2009 APS Annual Meeting Abstracts of Presentations

Abstracts submitted for presentation at the APS 2009 Annual Meeting in Portland, Oregon, August 1–5, 2009 (including abstracts submitted for presentation at the 2009 APS Pacific Division Meeting). The abstracts are arranged alphabetically by the first author's name.

Field assessment of non-toxigenic *Aspergillus flavus* strain K49 in competitive displacement of toxigenic isolates
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Non-toxigenic strains of *Aspergillus flavus* offer the potential to control aflatoxin contamination by competitive displacement of indigenous populations of *A. flavus* colonizing corn. Two sets of experiments were conducted to assess the competitiveness of strain K49 when challenged against two toxigenic isolates (F3W4 or K54) using a pin-bar inoculation technique. In 2007, corn ears were inoculated with six ratios of strain K49 and F3W4 in two experimental sites. A second study assessed the ability of equal densities of K49 when challenged with toxigenic strains F3W4 and K54 in 2007 and 2008. In the Stoneville site, when K49 comprised 10% of the inoculum, aflatoxin concentrations were reduced to ~500 ppb compared to 3500 ppb when inoculated with 100% F3W4. At the Elizabeth site, a 30% and 90% reduction in aflatoxin was observed when 10% and 50% of the inoculum was non-toxigenic strain K49, respectively. Strain K49 was capable of reducing aflatoxin contamination by ~90% when challenged with the high producing strain K54 or the moderate producer F3W4. In challenges with either F3W4 or K54, greater than 85% of isolates recovered were non-toxigenic, while 100% of the isolates were toxigenic when inoculated with F3W4 or K54 alone. These studies indicate that competition is affected by location and environmental conditions associated with the study, however, strain K49 had a similar degree of efficacy in competition with two toxigenic strains of varying potential for aflatoxin production.

Suppression of Phytophthora blight of cucumber and bell pepper with AG3 phosphonate
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Phytophthora blight or crown or root rot (*Phytophthora capsici* Leonian) is an important disease of a wide range of plant species including peppers, tomatoes, cucumbers and other cucurbits. AG3 phosphonate (Calirus 150, Bromine Compounds Ltd., Beer Sheva, Israel) is a new liquid formulation effective against Pythium damping-off of cucumber and clubroot of bok choy and cabbage. In this study, the effectiveness of AG3 phosphonate to suppress Phytophthora damping-off and root rot of cucumber or Phytopthora blight of bell pepper was determined in a peat-based mix or sandy-loam soil artificially infested with soil inoculum of *P. capsici* under growth room conditions. In an infested peat-based mix, AG3 phosphonate preplant amendment or postplanting drench treatments (0.05, 0.1, and 0.2% a.i.) and a 10-min seed-soak treatment (10.45% a.i.) significantly increased the percentage of healthy cucumber seedlings and reduced damping-off and root rot severity. The seed-soak and 0.2% treatments were the most effective treatments that consistently suppressed damping-off and root rot of cucumber even in a peat-based mix infested with high inoculum levels of *P. capsici*. The bell pepper transplants grown in an infested peat-based mix or a sandy-loam soil and received the single drench applications of AG3 phosphonate (0.05, 0.1, and 0.2% a.i.) showed significantly less incidence and severity of Phytophthora blight compared to the control plants receiving no treatment.

Quantitative trait loci associated with seedling and adult-plant resistance to oat crown rust caused by *Puccinia coronata*
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Phytopathology 99:S1

Crown rust is an economically important disease of oat worldwide, causing yield loss, reduction of test weight and seed quality, and increased lodging. Genetic resistance is an effective method to control crown rust. The oat line MN841801 has shown disease resistance to diverse populations of *P. coronata* for more than 30 years. The objective of this study was to identify and map the resistance in MN841801 at seedling and adult-plant stages using a population of 150 $F_{6:8}$ recombinant inbred lines of the cross of MN841801-1/'Noble-2'. The population was evaluated for crown rust resistance at the seedling stage using isolates avirulent on MN841801-1 and virulent on 'Noble-2'. Partial adult-plant resistance (APR) was evaluated in field and greenhouse experiments using two isolates virulent on both parents at the seedling stage but giving low infection on adult plants of MN841801-1. The seedling tests identified three loci for resistance on linkage groups MN3, MN6 and MN26. A total of nine quantitative trait loci (QTLs) were associated with APR. The two major APR QTLs overlapped with regions on MN3 and MN26 associated with seedling resistance. Comparison of our results with other crown rust resistance mapping studies indicates that seedling and APR resistance loci may be clustered in specific regions of the oat genome.

Evolutionary epidemiology of *Beet necrotic yellow vein virus* (BNYVV) in North America
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Analysis of the genetic structure of BNYVV populations from North America indicated that, before commercialization of resistant *Rz1*-cultivars, a wild type viral haplotype encoding the ACD p25-motif (RNA3) predominated in susceptible sugarbeets nationwide. Recently, rhizomania has emerged in resistant cultivars. This outbreak was associated with evolution of BNYVV from ACD to ALD (avirulent) to VLE (resistance breaking) p25-motifs in

design degenerate primers for PCR amplification from fungal DNA and cDNA. An open reading frame coding for a victoriocin precursor of 183 amino acids with calculated molecular mass of 20 kDa was amplified by PCR from *H. victoriae* genomic DNA. Sequence analysis indicates that victoriocin has a sequence motif similar to that found in scorpion short toxin/charybdotoxin and a consensus sequence similar to that of defensins. Although victoriocin is encoded by the fungal host, it resembles the virally-encoded killer proteins in that it is expressed *in vivo* as a preprotoxin precursor consisting of a hydrophobic N-terminal secretion signal, followed by a pro-region and terminating in a classical Kex2p endopeptidase cleavage site that generates the N-terminus of the mature victoriocin predictably in a late Golgi compartment. Overproduction of victoriocin in a virus-free strain transformed with the *victoriocin* gene mimicked the effects of virus infection on inducing overexpression and secretion of victoriocin in cultural filtrates.

Diversity of *Fusarium oxysporum* isolates infecting cortical tissues of chickpea roots

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Fusarium oxysporum (*Fo*) comprises host-specific pathogenic populations that cause mainly Vascular wilts a result of vascular infection of a host plant; and non-pathogenic but parasitic soil-inhabitant populations capable of colonizing the root cortex of host and non-host plants. The latter statement implies that any soil-inhabitant *Fo* isolate can potentially colonize the root cortex of any plant, but this assumption has remained largely unaddressed. Consequently, *Fo* populations resident in soil or colonizing the plant root cortex have received little attention. *Fo* f. sp. *ciceris* is a monophyletic group that causes Fusarium wilt of chickpea. In this study, we analyzed 42 putatively non-pathogenic to chickpea *Fo* isolates obtained from surface-disinfested chickpea tissues from Ethiopia, Spain, Mexico, Morocco and Syria. Based on phylogenetic analysis of the translation elongation factor 1-alpha gene, the non-pathogenic isolates displayed a certain degree of diversity. The highest diversity was found in isolates from Ethiopia, thought to be a center of diversity of chickpea. However, isolates from different areas were often closely related to previously well-characterized non-pathogenic cortex-colonizing *Fo* isolates obtained from chickpeas. Mexican isolates were the exception as they were placed in a distinct clonal lineage. These results suggest that only certain *Fo* genotypes have the ability to colonize the host root cortex and may represent a possible origin of host-specific pathogenic forms.

Current situation of citrus Huanglongbing in Guangdong, P. R. China

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Guangdong Province is an important citrus production region in China. Citrus Huanglongbing (HLB, yellow shoot disease) was observed in Guangdong probably in the late 1800's and the disease was first studied there. Since the 1990's, citrus production in Guangdong has gradually shifted from the coastal Chaoshan and Pearl River delta plains, where HLB was endemic, to the mountainous / hillock North and West areas, where citrus production was limited and HLB was little known. As citrus production expanded, reports of HLB followed. To understand the HLB situation in Guangdong, we collected symptomatic citrus samples from 16 cultivars in 12 prefecture cities in 2007. PCR with primer set OI1/OI2c was used to detect "*Candidatus* Liberibacter asiaticus" for HLB confirmation. Among the total of 359 samples collected, 241 (67.1%) were positive in "*Ca.* L. asiaticus", distributed in all 12 cities. Of particular importance is the confirmation of HLB in a mandarin cultivar "Shatangjie", which currently occupies two third of the citrus planting acreage in Guangdong. We also identified HLB in the less popular cultivars such as "Mashuijie" and "Cuntianjie" which have high potential for future citrus cultivations. Our data indicated that affected budwoods probably played a key role in the current spread of HLB. To avoid future HLB outbreaks, strict regulation of propagation materials should be exercised along with optimal orchard management.

Comparisons of plant cover estimates using APS *Assess* software and point-frame transects at Camp Guernsey, Wyoming

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The U. S. Army currently uses vegetative surveys to monitor ground cover and vehicle damage on training areas; however, methodologies for determining vegetative cover are not universal and vary among installations. These methods can be so labor-intensive and time consuming that repeated estimates per plot during the year become unrealistic. There is a need for techniques to estimate ground cover on training lands that are quantitative, accurate, inexpensive, and do not require extensive technical botanical skills. A commercially available software program, Assess (Lamari 2002), was used to analyze a series of digital images collected at Camp Guernsey, Wyoming. Three sites at Camp Guernsey were subdivided into twelve plots each. Ten random digital images were taken in each plot with a Nikon Coolpix® 4300 digital camera set on "normal" (1600 dpi resolution). Photos from Camp Guernsey were then analyzed using the Assess batch processing analysis feature. The analysis of digital photos using the *Assess* software is a very quick and accurate way to estimate ground cover. The collection and analysis of the photos took significantly less time than the point-frame method and the cover estimates were not significantly different during the spring and summer sampling periods. The results of this study show that the use of digital image analysis to determine vegetative cover can be an accurate, cost-effective way to monitor vegetative conditions on Army training lands.

A simple, reliable method for creating unmarked mutations in Gram-negative bacteria

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The ability to introduce site-specific unmarked deletions into a bacterium's genome is an important genetic tool. However, we found that creating the 'deletion allele' for the targeted gene using splice-overlap extension PCR or ligation independent cloning was problematic. As an alternative, we adapted the recently described DelsGate method, which employs a Gateway donor vector and the BP clonase reaction, for use in Gram-negative bacteria. First, we created a new vector, pDONR-SacTet, by introducing a cassette with *sacB* and a tetracycline resistance marker into pDONR201. Second, we reversed which site-specific primers normally are modified by the addition of *attB1*/*attB2* and the very rare I-*Sce*I recognition sequences. Third, we electroporated the circular plasmid carrying the deletion allele, rather than a linearized form, into *R. solanacearum*. Typical of SacB-assisted two-step allelic replacement, we then: a) selected transformants on agar plates supplemented with tetracycline or kanamycin and b) plated colonies on a minimal medium supplemented with 5% sucrose as the sole carbon source. Sucrose-sensitive colonies were screened for antibiotic sensitivity, and small pools were tested by colony PCR to detect those with the desired deletion. This method of creating deletion alleles is simple and robust, and should work for all Gram-negative bacteria that are amenable to SacB-assisted selection.

New records for the Brazilian Cerrado of leaf pathogens on *Jatropha curcas*

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Jatropha curcas is an euphorbiaceous plant that produces seeds with high oil concentration. The species is being extensively studied by Embrapa in several research centers throughout warmer and drier areas of Brazil to explore its potential as raw material for bio-diesel production. In Planaltina, Distrito Federal, leaf spots caused by a cercosporoid hyphomycete was observed and *Cercospora jatrophae-curcas* was the agent precisely identified. The symptoms consisted of well delimited light brown irregular necrotic spots where fascicles of sympodial cicatrized conidiophores were found in large numbers yielding simple obclavate conidia with an slight tint of pale brown. The dimensions of all structures agreed with those shown in the original species description. Also depressed yellowish leaf areas containing several acervuli were present on the host leaf. Two Colletotrichum species isolated are still being identified and tested. As *J. curcas* is still a crop under domestication in Brazil, it is important to take note of its associations with pathogenic fungi that may become limiting factors to the future growth of this potential commodity.

First record of *Jatropha* rust (*Phakopsora arthuriana*) in Central Brazil

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Jatropha curcas (Euphorbiaceae) is a potential source of biodiesel with ongoing research in Mato Grosso do Sul (MGS), Brazil. From March 2007 serious outbreaks of rust (*Phakopsora arthuriana* Buriticá & Hennen,

anamorph: *Malupa jatrophicola* (Arthur) Buriticá & Hennen) were detected in the municipalities of Dourados and Eldorado. It deserves attention the fact that until 1994 the fungus was known as *P. jatrophicola* Cummins, however, according to Hennen et al. [In: "Catalogue of the species of plant rust fungi of Brazil, 2005", also on line at http://www.jbrj.gov.br] Buriticá & Hennen in 1974 published *P. arthuriana* - "because *P. jatrophicola* (Arthur) Cummins published in 1937, technically refers only to an anamorph. Cummins (1937) transferred an anamorph name, *Uredo jatrophicola* Arthur, to the teleomorph genus. He did publish a description of the telia in English, but not in Latin as required by the Code. Later, Cummins (1956) published *P. jatrophicola* Cummins as new species, but the combination *P. jatrophicola* had been preoccupied, and could not be used as a name for a new species". – Symptoms description of the disease, and detailed illustration of the fungus specimen from MGS will be shown including both teleomorphic and anamorphic phases.

Impacts of Fusarium root inoculation on soybean plants
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Fusarium is a common fungal genus that has been implicated in soybean root rot, but its impact on yield is unclear. To estimate potential yield loss caused by *Fusarium* spp., fumigated and non-fumigated microplot experiments were established near Gilbert, IA. Microplots contained a single row of 20 plants. Sorghum seeds colonized with *Fusarium* isolates from soybean roots were used as inoculum. Fumigated plots were infested with 19 isolates representing seven species (*F. oxysporum, F. solani, F. graminearum, F. acuminatum, F. semitectum, F. equiseti, F. sporotrichioides*) and non-fumigated plots were infested with one isolate each of *F. oxysporum, F. solani, F. acuminatum,* and *F. graminearum*. Plants at R1 were evaluated for root rot, root and shoot dry weight, and yield was measured. In non-fumigated plots, inoculation increased root rot severity, but this increase was significant only for the *F. oxysporum* (120L9) and *F. graminearum* isolates. Root dry weight, yield and seed moisture did not differ among isolates, but shoot dry weight differed (P = 0.0126). In fumigated plots, *Fusarium* isolates differed (P = 0.0068) in aggressiveness, with *F. oxysporum* (120L9) causing the most severe root rot and *F. oxysporum* (34T5) causing least severe symptoms. Yield and seed moisture were unaffected. Late planting possibly affected root rot severity and plant growth in fumigated plots. Pathogenicity and aggressiveness of each *Fusarium* isolate are being tested in the greenhouse.

Further characterization of the MST12 transcription factor genes in *Magnaporthe oryzae*
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In *Magnaporthe oryzae*, the *PMK1* MAP kinase is known to regulate appressorium formation and plant infection. Homologues of *PMK1* have been shown in other phytopathogenic fungi to be important for plant infection. However, there are only limited studies on transcription factors and genes regulated by this MAP kinase pathway. In *M. oryzae*, one of its downstream transcription factors is *MST12*, which is essential for infectious growth. The *mst12* mutant was non-pathogenic but still formed appressoria. In yeast two hybrid assays, a weak interaction was detected between Pmk1 and Mst12. In CoIP assays, Pmk1 was co-purified with the Mst12-3FLAG fusion protein. When overexpressed with a strong, constitutive promoter, *MST12* could partially suppress the defects of the *pmk1* mutant. On penetration assays with onion epidermal cells, a few melanized appressoria were observed and none of them were able to penetrate and form infectious hyphae. Our preliminary data of Mst12-binding site assays suggest that Mst12 has a binding site similar to that of yeast Ste12. Microarray analysis has been used to identify genes regulated by *MST12*. Promoters of genes with significantly altered expression levels in the *mst12* mutant were analyzed for common regulatory elements. Sequences similar to yeast filamentation and pheromone response elements were identified in some of these genes. A few of them have been selected for verification by qRT-PCR and further functional characterization. Data on these genes will be presented.

Response of US *Cucumis melo* Plant Introductions to *Phytophthora capsici*
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Phytophthora capsici is distributed worldwide, and is an aggressive pathogen with a broad host range infecting solanaceous, leguminaceous, and cucurbitaceous crops. Over the past two decades, increased incidence of Phytophthora blight, particularly in eastern states, has threatened production of many vegetable crops. *Cucumis melo* (honeydew and cantaloupe), while especially susceptible to fruit rot, is also susceptible to crown/root rot. Currently, little is known about host resistance to *P. capsici* in *C. melo*. To assess resistance in *C. melo* seedlings, 318 *Cucumis melo* US Plant Introductions (PIs) from diverse geographic locations and two commercial hybrid cultivars (Athena and Dinero) were grown under greenhouse conditions. At the three to four leaf stage, seedlings were inoculated with a five isolate zoospore suspension (1.0×10^4) at the crown and monitored for six weeks. All the susceptible checks ('Athena' and 'Dinero') died within seven days post inoculation. Several PIs (PI 181748, PI 182964, and PI 273438) succumbed earlier than Athena and Dinero due to crown rot. Eighty seven PIs (27%) appeared to have some degree of tolerance to *P. capsici*. The level of resistance to *P. capsici* within individual PIs was variable. The 87 PIs selected from the primary screen are currently being re-screened and the results of this study will be presented.

Cloning of putative secreted protein genes from wheat infected by *Puccinia striiformis* f. sp. *tritici*
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Wheat stripe rust, caused by *Puccinia striiformis* f. sp. *tritici* (*Pst*), is a major disease of wheat worldwide. *Pst* genes encoding secreted proteins serving as effectors to interact with plant resistance genes are hypothesized to express specifically in haustoria. The objective of this study was to isolate secreted protein genes from *Pst* haustoria. Six putative secretion protein genes with partial sequences derived from the *Pst* haustorial cDNA library were selected to obtain full-length cDNA using the 5' rapid amplification of cDNA ends. The full-length of these cDNAs ranged from 543 to 1,152 bp encoding proteins of 175 to 378 amino acids without significant similarities with any accessions protein databases. We selected four of the genes for assaying their expression patterns in urediniospore, germinated urediniospores, and infected wheat tissues using quantitative real-time PCR. These genes had different expression patterns, but all tended increase their transcript level during the infection process. Two of them had the highest transcript level in infected wheat tissues and lower transcript levels in urediniospores and germinated urediniospores. The third gene had a higher transcript level in urediniospores. The transcript level of the fourth gene in urediniospores was slightly higher than in the infected wheat tissues, but much higher than in germinated urediniospores. This study has provided putative genes for further studies to identify *Pst* avirulence genes.

Role of exopolysaccharide in the biology *Enterobacter cloacae*
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The bacterium, *Enterobacter cloacae*, is the causal agent of Enterobacter rot of onion bulbs in storage. This disease results in a discoloration of the inner scales, but no maceration of the tissue. Exopolysaccharide (EPS) production can have a role in some plant-pathogen interactions, although its role is unknown in the *E. cloacae*-onion interaction. A mini-Tn*5* mutagenesis library of *E. cloacae* strain ECWSU1R was screened using Luria Bertani (LB) media modified with congo red dye, resulting in 61 mutants. Growth on congo red media allows for the differential identification of bacteria with potential disruptions in EPS production. These mutants were screened on LB, nutrient broth yeast extract and minimal medium agars for their ability to produce EPS. The mutants were inoculated into onion bulbs to determine if they differ in their pathogenicity relative to the wildtype strain. In addition, the mutants were screened for their ability to form biofilms, as well as determine their resistance to various salts compared to the wildtype strain. The insertions will be sequenced to identify which genes are being disrupted. Since EPS can have a role in the survival of bacterial pathogens, mutants are being assayed for their ability to resist desiccation compared to the wildtype strain.

A trans-Atlantic partnership for reducing the spread and impact of new and emerging viruses in ornamental crops
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The ornamental industry is considered to be a truly global industry as it involves significant movement of plant material across time zones, countries