S. Disease assessment relies on visual rating of disease severity, which can be inaccurate, imprecise, with poor repeatability and reproducibility. Accurate, precise assessments are important for comparing treatments to manage disease. A total of 26 raters assessed 2 sets of 40 images of pecan fruit valves infected with pecan scab and estimated the area diseased. The first set of images (E1) was assessed without using standard area diagrams (SADs), while the second set (E2) was assessed using a SAD set comprised of 10 black and white images of known disease severity. Estimates of E1 and E2 were compared to the actual disease severity, determined by image analysis. Use of the SADs improved rater precision, accuracy and repeatability among raters. Linear regression showed that rater precision improved between the unaided and SAD-aided assessments (r^2 = 0.44–0.94 and r^2 = 0.84–0.95, respectively). Lin's concordance correlation coefficient (ρ_c) demonstrated improved agreement as a result of using the SAD set (ρ_c = 0.28–0.95 for E1 compared to ρ_c = 0.68–0.96 for E2), and rater bias measured by location (μ) and scale (υ) shifts was reduced using SADs (E1, μ = –0.17–1.57; υ = 1.01–1.53 and E2, μ = –0.08–0.84; υ = 0.95–1.22, respectively). SADs improve rater estimates of pecan scab severity on fruit.

Evaluations and modifications of semi-selective media for improved isolation of *Agrobacterium tumefaciens* biovar 1 from cultivated walnut

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Phytopathology 101:S196

Agrobacterium tumefaciens, the causal agent of crown gall of walnut, is an aerobic, Gram negative bacterium belonging to the family Rhizobiaceae. Like many in this group, A. tumefaciens is a common inhabitant of soil and plant host tissue. Isolation from these complex environments is difficult even with the use of semi-selective media. Many semi-selective media have been developed for A. tumefaciens and more recent studies of Agrobacterium species have relied on the medium1A. In this study, six previously published semi-selective media were evaluated using a panel of A. tumefaciens biovar 1 and closely related Rhizobiaceae species isolated from walnut orchards in California. Based on the antibiotic and carbon-utilization profiles of twelve A. tumefaciens isolates, D1 and 1A recipes were modified by antibiotic amendment or substitution of the primary carbon source to increase isolation efficiencies and/or selectiveness out of a complex environment. The antibiotics ampicillin, trimethoprim, and vancomycin were evaluated in addition to the carbon sources proline, serine, theonine, and β-hydroxybutyric acid. Results from these experiments revealed significant variation in isolation efficiency within a genetically diverse group of A. tumefaciens isolates and allowed development of a more selective medium for diagnostics and quantification from soil.

Comparison of seed health methods for the detection of *Acidovorax avenae* subsp. *citrulli* in cucurbit seeds

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Phytopathology 101:S196

Bacterial fruit blotch (BFB), caused by *Acidovorax avenae* subsp. *citrulli* (Aac) is arguably the most destructive seed transmitted disease of cucurbits in many production areas. Factors possibly contributing to the damaging effects include the use of greenhouses in transplant production, lack of resistance, lapses in phytosanitary practices, conducive environmental conditions, and late onset of symptom development in the production system. Seed producers rely on seed assays as the final assurance of producing pathogen-free seeds making test reliability and sensitivity paramount. The current National Seed Health System (NSHS) accredited tests for BFB are either slow and expensive and/or require the use of multiple extremely toxic reagents. A safer, faster, real-time PCR method was first reported in 2009 and here we report on experiments comparing the method with other NSHS accredited methods. Using naturally infested seed lots, artificially infested seeds, and low level Aac spiked seed wash solutions, significant differences in detection were found among the methods. The Seminis Inc. PCR-Wash, and Syngenta SYBR Green methods detected Aac more frequently than the Seedling Grow-out (p = 0.027). The Aac spiked seed wash solutions revealed the Syngenta SYBR Green method was more sensitive at low infestation levels than the Seminis Inc. PCR-Wash method ($p < 0.01$). The DNA analysis of extractions from different cucurbit lots is reported.

Genome-wide identification of virulence factors of citrus canker pathogen *Xanthomonas citri* ssp. *citri* using a transposon mutagenesis strategy

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Bacterial citrus canker disease, which is caused by *Xanthomonas citri* ssp. *citri*, is one of the most devastating diseases on citrus. To investigate the virulence mechanism of this pathogen, a *X. citri* ssp. *citri* 306 mutant library was constructed by randomly mutagenesis using EZ::Tn5. Around 22,000 independent mutants were inoculated and screened in host plant citrus individually. And 216 mutants were identified which showed significant virulence change *in planta*. Southern blotting assay revealed only one mutant contains two EZ-Tn5 insertions. To determine the insertion site of transposon, a rescue cloning method was used to clone the flanking sequences of EZ-Tn5. 102 genes/loci were identified. Among them, 99 genes/loci were mapped on the chromosome; three and one genes/loci were mapped on the magaplasmid pXAC64 and pXAC33, respectively. Interestingly, 11 genes/loci were overlapped with the HrpG regulon which has been reported recently by our group. In addition to the known virulence factors such as type II, III and IV secretion systems, cell-cell signaling system and EPS, LPS biosynthesis systems, many novel genes were uncovered in this work. For example, three transcriptional regulators and 16 hypothetical genes were identified and their roles in virulence of *Xanthomonas* have not been determined previously.

Development of a species-specific PCR assay to identify the cereal cyst nematode Heterodera filipjevi

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Heterodera filipjevi is one of the most economically important cereal cyst nematodes that restrict wheat production in the world. It is found in winter wheat fields in Oregon, U.S.A. Accurate identification of cyst nematode species in affected fields is essential for providing effective management strategies. It is difficult and time-consuming to distinguish H. filipjevi from other closely related Heterodera species based on morphological characters. A species-specific PCR assay was developed to detect and identify H. filipjevi. A primer set was designed from the internal transcribed spacer (ITS) region of Heterodera rDNA. This primer pair was highly specific when tested on four other Heterodera species, seven non-Heterodera plant-parasitic and non-parasitic nematodes, and six fungal pathogens associated with wheat root diseases. This primer pair was also predicted to be specific by in silico analysis using the ITS regions of 35 other accessions of 14 Heterodera species and 26 accessions representing other 17 nematode species. Optimized PCR conditions were established and H. filipjevi was detected and identified by a specific PCR fragment of 170 bp. This PCR assay was rapid and reliable, and was able to detect single eggs and juveniles of H. filipjevi among all tested nematode species. The species-specific primers are currently being tested for use in a real-time PCR assay, which would enable us to simultaneously identify and quantify H. filipjevi from infested soils.

Effect of UV-A and UV-B on airborne conidia concentrations of *Erysiphe necator* in Eastern Washington

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To characterize the relationship between UV radiation and populations of airborne conidia of *Erysiphe necator* in grape vineyards in Eastern Washington, conidia were trapped under natural conditions using a Burkard volumetric air sampler positioned in a vineyard near Prosser, WA during the

2008 and 2009 growing seasons. Time series cross correlation analysis was selected as an appropriate technique to measure the lagged relationship between UV radiation series and conidia series. The results of this study described the negative relationship of UV radiation series data with conidia population series in current day (day_0) and the previous two days (d_{0-2}). The cross correlations of UVA with conidia concentrations were –0.306 in lag 0 and –0.182 in lag 2, and that of UVB with conidia were –0.311 in lag 0 and –0.235 in lag2 based on prewhitening series. Under natural conditions, low levels of UV radiation resulted in higher population of *E. necator* conidia, while high UV had the opposite effect. The thresholds of positive and negative effects of UVB and UVA were 0.4-0.6 W/m^2 and 11-15 W/m^2, respectively. However, effects of UV radiation on conidia can be influenced by temperature and relative humidity. Temperatures of 21–27°C can lowered the impact of UV radiation on conidia concentrations while high levels of relative humidity intensifed the effect of UV radiation.

The time lagged effects on the relationship between weather variables and airborne spore concentration of *Erysiphe necator*
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Conidia of *Erysiphe necator* were collected by Burkard volumetric sampler for developing spore concentration prediction models in Prosser, WA during 2008 and 2009. The model used spore population and meteorological parameters as independent predictors. Two time series analysis approaches (ARIMA and PDLREG) were applied to quantify that spore concentration was explained by previous values and weather variables. The current value of concentration was related to previous values, autocorrelations in this series were 0.918-0.870. ARIMA (4.0.0) model were appropriate to characterize the association between the current value and lag 1 to lag 4 values in concentration series. Polynomial distribution lag regression model described the delayed effect of weather variables on spore concentration during 7 days at polynomial degree of 2 (d = 2). Temperature related weather variables and average dew point were better predictors than average relative humidity, average leaf wetness and total daily rainfall. Temperature duration variables were superior to average air temperature, maximum air temperature and minimum air temperature. This study suggests that the delayed effect of weather variables and previous values of conidia concentration can improve the forecasting of conidia concentration.

Identification of type III secretion inhibitors in *Erwinia amylovora*, the causal agent of fire blight of apple and pear
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The type III secretion system (T3SS) is a potent virulence mechanism shared by a broad spectrum of gram-negative bacteria that infect both plant and mammalian hosts. Plant pathogenic bacteria utilize T3SS to inject effector proteins into plant host cells, thus manipulating the host immune response. It is reasonable to believe that disabling the T3SS function may provide another way of controlling bacterial diseases. High-throughput screening of chemical libraries have identified small molecule inhibitors that attenuate T3SS of mammalian pathogens, but no report so far for plant pathogenic bacteria. In this study, using GFP as a reporter, we screened and identified five chemicals that suppressed T3SS gene expression of *Erwinia amylovora* and three chemicals that delayed hypersensitive response (HR) in tobacco. One chemical was further characterized by conducting global gene expression assay with *E. amylovora* grown in *hrp*-inducing minimal medium treated with chemicals. Our results showed that majority of genes in *E. amylovora* T3SS pathogenicity islands including *hrpL* as well as several effectors including *avrRpt2* and *hopC* were down-regulated more than two fold. Surprisingly, expression of amylovoran biosynthesis genes was also suppressed, whereas siderophore biosynthesis genes were strongly induced by the chemical. These findings indicate that inhibitors against T3SS without interfering bacterial growth could be explored for controlling bacterial diseases.

Identification and pathogenic analysis of *Colletotrichum* species causing soybean anthracnose
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Anthracnose of soybean [*Glycine max* (L.) Merr.] is known to occur wherever soybeans are grown. The disease seriously affects agricultural economics especially in wet, warm and humid areas. The most common pathogen that causes soybean anthracnose is the fungus *Colletotrichum truncatum*. Several other *Colletotrichum* species have also been reported, including *C. coccodes*, *C. destructivum* (teleomorph, *Glomerella glycines*), *C. gloeosporioides* (teleomorph, *G. cingulata*), and *C. graminicola* (teleomorph, *G. graminicola*). It is important to screen, isolate and identify the *Colletotrichum* species that cause soybean anthracnose to study the disease epidemiology and to develop resistant soybean genotypes. In this study, we collected more than 80 *Colletotrichum* isolates from infected soybean petioles from different states and initially identified them by size and shape of conidia and appressoria and the teleomorphic state. Among them, 57 were curved-spored types and the rest were straight-spored types. In addition, molecular methods such as PCR combined with multi-gene phylogenetic analysis will be applied and compare to the results of morphological observations. Furthermore, isolates were tested for their pathogenecity on soybean cultivars. There were differences in isolates in their capacity to produce symptoms on soybean.

Fungi and oomycetes associated with a peach replant problem
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The objective of this study was to identify fungi and oomycetes associated with a peach replant problem at a field location at the UC Kearney Agricultural Center in California. Soil samples were exposed to a constant water bath temperature between 40 to 70°C and held for 30 minutes once the center of the sample reached the target temperature. Peach seedling were transplanted into the treated soils and grown for 6 weeks in a greenhouse. Plant growth parameters were measured while fungal and oomycete composition in the roots were analyzed by culture and culture-independent methods. An increase in seedling growth correlated with the soil treatment temperature. *Trichoderma asperellum*, *Trichoderma virens* and *Fusarium oxsporum* were the most abundant fungal strains isolated. Sequence-selective quantitative PCR analyses of these fungi showed that there were no significant associations between *Fusarium oxysporum* and plant growth parameters, yet *Trichoderma* species were associated with increased peach seedling growth. Culture-independent analyses identified a significant negative association between *Pythium vexans* and plant growth, indicating that it may be contributing to the replant problem.

Early warning method and system for cucumber diseases in solar greenhouses
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The disease early warning is an essential way for protecting eco-environment and improving the level of vegetable quality safety in solar greenhouses. We studied the early warning method and system in solar greenhouses with four parts as follows. Firstly, we combined the leaf wetness sensors and estimation model based on canopy relative humidity (RH threshold model) in to the leaf wetness duration (LWD) monitoring method. The errors were around 1~2 h; compared with the LWD that was over 3 h, the monitoring effects of the method were acceptable. Secondly, we developed the primary infection situation early warning models of important diseases, such as cucumber downy mildew (*Pseudoperonospora cubensis*), in solar greenhouses. The model was evaluated by over a 4-year (2006–2009) dataset in the field. The results showed that it could warn the primary infection and disease occurrence date with a probability of 95% and more than 2 days before disease appearance. Thirdly, considering the characteristics of multi-warning sources of cucumber downy mildew in solar greenhouses, the warning source traceability model for cucumber downy mildew in solar greenhouses was constructed for system ease-realization using chain-styled theory of disaster. At last, the cucumber disease early warning system in solar greenhouses was developed by Visual Studio 2005 and SQL Server 2000, which added more functions of ongoing decision making for revising cultural practices than traditional record-keeping systems. The method and system shows promise for increasing adoption for IPM in China, and can provide decision support for early warning of cucumber diseases in solar greenhouses.

Forest products protection: From chemical to biological roadways
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Wood is a renewable resource and plays an important role in the world economy; however, it is subject to attack from wood-degrading fungi and insects. Developing effective and low environmental impact technologies for wood pest control is a big issue in forest products industry. During the past years, various chemical products have been used as wood preservatives such as creosote, pentachlorophenol (PCP) and chromated copper arsenate (CCA).

In the current years, less toxic copper, boron-based biocides are mostly used for protection of wood products. At the same time, organic biocides have been developed and put in the market, and more and more chemical- or thermal-modified wood products have been available for certain use in building construction. For the future development, more studies are focused on biological ways in wood protection such as utilization of extractives from natural durable plants as biocides, biological protection of wood against insect, stain and decay damage, and genetic engineering trees for wood pest resistance. This presentation will review the history of using chemical biocides in wood protection and will discuss the challenges and future directions in the development and the use of biological products or process in forest products industry.

PCG1 encodes a novel splicing factor that is essential for pathogenesis in _Magnaporthe oryzae_

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Prp19 and its associated proteins play vital roles in assembly and disassembly processes of spliceosomes. In this study, *PCG1*, a novel gene essential to pathogenicity of *Magnaporthe oryzae* was identified by insertion mutagenesis. *PCG1* encodes a protein with similarity to hCCDC12, a component associated with hPrp19 in *Homo sapiens*. Deletion of *PCG1* resulted in severe defects in asexual development and loss of pathogenicity. Pcg1 protein was expressed throughout the infection processes and localized to the nucleus. Forty-six nuclear proteins were identified to be coimmunoprecipitated with Pcg1, more than 20 of which are similar to well-known components of spliceosomes. RNA-seq analyses revealed that intron retention is the major defect in the Δ*pcg1* null mutant. *MoPPF3*, a novel pathogenicity gene was identified among 421 genes, whose transcripts were not fully spliced in the Δ*pcg1* mutant. Interestingly, both Pcg1 and hCCDC12 could physically interact with MoCwf4 and its counterpart hCRNL from *H. sapiens*. Constitutive expression of *hCCDC12* could partially complement defects of the Δ*pcg1* mutant. In addition, *FgPcg1*, *Pcg1* ortholog in *Fusarium graminearum*, was also required for asexual development and pathogenicity, and these two genes could be functional interchangeable between the two fungi. Our data indicated that Pcg1 is a novel splicing factor controlling intron splicing efficiency of pre-mRNAs and plays vital roles in infection-related morphogenesis in pathogenic ascomycetes.

A critical amino acid of 6K2 protein of _Papaya ringspot virus_ for inducing wilting symptom on papaya plants

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The isolates of *Papaya ringspot virus* (PRSV), the major limiting factor for papaya production in tropical and subtropical areas, are classified as P-type (papaya-infecting) or W-type (non-papaya-infecting), according to their host reactions. Most strains of P-type virus induce mosaic symptoms on papaya, while strains causing wilting symptoms have also been recorded. In our previous investigation, from a mosaic P-type isolate P-YK, we created the recombinant virus P-WCI6K, which contained the region covering the partial CI, the full 6K2 and the partial NIaVPg proteins of a W-type isolate W-CI. Differing from the parental P-YK that induced mosaic symptom on papaya, P-WCI6K caused wilting symptom similar to the naturally occurring P-type wilting strains. To investigate the determinant for wilting, further recombinants were constructed. Results of the bioassay on papaya plants revealed that the genomic region of nts 6128-6509 of PRSV W-CI is responsible for inducing the wilting symptom. Most of the infected papaya plants with the wilting symptom promptly collapsed and died. Sequence analysis of 13 PRSV isolates revealed that the Tyr^{53} of 6K2 protein of W-CI is different from that (His) of other PRSV isolates. Bioassay of the mutant with point mutation (His to Tyr) of P-YK at the amino acid 53 of 6K2 protein also resulted in the wilting symptom. Our results indicated that a single amino acid change in 6K2 protein ($His^{53} \rightarrow Tyr^{53}$) is critical for changing the mosaic symptom into wilting symptom.

Solving the problem of sequence homology-independent breakdown of transgenic resistance by disarming viral gene silencing suppressor

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Papaya ringspot virus (PRSV) strain 5-19 can break down the transgenic resistance conferred by the CP gene of the mosaic strain PRSV YK and the double-virus transgenic resistance conferred by an untranslatable chimeric construct containing partial PRSV and *Papaya leaf-distortion mosaic virus* (PLDMV) CP genes. Our recent studies indicated that the stronger silencing suppressor HC-Pro of strain 5-19 suppresses the gene-silencing-mediated transgenic resistance in a sequence homology-independent manner. Hence, the emergence of a super strain like 519 is a serious threat to CP-transgenic resistance. To disarm the super strain, transgenic papaya lines carrying a construct containing an untranslatable full or partial HC-Pro sequence were generated. Transgenic lines highly resistant to 5-19 and YK did not accumulate both viruses beyond 28 days after challenge inoculation. In these lines low levels of mRNA and high levels of siRNA were detected, suggesting that their resistance was mediated by a post-transcriptional gene silencing (PTGS) mechanism. Levels of transgenic resistance are not positively correlated to copy numbers of the transgene. F3-2-2, a single-insert transgenic line with high siRNA accumulation, displayed broad-spectrum resistance also to PRSV strains from other geographical regions. Our results demonstrated that PTGS targeting viral gene silencing suppressor can solve the problem of sequence homology-independent breakdown of transgenic resistance.

An effector of Puccinia striiformis f. sp. tritici is expressed in haustoria and required for avirulence on wheat cultivar carrying resistance gene YrTr

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Stripe rust, caused by Puccinia striiformis f. sp. tritici (Pst), is a devastating fungal diseases that threaten wheat production worldwide. Genetic resistance is the preferred method to control the disease, but the effectiveness of race specific resistance is typically not durable due to the genetic plasticity of rust populations. In this study, we identified a Pst effector (PSTeTr) that was highly expressed in haustoria. The PSTeTr gene delivered by modified Pseudomonas fluorescens strain EtHAn into leaves of wheat cultivar Tres, which has resistance genes YrTr1 and YrTr2, induced noticeably more callose depositions than the empty pEDV6 vector and the vector inserted with other two Pst genes (Pstha2A5 and Pstha5A23). Silencing PSTeTr in leaves of Tres through BSMV-VIGS produced abundant uredia in most leaves inoculated with a Tres-avirulent race, while the leaves infected by BSMV::MCS with the control constructs with no Pst gene and with PSTha12O3 sporulated sporadically. In contrast, silencing PSTeTr in other resistant wheat cultivars with Yr5 or Yr10 did not change the resistance reactions. The results indicate that PSTeTr is required for avirulence on wheat cultivar Tres.

Detection and damage analysis of _Acidovorax avenae_ subsp. _avenae_ in proso millet

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The bactericum, *Acidovorax avenae* subsp. *avenae* causes several important plant diseases including bacterial stripe of rice, bacterial stalk rot of corn, bacterial leaf blight of oats, and red stripe of sugarcane and millet. Three sets of polymerase chain reaction (PCR) primers was designed for use in a BIO-PCR assay for detection of *A. avenae* subsp. *avenae* in proso millet. Primer set designed from a 619bp fragment of the internal transcribed spacer region (ITS) of the 16S-23S rDNA of *A. avenae* subsp. *avenae* strain MY1. Investigated a growth difference for the 7-days, 14-days, and 21-days seedling inoculated the *A. avenae* subsp. *avenae*, but there was no significant differences in inoculation time. However in proso millet variety test, they show difference infected kernel rate, and seed weight.

Roles of NtERF5 in N-gene mediated TMV resistance

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Resistance in tobacco to Tobacco mosaic virus (TMV) mediated by the N gene involves a hypersensitive response (HR) which prevents further spread of TMV from infected cells. Over-expression of the 1a protein of Cucumber mosaic virus in N-gene tobacco neutralized a novel resistance specific to TMV-GFP and ethylene-response factor 5 (NtERF5), which interacted with the 1a protein in the yeast two-hybrid system, played a crucial role in the defense mechanism against TMV spread. NtERF5 expression was controlled by a salicylic acid (SA)-independent pathway of the N gene resistance. We also found that NtERF5 interacted in the yeast two-hybrid system with the tobacco Myb1 gene, which also was induced by TMV during the HR. NtERF5 and

Myb1 co-localized in the nucleus and on tonoplast membranes in Nicotiana benthamiana cells using confocal microscopy. We generated NtERF5 and/or Myb1- singly or doubly silenced transgenic tobacco. Interestingly, TMV could move systemically to upper leaves in NtERF5-silenced tobacco plants. TMV moved much faster to upper leaves of NtERF/Myb1-doubly silenced tobacco plants at 25°C while TMV was restricted to the inoculated leaf tissues of Myb1-silenced and non-transformed tobacco plants. Taken together, these results from transgenic studies supported strongly the conclusion that NtERF5 is a novel protein in the SA-independent pathway of the N gene resistance mechanism and associates with the tobacco Myb1 gene in TMV resistance.

BBWV2-resistant transgenic Nicotiana benthamiana expressing a virus-derived hairpin RNA

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Broad bean wilt virus 2 (BBWV2), one of the important pepper-infecting viruses, causes economically severe loss of pepper production in Korea. BBWV2 consists of a single-stranded, positive sense RNA, bipartite genome and is taxonomically classified in the genus Favavirus (the family Comoviridae). Sequence alignments showed highly conserved sequences in 5′ non-coding regions (NCRs) of RNA1 and RNA2 in all BBWV2 strains characterized so far. Based on this observation, we constructed transgenic Nicotiana benthamiana plants expressing an inverted repeat harboring a 210 bp cDNA fragment of the conserved 5′ NCR of BBWV2. The transgenic N. benthamiana plants inoculated with BBWV2 did not produce any symptoms in non-inoculated leaves. BBWV2 RNAs were not detected by RT-PCR analysis from tissues of both the inoculated leaves and upper leaves of the transgenic N. benthamiana plants. Accumulation of virus-derived small interfering RNAs was detected in the inoculated leaf tissues of the transgenic N. benthamiana plants, indicating RNA silencing is responsible for the resistance to BBWV2.

Identification of a candidate resistance gene to *Phakopsora pachyrhizi*, the causal agent of soybean rust, in the alternative host kudzu, *Pueraria* spp.

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Soybean rust (SBR), caused by the biotrophic fungus *Phakopsora pachyrhizi*, is a potentially destructive disease to U.S. soybean (*Glycine max*) production. The fungus over-winters in the southeastern U.S. on exotic, invasive kudzu species (*Pueraria* spp.), providing initial inoculum for each season that has the potential to disperse to northern soybean producing areas. A more precise understanding of resistance in kudzu can improve estimates of initial inoculum for forecast models. Five major sources of SBR resistance have been identified in soybean, *Rpp1* to *Rpp5*. A resistance candidate gene, *Rpp4C*, belonging to the CC-NBS-LRR gene family has been associated with *Rpp4* resistance. We predicted SBR resistance in kudzu also might be associated to *Rpp4C* orthologues, in particular for a kudzu accession (FLAL15), which shows a SBR resistance reaction similar to *Rpp4*. Primers targeting the conserved NBS and LRR domains of the soybean *Rpp4C* resistance gene were used to amplify *Rpp4* candidate gene orthologues from our SBR-resistant kudzu accessions. Amplicon analysis revealed multiple sequences for at least 5 *Rpp4*-orthologue candidate genes, named *Rpp4K1* to *Rpp4K5*, sharing from 85% to 91% nucleotide identity to the *Rpp4C* genes. Efforts to demonstrate association between the genotypic profile of each kudzu accession with the SBR susceptibility phenotype are being pursued.

Engineering an infectious cDNA clone of an Arizona Pepino mosaic virus isolate

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Pepino mosaic virus (PepMV), a member of *Potexvirus* genus, is a significant pathogen in greenhouse tomato production worldwide. To better understand the virus and the disease it causes, we initiated molecular characterization of an Arizona isolate (PepMV-AZ). Cloning and sequencing analysis of selected genomic regions indicates that PepMV-AZ is most closely related to members of the PepMV-EU clade, suggesting a possible European origin of PepMV-AZ. Viral RNA was extracted from purified virion preparations and used as a template to synthesize full-length infectious cDNA. The first strand cDNA synthesis was primed with an oligo$(dT)_{40}$ primer that also contains a *Not*I restriction site at the 5′ end. The second strand cDNA synthesis was primed with an oligonucleotide that contains a T7 promoter sequence at the 5′ end and 32 nucleotides identical to the 5′ terminal sequence of PepMV-AZ genomic RNA. The full-length infectious cDNA was cloned into a *Sma*I-linearized puc18 plasmid. Infectious transcript was synthesized *in vitro* by T7 RNA polymerase following linearization of the plasmid by *Not*I. Inoculation of the infectious cDNA transcript to experimental hosts resulted in wild type symptoms. Functional analysis of PepMV genes is conducted using this newly developed infectious cDNA clone of PepMV-AZ.

Cloning and sequencing analysis of two *Banana bunchy top virus* genomes in Hainan, China

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Banana bunchy top virus (BBTV) causes one of the major diseases in banana. The genome of BBTV consists of six segments of single-stranded DNA of approximately 1 kb in length. We found two BBTV isolates in Haikou, Hainan, China, one with a satellite DNA and one without. To further characterize these two isolates, the genomes of the two isolates were cloned from total DNA extracted from symptomatic tissues. The Haikou-2 isolate contains six genomic segments and one satellite DNA of 1103, 1067, 1058, 1041, 1013, 1082 and 1093 nucleotides in sizes, respectively while the HaiKou-4 isolate contains only six genomic segments of 1103, 1055, 1059, 1041, 1013 and 1081 nucleotides in sizes, respectively. Both the HaiKou-2 and HaiKou-4 isolates belong to the member of the Asian group. Typical of other babuviruses, each genomic segment encodes a single open reading frame and contains the highly conserved stem-loop and major common region. Further sequence analysis showed that DNA1 segments of the two isolates are most conserved while the DNA2 segments are most diverged in comparison with other segments. In addition, the satellite DNA of the HaiKou-2 isolate encodes a 33.1 kDa replication initiation protein similar to the one encoded by its DNA1. However, this protein is only 31.99% homologous with the Rep protein encoded by DNA1.

Production of single chain antibodies against '*Ca.* Liberibacter asiaticus

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Citrus huanglongbing (HLB), associated with '*Ca*. Liberibacter asiaticus' (CaLas), is the most serious disease of citrus in Florida and Brazil. Antibodies are the most widely used tool to detect pathogens, and they are also uniquely useful as experimental reagents. Psyllids that contained more than 10^8 CaLas were used to immunize BALB/C mice. mRNA from the spleens of the immunized mice was purified and converted into cDNA library. Antibody gene repertoires were PCR-amplified using 23 primers for the heavy chain variable region (VH) and 21 primers for the light chain variable region (VL). The VH and VL were joined by overlap extension PCR, and the completed scFv inserts were ligated into the phage vector pKM19. Forty-five clones were picked at random from the library and tested by PCR. All tested clones contained scFv inserts of about 750 bp. The BstN1 fingerprints of 23 scFvs randomly selected inserts were each unique and we estimate that the library contains 1.3×10^7 independent clones with full-length scFv inserts. Several proteins expected to be exposed on the bacterium's surface were expressed, purified, and used as capture antigens. ELISA data show that several different scFvs with specificity for different CaLas proteins were obtained. These include the enzyme producing polysialic acid capsule polysialic acid (PSA), a component of a type IV pilus (TFP), and the major outer membrane protein (OMP6f). Screening is in progress with other antigens. The utility of these scFv antibodies will be discussed.

Reduced infection of wheat spikelets inoculated with ascospores of *Gibberella zeae* in the presence of fungal mating pheromone peptides

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Anti-fungal peptides are an emerging area of antibiotic therapy with application for control of wheat head blight, caused by *G. zeae*. In past

research we demonstrated that mating pheromone peptides derived from *G. zeae* and other ascomycetous fungi, and combinatorially selected peptides can inhibit or disrupt germination of pathogen ascospores. We are now assessing the potential for mating pheromone and combinatorial peptides to protect wheat spikelets from infection. For assessment, peptides were synthesized and applied to individual spikelets in combination with a water droplet containing infectious ascospores. In initial experiments, only 1% of spikelets became infected in the presence of 20 μM Pgz, the mating pheromone peptide derived from *F. graminearum*. Pathogen mycelial growth on spikelets was also severely reduced at this peptide concentration. A representative combinatorial peptide also significantly reduced spikelet infection. Protective effects of all peptides declined with decreasing concentration. We are currently evaluating the protective efficacy of additional mating pheromone peptides and their derivatives when compared to Pgz as a standard treatment. Assessments are being made over a range of concentrations to identify the best peptides for larger-scale greenhouse trials. If effective, inhibitory peptides could be applied as a protective spray to wheat during flowering or alternatively, deployed in transgenic wheat.

Biological control of Fusarium head blight in wheat caused by *Gibberella zeae* - a two-year, multi-location study

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Biological agents for controlling Fusarium head blight (scab) and deoxynivalenol (DON) accumulation in grain are needed to augment host resistance and chemical fungicides or to serve as the primary management tool where the other strategies are not available. Field experiments were conducted in 2009 and 2010 at six sites to evaluate two biocontrol materials, one being a mixture of two yeast strains, *Cryptococcus flavescens* OH 182.9 (NRRL Y-30216) and *C. aureus* OH 71.4 (NRRL Y-30213), that were co-cultured in the same fermentor. The other was Taegro, a commercial product containing *Bacillus amyloliquefaciens* FZB24. The biocontrol materials were tested as stand-alone treatments or applied in combination with a commercial fungicide Prosaro 421 SC (prothioconazole plus tebuconazole). When applied alone, the biologicals reduced scab levels relative to the control in some individual experiments and averaged across experiments. The biological treatments, however, were not as efficacious as the fungicide alone. Although the various biocontrol-fungicide combinations did not confer any significant advantage over a single fungicide application in regard to controlling scab, applying either biocontrol material as a sequential treatment several days after the fungicide was more effective than the fungicide alone in reducing DON content in the harvested grain. Presumably, this advantage was due to the biological agents inhibiting late infections.

Field evaluation of an anthracnose forecaster to spray fungicide for hot pepper during 2005–2010

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Hot pepper anthracnose has been an important disease in Korea. To evaluate an anthracnose forecaster, field studies had been conducted in South Korea during 2005–10 measuring hourly temperature and leaf wetness for the model input. Percentage of diseased fruits by anthracnose (incidence) and yield were compared among the three treatments, unsprayed control, disease forecaster, and 7–10 days interval spray. According to the model advice when the model-determined infection risk (IR) exceeded a value of 3, we sprayed three to eight times at the plots of disease forecaster treatment. Whereas, we sprayed seven to nineteen times at the plots of interval spray treatment. The incidences of unsprayed control were 14–91%, those of interval spray were 0.3–15.4%, and those of disease forecaster were 3.1–52%. The control values of interval spray were mainly 79–98%, and those of disease forecaster were mainly 32–87%. Compared to the yields of unsprayed control, the yields of interval spray were 100–420% and the yields of disease forecaster were 91%–280%.

Incorporation of peanut rhizobia with plant growth promoting rhizobacteria as biocontroller effectively against the seed borne fungi, *Aspergillus niger*

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Phytopathology 101:S200

The inhibition of the seed borne pathogenic fungus *Aspergillus niger* that causes root rot diseases in peanut was investigated using soil-isolated Plant Growth Promoting Rhizobacteria (PGPR) as biological controllers. The best 4 PGPR isolates that were selected were A20, A45, A62, and A106. The sequence of 16S rDNA genes of these selected strains indicated that A20, A45, A62, and A106 were highly homology to *Bacillus megaterium* strain AM1C7 (99%), *B. subtilis* strain Setapak 8 (99%), *B. subtilis* subsp. *subtilis* strain SB 3130 (99%), and *Pseudomonas* sp. NJ-61 (95%), respectively. The strains A20, A45, A62, and A106 were able to inhibit *A. niger* growth at 42.5%, 51.42%, 67.81%, and 44.53%, respectively. Antifungal activities were found clearly in cell-free supernatants of A20 and A62. Interestingly, the antifungal activity of isolates A45 and A62 was proteinase k resistant. This implied that the mode of action against the fungus from these isolates was not from protease enzyme. All of the PGPR isolates could produce indole-3-acetic acid (IAA), an auxin hormone. IAA hormone produced from PGPR isolates could promote peanut root growth. When either isolate A20 or A45 (10^8 cells per ml) was co-inoculated with *Bradyrhizobium* sp. TAL 173 (10^8 cells per ml), the peanut root rot disease caused by *A. niger* (10^5 and 10^6 spores per seed) could be inhibited. Therefore, application of co-inoculum of rhizobia-PGPR is likely a viable agricultural technology to increase nitrogen fixation and reduce fungicide usage.

First report of a bacterial disease in Australian cedar (*Toona ciliata*)

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Phytopathology 101:S200

The aim of this study was to isolate and identify the causal agent of a foliar disease of Australian cedar (*Toona ciliata*), which is important due to the physical characteristics of the wood and is found in nurseries in Brazil. Exudation test, isolation, pathogenicity test and reisolation were performed, fulfilling Koch's postulates. The isolates were subjected to biochemical tests to determine the genus of the pathogen. Molecular identification of the genus of bacteria consisted of the amplification of genomic fragments of approximately 1400 bp, using primer ERIC by polymerase chain reaction (PCR), sequencing and phylogenetic analysis. The isolated colonies were whitish slimy-looking, The bactéria were Gram negative, strictly aerobic negative for utilization of carbon from arginine, asparagine and did not produce fluorescent pigments on King B medium. They were positive for glucose as carbon source, gas production, and starch hydrolysis. The bacteria induced HR on tomato and pepper. The comparison of the DNA sequence of the isolates with those available in *GenBank* indicated that the bacterium in question in the genus *Xanthomonas*, but is not identical to any known species.

The effect of phase variation on the interaction of Salmonella enterica sv. Typhimurium with tomatoes

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Several recent outbreaks of gastroenteritis have been linked to non-typhoidal Salmonella within fresh fruits and vegetables. For interactions with plants, Salmonella relies on an array of surface structures. Phase variation is thought to regulate the production of these surface structures and has been associated with increased virulence in Salmonella. We hypothesize phase variation may affect the ability of the bacterium to persist in plants. We assayed the attachment of wild type Salmonella enterica sv. Typhimurium and its phase variants to tomato surfaces. A cellulose-deficient mutant and high frequency phase variant attached to tomato surfaces at lower rates than the wild type that consistently produces extracellular polymers. Using RIVET, the roles of two genes (yihT and agfB) involved in the production and assembly of surface structures were tested inside tomatoes. yih operons are involved in capsule assembly and translocation. agfB is a curlin nucelator protein involved in fimbriae assembly. RIVET reporter constructs in yihT and agfB in both wild type and phase variant backgrounds were infected into tomatoes and recovered after a week. The expression of yihT was increased in high-frequency phase variants compared to wild type. In the low-frequency phase variants, reduced level of expression of both yihT and agfB genes compared to wild type was observed. These results will be investigated to determine if this difference in expression confers an advantage to survival within tomatoes.

Molecular cloning and characterization of the immunosuppressive protein from the surface coat of *Steinernema glaseri*

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Phytopathology 101:S200

Entomopathogenic nematodes (EPNs) are parasitic natural enemies of many agricultural pests which are widely used as biological control agents. They can suppress or evade the host immune defense upon entry into insects. The surface coat of *Steinernema glaseri* was proved to play important roles in defeating the host immune system. In this work, a protein fraction with immunosuppressive activity was separated by electro-elution and further analyzed with 2D-electrophoresis. LC-MS/MS analysis of protein spot from 2D-electrophoresis gave five peptides. Based on the sequences, specific primers were designed and the full-length cDNA sequence of the encoding gene was cloned. The deduced protein, Sg-E1, was then expressed in *E. coli*. Using immuno-gold transmission electron microscopy, native Sg-E1 was confirmed to be located on both nematode cuticle and surface coat. Furthermore, Sg-E1 was detected in host hemolymph after infection of *Galleria mellonella* with *S. glaseri*, indicating that Sg-E1 was secreted into insect hemocoel and involved in infection. This is the first report of the cloning and characterization of a surface coat protein in EPNs. Our findings help to illuminate the mechanism of EPNs defeating the host immune system.

Genome and transcriptome analysis of *Geosmithia morbida*

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Thousand cankers disease of black walnut is the result of aggressive feeding by the walnut twig beetle (*Pityophthorus juglandis*) and canker formation around galleries caused by *Geosmithia morbida*. The disease is widespread in the western United States and was detected in the native range of black walnut in Tennessee in 2010. *G. morbida* is the first phytopathogenic species reported in this genus and in the Bionectriaceae. We started a genomic and transcriptomic analysis of *G. morbida* to better understand its gene complement and for comparison of its genome to other fungal pathogens in the Hypocreales. Total DNA of isolate CBS124663 was sequenced in a half plate reaction using 454 GS FLX (Roche) sequencing technology. The other half was used to sequence the transcriptome from *G. morbida* growing under six different environmental conditions including nutrient and temperature differences and in the yeast phase. Genome sequencing resulted in 779,553 reads that assembled into 27,933 contigs. This represented 16 million of non-redundant base pairs, or approximately 1/3 of the predicted genome size. A total of 15,254 sequences (7,697 contigs and 7,557 singletons) were auto-matically annotated using the Blast2GO similarity tool (http://www.blast2go.org/). Another 3.4 Mbp of non-redundant sequences of the fungus transcriptome were assembled into 5,773 contigs and that were subsequently mapped into 6,867 contigs of the *G. morbida* genome.

Population structure of *Geosmithia morbida* in the United States is complex

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Phytopathology 101:S201

Thousand Cankers Disease of black walnut (*Juglans nigra*) was first described in 2009, but was likely present in Utah and Oregon since at least the early 1990's based on walnut twig beetle (WTB) collections and historical records of walnut mortality. *Geosmithia morbida*, the causal agent of the disease, is now widely distributed in the western United States on several walnut species and was recently found in Tennessee. The origin of the WTB and *G. morbida* has not been proven, although both are likely native to Arizona walnut (*Juglans major*) in southwestern U.S.A. We sequenced the rDNA internal transcribed spacer (ITS) region and the beta-tubulin gene of 100 *G. morbida* isolates from a broad geographic area and from different walnut species. At least nine ITS and three beta tubulin haplotypes have been identified indicating the population is complex and diverse. Haplotype variability was not correlated with geographic sites or hosts from which isolates were collected. In some cases more than one haplotype was collected from cankers on individual branches. Four haplotypes were identified from 9 isolates collected in Tennessee. To further elucidate the relationship among isolates, five housekeeping genes are being used in a multilocus sequence typing (MLST) analysis. We have also constructed a DNA library enriched for microsatellites. Seventeen candidate loci for polymorphism are currently been screened in a panel of 10 *G. morbida* isolates from different geographical locations.

Virulence and molecular comparison of *Puccinia striiformis* f. sp. *tritici* populations in China and the United States

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Stripe rust, caused by *Puccinia striiformis* f. sp. *tritici* (*Pst*), is one of the most important diseases of wheat in both China and the U.S. The *Pst* populations of these countries were compared for virulence patterns on wheat genotypes used to differentiate races of the pathogen, and genotypes using simple sequence repeat (SSR) markers. From 86 Chinese isolates, 58 races were identified based on reactions on the 17 Chinese differentials and 52 races were identified based on the 20 U.S. differentials. The selected 51 U.S. isolates, representing 50 races based on the U.S. differentials, were identified as 42 races using the Chinese differentials. A total of 132 virulence patterns were identified from the 137 isolates based on their reactions on both Chinese and U.S. differentials. From the 137 isolates, SSR markers identified 102 genotypes, of which 71 from the Chinese isolates and 31 from the U.S. isolates. Virulence and SSR data had a low ($r = 0.38$), but significant ($P = 0.01$) correlation. The Chinese and U.S. populations had similar levels of diversity based on Kosman indices. Principal analysis using the SSR data separated the two populations more clearly than using the virulence data. A non-rooted tree generated using the molecular data indicated that the Chinese and U.S. populations have evolved independently, but may share the same origin, which was also supported by the low value (0.13) of differentiation and high value (1.74) of gene flow.

Construction of recombinant fluorescent *Pseudomonas* spp. for suppression of soilborne pathogens

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Take-all, caused by *Gaeumannomyces graminis* var. *tritici*, and Rhizoctonia root rot, caused by *R. solani* and *R. oryzae*, are among the most important soilborne diseases of wheat in the Pacific Northwest. Because of the lack of resistance to these and many other soilborne diseases, wheat roots rely on antagonistic rhizosphere microorganisms as a first line of defense against these diseases. Many of these antagonists lack activity against a wide range of pathogens. The purpose of this study was to construct recombinant fluorescent *Pseudomonas* spp. that produce multiple antibiotics and to determine their activity against soilborne pathogens. We stably inserted the biosynthesis loci for different antibiotics into various biocontrol strains of *P. fluorescens*. All recombinant strains produced both their indigenous antibiotic and that encoded by the introduced genes, but the level of antibiotic production varied significantly depending on the transgenes introduced and the recipient *P. fluorescens* strain. In general, recombinant strains inhibited target pathogens better that did the respective wild-type strain, but inhibition varied by strain. Our results indicate the need to carefully screen strains and antibiotic combinations to obtain recombinants that retain the traits of the parental strain and exhibit broader activity due to the transgenes.

Further spread of and domination by Bemisia tabaci biotype Q on field crops in China

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The sweetpotato whitefly, Bemisia tabaci (Gennadius) (Hemiptera: Aleyrodidae), causes severe crop losses to many crops. The worst of these losses are often associated with the invasion and establishment of biotypes B and Q of this pest. The previous research in 2007 showed that biotype Q occurred with other biotypes in most field populations in China. To know about the current status of the biotypes composition in the field, an extensive survey covering mainly eastern parts of China was conducted in 2009 to determine the current distribution of B. tabaci biotypes. Using PCR primers specific for the mtCOI (mitochondrial cytochrome oxidase I) of biotypes B and Q and gene sequencing, we determined the biotypes composition in 61 whitefly populations and their distribution across 19 provinces in China. Our research revealed that only biotypes B and Q have been found in the field in 2009 in China. Among them, biotype Q was dominant in 44 locations

(100.0%) and biotype B was dominant in 17 locations (100.0%). The current survey indicates that biotype Q has rapidly displaced biotype B in most locations in China.

What we can learn from high similarities of molecular mechanisms between barley host and nonhost resistances to Blumeria graminis
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Phytopathology 101:S202

Nonhost resistance is strong, effective, and durable. Powdery mildew pathogens of Blumeria graminis f. sp hordei (Bgh, adapted to barley) and B. graminis f. sp tritici (Bgt, nonadapted to barley) are ideal systems to explore the mechanisms of nonhost resistance. We inoculated barley seedlings with Bgt and Bgh, separately. Leaves were collected at 0, 6, 12, 24, 36 hours after inoculation. We compared expression profiles of barley host and nonhost resistances based on 849 regulated genes. Expression patterns between host and nonhost resistances are similar, especially at time points of 24 and 36 hours, which the correlation coefficient indexes are 0.9322 and 0.9474, respectively. Based on our microarray data, we selected 389 up-regulated genes and tested their resistances to Bgh and Bgt by transient induced gene silencing. We found that 16 genes are resistant significantly to both Bgh and Bgt, but no gene is specifically resistant to Bgt only. This is not surprising because most reported mechanisms of nonhost resistance are overlapped with that of host resistance. Question is that the overlapped mechanisms cannot explain the distinct differences between host and nonhost resistances. So, we infer that major components of nonhost resistance are possibly constitutively expressed or slightly induced but non-detectable in our array experiments. Currently, we are systematically screening the major components of nonhost resistance in barley and wheat by high-throughput yeast hybrid protein-protein interactions.

Evaluation of chemicals for control of citrus canker, *Xanthomonas citri* subsp. *citri*
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Phytopathology 101:S202

Citrus canker, caused by *Xanthomonas citri* subsp. *citri* (*Xcc*), is one of the important diseases of citrus worldwide. Selected chemicals were evaluated for control of *Xcc* on citrus in greenhouse with three replicates using a randomized complete block design. Two antibiotics (oxytetracycline and streptomycin), one *Bacillus subtilis* (BS) extract, one copper-based bactericide and salicylic acid were sprayed three times at one-week intervals before or after inoculation of *Xcc* on grapefruit leaves. Each chemical/extract was tested twice with 10 to 15 leaves on one branch per treatment. *Xcc* colony-forming units (CFU) and bacterial titers were quantified from a single lesion of each treatment. Oxytetracycline, the copper-based bactericide and the BS extract consistently inhibited the bacterial growth, decreased CFU by 88.3%, 1.81% and 74.2%, respectively, when compared to the water-treated control. The effectiveness against *Xcc* bacterium was 4-fold increase with the spray applications of these chemicals before versus after *Xcc*-inoculation. Oxytetracycline was better than streptomycin or copper-based bactericide for the control of citrus canker. The *Xcc* bacterium did not multiply in citrus leaves pre-treated with oxytetracycline before *Xcc*-inoculation. Adequate application of effective chemicals, such as oxytetracycline may successfully control citrus canker.

Assessment of copper resistance in populations of *Pseudomonas syringae* pv. *phaseolicola*, the causal agent of halo blight on snap bean
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Phytopathology 101:S202

Halo blight infects foliage and pods of beans, and is a major bacterial disease on beans worldwide caused by *Pseudomonas syringae* pv. *phaseolicola* (Psp). Psp is most destructive in areas such as Florida where temperatures are moderate and abundant inoculum is present. Application of fixed copper is the primary method used by growers to reduce the damage. However, the development of copper-resistance in the pathogen has become a primary concern in managing this disease. Samples of snap bean with typical halo blight symptoms were collected from commercial fields in 2010 at Homestead, Florida. Bacterial isolates were obtained by culturing symptomatic leaf tissues on nutrient agar. A total of 36 isolates were subsequently identified as Psp by sequencing the 16S DNA gene. The pathogenicity of these isolates was confirmed by foliar and pod inoculation. All 36 of the isolates grew vigorously in nutrient broth (NB), but in NB containing 1.2 g Kocide 3000 per liter, 7 isolates failed to grow. The CFU per ml of the remaining 29 isolates increased as much as10000 times in 24 h. The survival and growth of the 29 isolates in NB + Kocide corresponded to that on NA + Kocide and CYE + CuSO4 or Kocide. Results from greenhouse experiments indicated that addition of mancozeb improved the efficacy of fixed copper for control of halo blight on snap bean caused by resistant Psp. Assays on NA or CYE agar plates with and without copper could be used for rapid assessment of copper resistance in Psp.

Analysis on population sources of the first generation *Loxostege sticticalis* L. (Lepidoptera: Pyralidae) moth in China
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Phytopathology 101:S202

The beet webworm moth, *Loxostege sticticalis* L. (Lepidoptera: Pyralidae), is a worldwide pest mainly distributing on the Eurasia Continent and North America. Its first generation outbroke in 2008 and caused massive losses to the farming and animal husbandry industries in China. The migration behavior of the insect pest were observed with a vertical-looking radar, a simultaneously-operated searchlight trap and surface light trap at the radar station in Xilinhot, Inner Mongolian Autonomous Region. The population sources were studied by the means of wind field analysis and trajectory analysis. The results showed that the first generation moths in North and Northeast China at the end of July mainly came from the central and east regions of the Republic of Mongolia, the China-Mongolia and China-Russia boarder. The peaks of the moths occurred in most regions of North China at the early August were mainly caused by the moths dispersed by the north-easterly and south-easterly airflows. The moths occurred on 6 August at Kiamusze of Heilongjiang province mainly originated from Russia and the China-Russia border. Therefore, it is an effective way to control the international pest by strengthening the cooperative with Mongolia and Russia and to study on the migration behavior and population sources of the L. sticticalis moth in large-scale.

Evolution of mode of infection in the rice blast fungus and allied species
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Phytopathology 101:S202

The family Magnaporthaceae contains devastating fungal cereal and grass pathogens, such as *Magnaporthe oryzae* (rice blast fungus, formerly known as M. grisea), *M. poae* (summer patch pathogen of turf grasses), and *Gaeumannomyces graminis* (take-all fungus of various cereals and grasses), which are popular model organisms in fungal biology and host-pathogen interaction studies. Despite their ecological and economic importance, the phylogenetic relationships among the constituent species remain ambiguous due to the lack of convincing morphological characters and paucity of molecular data for the majority of the non-model species in the family. In this study, our multilocus phylogeny suggests that both *Magnaporthe* and *Gaeumannomyces* are polyphyletic genera. The phylogeny also provides insights into fungal biology and pathogenesis. *Magnaporthe oryzae* formed a basal clade, while *M. poae* and *M. rhizophila* formed another well-supported clade with *G. incrustans*, *G. graminis* and *M. salvinii*. The basal species infects both root and aerial parts of plant host, while the aerial infection capacity seems to be lost in the taxa of the latter clade. The study indicates that anamorphic and ecological features are more informative than the teleomorphic characters in defining monophyletic groups among these taxa.

A DNA virus in grapevine and its association with vein-clearing and vine decline syndrome
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More than sixty viruses that are known to infect grapevine all contain RNA as their genome. A novel DNA virus was discovered in grapevine upon deep sequencing of cDNA libraries that were constructed from small RNAs of grapevines. Results from polymerase chain reaction (PCR) assays indicated that the DNA virus was closely associated with the vein clearing and vine decline syndrome that affects vineyards in the Midwest of U.S.A., and thus is given a provisional name Grapevine vein clearing virus (GVCV). Three overlapping DNA fragments were amplified from the symptomatic vine and subject to sequencing from both directions by primer walking. The whole genome of GVCV was assembled and represents a circular DNA of 7,752

base pairs. Four open reading frames (ORFs) are predicted on the sense genomic strand. The sequence of GVCV is most closely related to the genomes of badnaviruses in the family Caulimoviridae. GVCV has been detected in six grape varieties that show similar syndrome in Missouri, Illinois and Indiana. Restriction Fragment Length Polymorphism (RFLP) and sequencing of DNA fragments revealed a great genetic diversity of GVCV populations in commercial vineyards. Interestingly, GVCV was also found in wild grapevine species in their native habitats in Missouri. Discovery of this novel DNA virus allows us to investigate its epidemics and damages to the grape production, and its causal relationship with the syndrome.

Interactions of post emergence herbicides, strobulurin fungicides, and Rhizoctonia root rot of soybean

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Rhizoctonia root rot, caused by *Rhizcontonia solani* is a major disease of soybeans in the north-central United States. The emergence of glyphosate resistant weeds and the payment premium for growing non-GMO soybeans has increased the use of non-glyphosate herbicides on soybeans. However, some of these herbicides may increase the severity of root rot caused by *Rhizoctonia solani*. Field and greenhouse studies were conducted to evaluate the potential interaction among glyphosate-tolerant soybeans, post emergence herbicides (glyphosate, acifluorfen, lactofen, imazethapyr), and fungicide seed treatments (azoxystrobin, pyraclostrobin, trifloxystrobin). Inoculum of *R. solani* (AG 2-2) was planted along with the treated seed. Herbicides were applied at recommended field rates at the V4 growth stage. Plant stand was counted after seedling emergence, and soybean roots samples were collected two weeks after herbicide application. Based on the data from two locations (Urbana, IL and Monmouth, IL), plant stand was significantly increased in the fungicide treated plots compare to non-treated plots. Analysis of variance revealed significant treatment effects on root rot severity, and lactofen treated plants showed the highest disease severity levels and reduced yields. The azoxystrobin seed treatment provided the best protection against Rhizoctonia root rot disease. There was no significant difference of root rot severity between glyphosate treated and non-treated plants.

Identification of the pathogens caused greenhouse strawberry root and crown diseases in Beijing area, China

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Phytopathology 101:S203

There has been a particularly increasing number of strawberry cultivation in greenhouse in Beijing suburbs in recent years. However, root and crown diseases causing wilt and even death of strawberry have become a serious problem, which affects the strawberry production significantly. It was unknown which pathogens caused these diseases in strawberry. Pathogen identification was investigated prior to the efficient control of the diseases in strawberry. Sampling was carried out from diseased root and crown of strawberry in greenhouses from five different locations in Beijing during Oct. 2010 to 2011. Pathogen was isolated with regular tissue isolating method and the resulting isolates were purified by isolating single conidia. Morphologically characterization indicated that the isolates belonged to three respective genera: *Colletotrichum*, *Fusarium* and *Cylindrocarpon*. Reappearance of the symptoms on healthy strawberry plants 3–4 weeks after inoculation with the conidial suspension to the crown also confirmed the pathogen identification. Phylogenetic characterization of rDNA ITS was performed in the isolate of *Colletotrichum*, demonstrating that the sequence of amplified PCR product with primers ITS1/ITS4 shared a 99% identity with that of *Colletotrichum gloeosporioides* in GenBank. Herein, one of the pathogens that cause crown disease of greenhouse strawberry from the collected isolates was identified as *C. gloeosporioides*. As such, species identification of other isolates is undergoing.

Causes of genetic diversities of plant viruses in Yunnan

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There exist abundant resources of plant virus in Yunnan. So far, 13 families, 18 genus and 65 species of plant virus have been found, which account for 50% of the plant virus species of China. The genus Begomovirus has 20 species, Tospovirus has 6 species, Potyvirus has 12 species. 26 isolates of Tomato yellow leaf curl China virus (TYCCNV) were found in Yunnan. Sequence analysis of TYCCNV DNA-A showed all these isolates have the identities of 88.7%–97.4%. There are four causes of genetic diversity of plant virus in Yunnan. Firstly, the geoecological diversity, seven typical climate regions exist from tropical climate to high plateau cold climate. Secondly, the host plant biodiversity, 17000 plant species of Yunnan, account for 62.9% of the total plant species of China, distribute at the diverse climate regions. The rich plant resources are good host plants of many different plant viruses. Thirdly, the population and diversity of the virus-transmitting vector insects such as aphid, whitefly, thrips, leaf hopper are abundant due to the favorable habitats. Forthly, new plant virus isolates or strains are emerging based on the mutation, recombination and reassortment of plant virus. The rich genetic diversity of plant virus in Yunnan provides good natural models for research of plant virus origination and evolution, interaction among plant virus and host plants, virus-transmitting insects.

Development and dispersal of chasmothecia of *Erysiphe necator* and *Podosphaera clandestina*, causal agents of powdery mildews of wine grape and cherry

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Phytopathology 101:S203

In eastern Washington chasmothecia are the only known form of perennation for the powdery mildews of grape (*Vitis vinifera* L.) and cherry (*Prunus avium* L.), caused by *Erysiphe necator* and *Podosphaera clandestina* respectively. A 2-yr study was initiated to determine the temporal formation of chasmothecia on infected foliage as well as primary and seasonal modes of dispersal. Chasmothecia development on leaves was tracked from mid-Jul through mid-Oct. Chasmothecia of *P. clandestina* were first formed shortly after harvest in early Jul and those of *E. necator* in late Aug or mid-Sep. Chasmothecia numbers of both pathogens peaked near leaf fall in Oct. Wooden posts 3 m in height equipped with glass slides (orientated vertical and horizontal) coated with silicon grease and filter paper cones were positioned around the periphery of the vineyard and orchard to study dispersal of chasmothecia by air currents. Ascospore viability was assessed using the methods of Cortesi *et al.* (1997). Peak dispersal for both types of chasmothecia occurred in Oct and was significantly correlated with precipitation and wind speed. The viability of ascocarps ranged from 38% to 92% for *E. necator* and 56% to 96% for *P. clandestina*. Chasmothecia appear to be loosened by precipitation and then dispersed by rain splash or air currents. In eastern Washington, an area characterized by sustained periods of high winds, dispersal by the latter mechanism may be epidemiologically significant.

Aapoptosis of insect cells Sf9 and Spex-VII leaded by cantharidin

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Phytopathology 101:S203

To investigate the of toxic mechanism of cantharidin (C10H12O4, CTD) against Lepidoptera insects, the morphological changes of cell livability and cell cycles arrest of insect cell lines Sf9 and Spex-VII were observed after treatment by CTD in vitro by means of transmission electron microscope (TEM) and flow cytometry. The results showed that the two lines presented apoptosis features treated with CTD as chromatin condensation, nucleic fragmentation and apoptotic body formation etc. Typical characteristics of cell apoptosis were observed in 48h by TEM in Sf9 cells treated with 3.13 μg/ml and 6.25 μg/ml and in Spex-VII cells with 25 μg/ml of CTD. Flow cytometry detection showed that cells proliferation and growth were inhibited after CTD treatments with the dosage ≥3.13 μg/ml on Sf9 cells, and ≥12.5 μg/ml on Spex-VII cells respectively. Under the condition of 12.5 μg/ml CTD for 48h, majority of Sf9 cells showed as cells cycle arrest at G0/G1 phase. Majority of cells treated by CTD exist at the early apoptosis. As the treated concentration of CTD increased the ratio of necrotic cells increased.

Survival of *Cercospora sojina* on soybean leaves in Illinois

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Historically, frogeye leaf spot (FLS; caused by *Cercospora sojina*) of soybean has been observed more frequently in the southern U.S. than the North Central U.S. However, in recent years, FLS field observations have been on the increase in the North Central U.S., including Illinois. To better understand the survival ability of *C. sojina* in Illinois, a field study was conducted across three locations: Monmouth (northern Illinois), Urbana (central Illinois), and Dixon Springs (southern Illinois). At each location, soybean leaves affected by FLS were placed at depths of 0, 10, and 20 cm and retrieved after 12, 19, and 24 months. To determine the viability of *C. sojina* in the collected leaves, a greenhouse bioassay was developed. Survival of *C. sojina* declined with time equally at all three locations through 19 months. After 24 months, *C.*

sojina from leaves collected from Monmouth and Urbana was no longer active, but was still active in leaves collected from Dixon Springs. Depth of leaf placement had no effect on survival of *C. sojina*. These results suggest that planting a non-host crop for two years in central and northern Illinois will reduce the level of *C. sojina* inoculum to a negligible amount, but non-host crops may need to be planted for a longer duration in southern Illinois to achieve the same effect.

Isolation, purification and identification of the antifungal protein produced by a newly isolated *Bacillus subtilis* strain

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Phytopathology 101:S204

Bacillus subtilis is able to produce many antagonistic substances against plant pathogens. A wild type bacterium producing antifungal protein has been isolated and identified as *B. subtilis*. The *B. subtilis* strain which was named F3 exhibited good growth inhibition against *Monilinia fructicola*, so did the culture filtrate of it. The antifungal substance of F3 was crudely prepared by ammonium sulphate precipitation of the culture filtrate. Farther purification was developed by chromatographic separation with Sephadex G-50, TEAE-Sephadex A-25 anionexchange and Sephadex G-100. The first eluting peak of Sephadex G-100 chromatographic displayed obviously antifungal active against *M. fructicola* and showed one protein band in SDS-PAGE. The active chromatographic component was analyzed with MALDI-TOF/TOF mass spectrum. NCBI protein database was searched by Mascot searching tool with the MS analysis data. Searching results show that the top protein score is 88 which means significant ($P < 0.05$) for gi|124502416, flagellin [Bacillus subtilis]. Flagella contribute to the virulence of pathogenic bacteria and flagellin gene is a good biomark for bacterial detection. The antifungal protein of *B. subtilis* strain F3 was identified as flagellin may indicate that flagellin was a new kind of *B. subtilis* antifungal protein. Thanks to funding project for academic human resources development in institutions of higher learning under the jurisdiction of Beijing municipality (PHR201107135).

Development and application of a TaqMan real-time PCR assay for rapid detection of *Magnaporthe poae*

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Phytopathology 101:S204

Turfgrasses are ubiquitous in urban landscape, athletic fields, golf courses, and residential lawns. Approximately 1.9% (16.3 million hectares) of the total continental U. S. area is covered by turfgrass. One of the most important diseases of turfgrass in North America is summer patch that is caused by the root-infecting fungus *Magnaporthe poae* Landsch. & Jacks. The pathogen affects the roots, crowns and rhizomes of several cool-season grasses under favorable environmental conditions. Detection and identification of *M. poae* are notoriously difficult and time-consuming using the conventional culture-based method that usually takes three weeks or longer. In this study, a culture-independent TaqMan real-time PCR assay has been developed for *M. poae* that enables pathogen detection from the field samples within a few hours. The assay was validated with the target pathogen, its closely related fungal species and a number of other microorganisms that inhabit the same host and soil with the target. It was used to evaluate the effectiveness of fungicide treatments. Compared with traditional diagnostic methods, we found that the TaqMan real-time PCR assay is more sensitive, accurate and fast. This assay will facilitate turfgrass management by early and accurate diagnosis of summer patch and will reduce pesticide input.

Ophiostomatoid fungi associated with bark beetles infesting conifers in China

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Phytopathology 101:S204

Bark beetles are important forest insects and are known to live in close association with ophiostomatoid fungi, especially species of Ophiostoma, Grosmannia and Ceratocystiopsis, and their asexual states. Several species are important plant pathogens and many can cause sapstain on freshly cut wood lumber. In China, there are more than 195 million hectares of natural forest mainly consisting of conifers, and about 170 species of conifer-infesting bark beetles are known. However, little is known regarding the fungi associated with these bark beetles there. During the course of the past eight years, surveys of ophiostomatoid fungi associated with economically important bark beetles such as Tomicus spp. and Ips spp. have been conducted in South-western and North-eastern China. A total of 750 isolates of Ophiostoma, Grosmannia, Leptographium, Pesotum and Graphium have emerged. They have been identified based on morphological characteristics and these identifications are being confirmed using DNA sequence comparisons. Species such as Ophiostoma abietinum, O. ips, O. piceae, O. quercus, O. setosum, Pesotum fragrans, Leptographium pineti, Grosmannia yunnanensis, Graphium pseudormiticum and eight newly described Leptographium species were characterized and described. A further four Ophiostoma spp. and one Pesotum sp. appear to represent novel taxa, and further study will be needed. The results of this study have greatly expanded our current knowledge of the ophiostomatoid fungi occurring in China.

Field disease reaction of rice cultivars and elite lines in Texas

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Phytopathology 101:S204

The development and use of improved disease resistance rice cultivars remains of foremost importance to rice producers. Field evaluation of disease resistance under local environments is essential toward this effort. More than 48 cultivars and Texas elite breeding lines were evaluated for resistance to sheath blight (*Rhizoctonia solani*), bacterial panicle blight (*Burkholderia glumae*) and narrow brown leaf spot (*Cercospora janseana*), the three major rice diseases in Texas, in five trials located at two locations of Texas in 2009 and 2010. Sheath blight was introduced by inoculation while narrow brown leaf spot came from natural infection. Bacterial panicle blight developed from either natural infection or bacterial inoculum at the heading stage. All but several cultivars and lines were susceptible or very susceptible to sheath blight with disease severity ratings of 5 or above on a 0–9 scale. CL142-AR, Jasmine 85, Milagro Filipino, Templeton, Rondo, and the hybrid XL723 showed partial resistance to sheath blight. In reaction to bacterial panicle blight, all cultivars and lines were susceptible except Catahoula, Jupiter, Spring, XL723, and the two TX elite lines, RU0703190 and RU0703144, which showed partial resistance. Except CL181-AR, Jazzman, Sabine and Sierra, all others were resistant or moderately resistant to narrow brown leaf spot. CL151, Presidio, Tesanai 2, and RU0703190 had grain yields that ranked among the highest.

Field evaluation of a beneficial *Bacillus* strain for biocontrol of sheath blight in rice

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Sheath blight caused by *Rhizoctonia solani* is the most important rice disease in the southern United States. Rice farmers heavily depend on fungicides for control of sheath blight. A field experiment was conducted at two locations to evaluate the efficacy of *Bacillus subtili*s strain MBI-600 and its combined use with a reduced rate of azoxystrobin for management of sheath blight. MBI-600 is the active ingredient in the biopesticide, Integral, and was among the most effective bacterial strains that were screened against sheath blight in our previous in vitro and greenhouse evaluations. *R. solani* inoculum was introduced into plots at panicle differentiation. Foliar applications of MBI-600 and azoxystrobin (Quadris 2.08 SC) were made at the boot stage. MBI-600, applied to both seed and the foliage at 10^9 CFU/ml, resulted in a significant reduction in sheath blight severity over the untreated control. The combined use of MBI-600 with azoxystrobin at 0.08 kg a.i./ha further reduced disease severity. The efficacy of this combined treatment was comparable to that of azoxystrobin at 0.16 kg a.i./ha. The combined treatment tended to have numerically higher grain yield than the untreated control and have similar yield to azoxystrobin at 0.16 kg a.i./ha. The combined use of the beneficial *Bacillus* strain with a rate-reduced fungicide may provide a practical means to minimize yield losses caused by sheath blight while reducing the usage of fungicides on rice.

Suppression of soilborne diseases in watermelon and rice with brassica biofumigation crops

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Phytopathology 101:S204

Fusarium wilt caused by *Fusarium oxysporum* f. sp. *niveum* (FON) and sheath blight caused by *Rhizoctonia solani* are, respectively, the most important soilborne diseases in watermelon and rice worldwide. Chemical control of these diseases is effective. However, chemical control is also costly and may have negative environmental consequences. A research program was initiated

to develop a biofumigation approach as an alternative for management of these diseases. *In vitro* assays were performed on agar plates to evaluate the effects of volatile activity of the macerated tissues of *Brassica* and other crops on the growth of FON and *R. solani*. Of 37 crops evaluated, 15 *Brassica* crops significantly inhibited FON, 17 inhibited *R. solani*, and seven of them, including the mustards 'Brand 199', 'Florida Broadleaf' and 'Sheali Hong', reduced the growth of both pathogens up to 50%. When amended into potted soil in the greenhouse, 12 *Brassica* crops reduced Fusarium wilt incidence by 24 to 72% and increased plant weight by 23 to 183%. Mustard 'Brand 199', 'Florida Broadleaf' and 'Sheali Hong' were most effective in reducing wilt incidence (up to 60%) compared to the nonamended control in microplot and field trials. Use of *brassica* crops may offer a new alternative management for Fusarium wilt in watermelon and sheath blight in rice.

Severe outbreak of bacterial panicle blight across Texas Rice Belt in 2010

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Bacterial panicle blight symptoms have been observed in rice fields in Texas for many years but it was not until 1996 that *Burkholderia glumae* was identified as the causal agent. Although it is generally considered a minor disease, there have been years when significant losses to yield and milling quality occurred. In 2010, a severe outbreak of this disease occurred throughout the Texas Rice Belt. The disease caused partially filled or aborted grains, resulting in an estimated 10 to 20% yield loss and reduced milling quality. The disease was most severe in the cultivars CL111, CL261, Cocodrie, and Francis but was relatively less severe in CL151, Jupiter, Neptune, Presidio, and the hybrid XL723. The disease also was present in the ratoon (second) crop but caused no serious damage. *B. glumae* was consistently isolated from the symptomatic panicle samples collected from across the Texas Rice Belt. Of 47 isolates of *B. glumae* collected, 10 were selected for pathogenicity assays and all were shown to be pathogenic. Two of the pathogenic isolates, one from the main crop and the other from the ratoon crop, were further verified to be *B. glumae* using PCR. This is the first report of bacterial panicle blight of rice in the ratoon crop in the United States resulting from an epidemic outbreak of this disease in Texas. This disease poses a threat to rice production since there are no chemicals or highly resistant cultivars currently available for management of this disease.

Molecular characterization of a Chinese isolate of Chickpea chlorotic stunt virus infecting pea

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Phytopathology 101:S205

Chickpea chlorotic stunt virus (CpCSV) causes yellowing and stunting diseases in cool-season food legumes in West Asia and North Africa. The complete genomic sequence of a Chinese isolate of CpCSV infecting pea (CpCSV-CHN) was determined for the first time. CpCSV-CHN was 6021 nts in length, its genome organization was very similar to CpCSV-Eth. Sequence identity analysis showed that CpCSV-CHN was most closely related to, but distinct from CpCSV-Eth. The full length of CpCSV-CHN shared 82.5% nucleotide sequence identity with CpCSV-Eth. ORFs, 0, 1, 2, 3, 4, 5 and the corresponding encoded proteins (P0, P1, P1-P2, P3, P4, P3-P5) of CpCSV-CHN had homology ranged from 66.7% to 93.1% and from 74.1% to 91.5%, respectively, with that of CpCSV-Eth. Except the conserved P3, identity in aa sequence of any gene product of CpCSV-CHN with that of CpCSV-Eth was <90%. Based on the sequence and phylogenetic analysis, we proposed CpCSV-CHN a new virus, and this was the first report of CpCSV in China.

Development of a new methodology for identification of rice cultivar's resistance to rice stripe disease

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Phytopathology 101:S205

Rice stripe disease, caused by Rice stripe virus (RSV), is one of the most serious rice diseases in temperate and subtropical regions of the world. Culturing resistant varieties is one of the most economically and environmentally effective control strategies. Since the selection method had been restricting the development of breeding process, a new methodology was developed to identify the rice stripe disease resistance in rice varieties, named tiller inoculation. The resistance to rice stripe disease was analyzed in Zhendao 88 and Wuyujing No.3 by tiller inoculation and inoculating at the seedling stage, separately. The results showed that the identification effect was uniform in two methods. It indicated the reliability and precision of tiller inoculation. The breeding process and genetic research for rice stripe disease resistance should be accelerated by using this method in the early generation selection.

Pathogenicity analysis of secretory protein of the rice blast fungus and interaction study using rice cell suspension culture

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Phytopathology 101:S205

The rice blast fungal strains CH-63 and TH-16 with 2 different pathogenic factors were cultivated in a liquid culture medium under nitrogen starvation. After 2 d of cultivation, the protein secreted by the fungal strains was extracted by ammonium sulfate sedimentation and purified by dialysis. A rice plant was infected with the secreted protein of the rice blast fungal strains through inoculation or soaking method. A necrotic lesion was observed in the rice leaf and stem at the inoculated position; the lesion diameter was 2 to 4 times that of control. Soaking resulted in severe browning of the rice radicle, and the inoculated plant height was only half of that of control. In the rice plant inoculated with the secreted protein, the rice disease symptoms disappeared or weakened after treatment with protease K. This finding further confirmed that the secreted protein was the major pathogenicity factor. Twenty-four monogenic rice lines developed at International Rice Research Institute (IRRI) were inoculated with the secreted protein and then categorized into resistant, intermediate resistant, and susceptible groups based on the size of necrotic lesion observed on the rice leaf tissue after 72 h of inoculation. The pathogenicity of rice blast fungal strains CH-63 and TH-16 was compared; the CH-63 strain showed higher pathogenicity than the TH-16 strain. The difference between the pathogenicity of these 2 strains was proved by trypan blue staining.

Molecular mapping of new genes for stripe rust resistance in spring wheat genotypes PI 178759 and PI 183527

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Phytopathology 101:S205

New genes are essential for breeding wheat cultivars with effective resistance against stripe rust, caused by *Puccinia striiformis* f. sp. *tritici* (*Pst*). Spring wheat PI 178759 and PI 183527 were identified to have high-levels of resistance in fields in 2004 to 2010. Further testing under controlled greenhouse conditions with individual races showed that PI 183527 had a typical high-temperature adult-plant (HTAP) resistance while the HTAP resistance in PI 178759 was more temperature-sensitive. The genotypes were crossed with susceptible spring wheat Avocet. Genetic analysis identified two genes in PI 178759 and one gene in PI 183527 using the phenotypic data of F_2 and F_3 tested with individual *Pst* races under the controlled greenhouse conditions and of F_3 tested in the field under natural infection of the pathogen. Molecular mapping using resistance gene analog polymorphism and simple sequence repeat (SSR) markers located the three genes on the long arm of chromosome 7B. The two genes in PI 178759 were 39.1 cM away. One of the PI 178759 genes was linked in repulsion with the PI 183527 gene. Both genes were flanked by SSR markers *Xbarc182* and *Xcfa2040*. Allelism testing is underway to determine if the two genes are at different loci. Based on the types of resistance, the genes in PI 178759 and PI 183527 appeared to be different from previously identified genes and should be useful for developing wheat cultivars with durable resistance to stripe rust.

Metagenomic analysis of *Candidatus* Liberibacter asiaticus in naturally populated psyllids (*Diaphorina citri*) using BAC libraries

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Phytopathology 101:S205

Candidatus Liberibacter asiaticus (Las) is the most prevalent species of three species of *Ca*. Liberibacter causing citrus huanglongbing (HLB) in the world. The Las genome sequence published in 2009 was obtained from a single Las-infected psyllid using metagenomic approach. Studies of genetic diversity suggest a strong potential of various populations of Las bacteria exiting in different hosts. In this study, three BAC libraries were constructed using whole genomic DNA from thousands of *Diaphorina citri* collected from the HLB-affected citrus plants in the fields. A total of 61,440 clones were obtained from three libraries constructed by partial digestions of *Bam*HI and *Hin*dIII, respectively, or random shear. Superpools and pools of DNA from the *Bam*HI BAC library were screened by conventional PCR using Las-specific primers. Thirty sets of the Las-specific primers were designed with a

distance from 30-50 kb based on the Las-psy62 genome. Positive BAC clones were identified and subjected to end sequencing. The results indicated 54 overlapping clones that matched the Las genome sequence with a size range from 20 kb to 140 kb. PCR confirmation of the end sequence using infected vs. non-infected plants or insects with the primer sets designed from the end sequences revealed 7 overlapping clones contained new sequences that were missed in the psy62 genome, while 15 clones did not match the Las sequence, suggesting a potential chimera in these BAC clones.

Sweet bunden of sugarberry - a novel ampelovirus found in *Celtis laevigata*
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Phytopathology 101:S206

Virus –like symptoms are observed in several Celtis species across the Southern United States. The most striking of symptoms are seen on sugarberry (Celtis laevigata) where bright yellow mottling appears in late spring and becomes more prominent as the season progresses. Here we report a new virus, closely associated with the bright mottling symptoms. The new virus has a monopartite, single-stranded RNA genome consisting of approximately 17 kb. Protein pairwise comparisons and phylogenetic analysis show that the virus is most closely related to Grapevine leafroll-associated virus-3, the type member of the genus Ampelovirus, family Closteroviridae. The amino acid identities between the two viruses range from 53% for the RNA dependent RNA polymerase to 26% for the coat protein homolog signifying that the new sugarberry virus is a novel member of the genus. Detection protocols have been developed and the virus was detected is several other Celtis species showing yellow mottling symptoms. High numbers of mealybugs, known vectors of ampeloviruses, are often seen on affected trees and transmission studies are underway to determine whether they can transmit the virus.

***Fusarium verticillioides* infection of maize seedlings and the corresponding movement of fungus, fumonisins, and biomarkers of exposure**
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In earlier studies using maize seedlings grown from kernels inoculated with *Fusarium verticillioides*, fumonisin B1 (FB1) was preferentially accumulated in leaf tissue compared to FB2 and FB3, whereas, in plants watered with purified toxins there was no accumulation of fumonisins in the leaves. The present study was designed to validate the effects observed previously, but utilized FB producing and non-producing strains of *F. verticillioides*. This study correlated fungal infection with accumulation of FB1, FB2 and FB3 and sphingolipid biomarkers in plant tissues. Different maize lines were used to determine their resistance to FB and sphingolipid biomarker accumulation and the development of disease symptoms when exposed to pathogenic and non-pathogenic strains of *F. verticilliodes*. As seen in the previous studies, FB1 accumulated to a higher level than FB2 or FB3, in those plants exposed to FB-producing strains of the fungus. In addition there was clear evidence of ceramide synthase inhibition based on accumulation of sphingoid bases and sphingoid base 1-phosphates in root and leaf tissue. As expected, resistant maize lines showed fewer effects from pathogenesis and less FB1 and biomarker accumulation compared to the susceptible lines.

The SlyA/MarR family regulator Hor regulates HrpL regulon T3SS genes in a HrpL independent manner
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The *hrp* genes of *Dickeya dadantii* 3937 encode a type III secretion system (T3SS) which is essential for its full virulence. Previous studies of the T3SS regulation in *D. dadantii* revealed that the expression of the *hrp* genes is regulated by HrpL, an alternative sigma factor, through the HrpX-HrpY-HrpS-HrpL and GacS-GacA-*rsmB*-RsmA pathways. In this work, we identified a novel T3SS regulator of the MarR/SlyA family, Hor, which regulates *hrp* regulon genes through a HrpL independent pathway. We demonstrated that the expression of *hrpL* was enhanced in a *hor* deletion mutant, due to an enhanced *hrpS* expression and a reduced *rsmA* expression. However, the expression of *hrpA* and *hrpN*, two *hrp* genes in the HrpL regulon, was greatly reduced in the *hor* mutant. Interestingly, concomitant with its up-regulation of the T3SS, Hor exerts a negative regulatory effect on the production of extracellular enzymes such as pectate lyase (Pel), cellulase (Cel), and protease (Prt) in *D. dadantii*. These results indicate that Hor plays a role in coordinate regulation between two virulence factors, T3SS and extracellular enzyme production. Finally, we demonstrate that Hor also controls bacterial swimming motility, pellicle formation, and bacterial virulence in *D. dadantii*.

Characterization of the host defense response induced by the flagellin protein of *Candidatus* Liberibacter asiaticus
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The pathogen-associated molecular patterns (PAMPs) are recognized by plant receptors leading to PAMPs-triggered immunity. The highly conserved N-terminus domain of flagellin (Flg22) is an extracellular PAMP that is recognized by most plant species. In the genome of '*Candidatus* Liberibacter asiaticus', the causal agent of citrus Huanglongbing, there is only one copy of flagellin-encoding gene in contrast to four copies of them in *Sinorhizobium meliloti.* Transient expression of the Las-flagellin using PVX expression vector induces an immunity response with cell death in *Nicotiana benthamiana*. The flagellin consists of 22 amino acids near N-terminus (-DRVSSGLRVSD AADNAAYWSIA-), sharing the conserved Flg22 domain. By comparison with the nonfunctional homologue from *S. meliloti*, three divergent amino acids were identified. To test if this PAMP exited in citrus, 10 µM commercial Flg22 (RP19986, GenScript) and FLg22-Las were infiltrated into young tissue of citrus leaves, respectively. The results indicated these peptides induced hypersensitive response in young citrus leaves. This is first evidence of citrus defense response to the phloem-limited '*Ca.* L. asiaticus'.