Maxwell 1 . 1 Dept. Plant Path., Univ. of Wisconsin-Madison, 2 EMBRAPA CNPAF, Caixa Postal 179, 74000 Goiania, Brazil, and 3 Dept. Plant Path., Univ. of Florida, Gainesville, FL.

Bean golden mosaic (BGM-BZ) has become a major constraint to bean production in Brazil. The causal agent is whitefly-transmitted and disease symptoms are similar to those caused by a mechanically transmissible geminivirus, bean golden mosaic virus (BGMV). Unlike BGMV from the Caribbean and Central America, however, the BGM-BZ virus has not been mechanically transmitted. To confirm that BGM-BZ is caused by a geminivirus, light and electron microscopic studies were completed. Infected plants had inclusion bodies consistent with a geminivirus. All attempts to mechanically transmit BGM-BZ virus using inoculum from field-collected and/or whitefly-inoculated greenhouse grown plants were unsuccessful. A virus mechanically transmitted from field-collected leaves was identified as bean rugose mosaic virus.

442

MOLECULAR CHARACTERIZATION OF GEMINIVIRUSES CAUSING BEAN GOLDEN MOSATC. R. L. Gilbertson 1 , J. C. Faria 1 , F. Morales 2 , S. A. Leong 1 , D. P. Maxwell 1 , and P. G. Ahlquist 1 . 1 Dept. of Plant Path., Univ. of WI-Madison, and 2 CIAT Cali, Columbia.

Because recent evidence indicated that genetic variation might exist among geminiviruses causing bean golden mosaic (BGM), a molecular approach was taken to characterize BGM virus isolates from Brazil (BGMV-BZ) and Guatemala (BGMV-GA). DNA-DNA hybridization indicates that BGMV-BZ is surprisingly divergent from a previously characterized BGMV isolate from Puerto Rico (BGMV-PR), and that BGMV-GA contains sequences related to BZ and PR isolates. Double-stranded viral DNAs from infected plants were used to make full-length clones of DNAs A and B for BGMV-BZ and partial clones for BGMV-GA. Extensive DNA sequence analysis of BGMV-BZ clones showed sequence similarities of 60-85% with BGMV-PR. Limited comparisons with BGMV-GA clones showed 70 and 95% sequence similarity with BGMV-PR and BGMV-BZ, respectively. These results indicate that considerable differences exist among these BGMV isolates.

443

SURVEY OF GRAPEVINE STEM PITTING IN NEW YORK AND ISOLATION OF DSRNA FROM A GRAPEVINE SELECTION INFECTED WITH STEM PITTING. O.I. Azzam and D. Gonsalves. Plant Pathology Dept., N.Y.S. Agr. Expt. Sta., Cornell University, Geneva, N.Y. 14456

Rupestris stem pitting (SP) is a virus-like disease widespread throughout New York. A survey showed that 170 out of 257 tested grapevines indexed positive for SP using graft inoculations to the woody indicator, Rupestris St. George. Infected St. George developed pitting on the woody cylinder, usually below the inoculum bud. Stem pitting was diagnosed in European, American-French hybrids, and American type cultivars. However, many of these SP-infected grapevines did not show pitting on the woody cylinder. Isolations of dsRNA were attempted from healthy grapevines and from grapevines of a selection that had indexed positive for SP but tested negative for grapevine leafroll virus (GLRV), corky bark (CB), and three nepoviruses. DsRNA was recovered from SP-infected but not from healthy plants. Extracts made from leaf and bark tissues from SP-infected plants yielded similar dsRNA patterns. DsRNA patterns associated with stem pitting differed from those associated with GLRV and CB.

444

BIOCHEMICAL AND SEROLOGICAL CHARACTERIZATION OF CLOSTEROVIRUS-LIKE PARTICLES ASSOCIATED WITH GRAPEVINE LEAFROLL DISEASE. J. S. Hu and D. Gonsalves Dept. of Plant Pathology, Cornell Univ., NYSAES, Geneva, New York 14456.

The molecular weight of virus coat protein (NY-1 isolate) was $ca.43 \times 10^3$ daltons in SDS-PAGE analysis; the protein reacted with specific polyclonal and monoclonal antibodies in Western blotting tests. The possibility that the protein is a dimer has not been completely ruled out. A large dsRNA molecule $(ca.10 \times 10^6 \ \mathrm{Mr})$, along with several low molecular weight dsRNAs, was consistently isolated from leafroll diseased grapevines. Polyclonal antisera to two European and to two US leafroll isolates were used to determine the serological relatedness of different isolates in a protein A-gold labelling immuno-electron microscopy. Results indicated that serologically distinct serotypes existed, and mixed infection of grapevines with different serotypes was common. High titer monoclonal antibodies to NY-1 isolate were produced and used in double diffusion, ELISA, ISEM, and Western blotting assays. A new antiserum to a California leafroll isolate was produced and used in ELISA for detection of virus from crude preparations.

445

BIOLOGICAL AND SEROLOGICAL PROPERTIES OF FOUR STRAINS OF ZUCCHINI YELLOW MOSAIC VIRUS. H.L. Wang, D. Gonsalves, R. Provvidenti, and T.A. Zitter. Plant Pathology Dept., N.Y.S. Agr. Expt. Sta., Cornell University, Geneva, N.Y. 14456.

Four strains of zucchini yellow mosaic virus, ZYMV-CT (Connecticut). FL (Florida), -FR (France), and -TW (Taiwan), were characterized and compared. All four strains could be distinguished by symptoms incited on melon, cucumber, zucchini squash, Black turtle #2 bean, Red Kidney bean, Ranger pea, and Chenopodium quinoa. ZYMV-CT, -FL, and -TW incited severe symptoms on melons, cucumbers and squash, whereas -FR caused only mild symptoms under similar conditions. ZYMV-CT, -FL, and -TW were transmitted by the green peach and cotton aphids with different efficiencies, but ZYMV-FR was not transmitted by either aphid species. Polyclonal antibodies produced to the four strains gave strong cross reactions with all strains. However, cross-absorption of antisera indicated the existence of different antigenic determinants among strains. Monoclonal antibodies (Mab) were produced to ZYMV-CT, -FL, and -FR. In indirect enzymelinked immunosorbent assay, some Mab reacted only to ZYMV-FR, while others reacted to ZYMV-CT, -FL, -TW, but not to -FR.

446

SCREENING OF CEREAL PROTOPLASTS FOR RESISTANCE TO BARLEY STRIPE MOSAIC VIRUS. Yu-Zhi Zheng and Michael C. Edwards, USDA-ARS Cereal Crops Research Unit and Dept. of Plant Pathology, North Dakota State University, Fargo, ND 58105.

Protoplasts were isolated from barley and oats using a discontinuous gradient centrifugation procedure. Yields of up to 4×10^6 protoplasts per gram of tissue were achieved with a viability of up to 50% after 7 days of incubation. Protoplasts from both susceptible and resistant plants were inoculated with RNA purified from two BSMV strains, CV52 (ND18) and CV42 (ND159). Protoplasts from the susceptible cultivar Black Hulless were susceptible to both BSMV strains, as indicated by FITC staining and ELISA. Protoplasts isolated from barleys resistant to CV42, but not CV52, remained resistant to CV42. A small percentage of protoplasts from oats normally resistant to CV52, but not CV42, were susceptible to CV52. Percent infection of protoplasts varied and depended greatly upon the inoculation conditions. Up to 95% of the viable cells became infected under optimum conditions.

447

DECLINE OF ORIENTAL PERSIMMON. S.W. Scott, Dept. Plant Pathology and Physiology, Clemson University, SC 29634 and Jerry A. Payne, USDA/ARS S.E. Fruit and Tree Nut Research Laboratory, Byron, GA 31008.

A planting of 17 cultivars of Oriental persimmon (Diospyros kaki L.)intended to evaluate the suitability of the species as a fruit crop in the south-eastern United States has suffered extensive tree death (22 trees remain alive from an initial population of 238) during the 5 years of its existance at Byron, GA. Symptoms associated with the decline and preceeding the death of trees are: veinal necrosis in leaves, premature defoliation, bud death, and the death of individual scaffold branches. Crystals of isometric viruses were revealed by electron microscopy of fixed and embedded tissue from diseased leaves. Concentrated leaf dip preparations showed a few isometric particles. Sap-inoculation of herbaceous hosts using juvenile leaf tissue ground in 2% nicotine produced symptoms in Chenopodium quinoa, C. amaranticolor, and Prunus persica. This is the first report of a virus in persimmon in the U.S.A.

448

EVALUATION OF CULTURE-INDEXING AND TWO IMMUNOASSAYS FOR DETECTION OF XANTHOMONAS CAMPESTRIS PV. PELARGONII IN GERANIUM. K. K. Rane and R. L. Wick, University of Massachusetts, 240 Beaver St., Waltham, MA 02154.

Culture-indexing (CI) was evaluated for sensitivity in detecting low levels of <u>Xanthomonas campestris</u> pv. pelargonii (Xcp) in the florist's geranium (Pelargonium x hortorum). Groups of 40 plants were inoculated with either sterile buffer or Xcp (approx. 200, 20 and 5 cfu/plant). Twenty-four hours later, 20 plants from each treatment were culture-indexed and the remaining 20 plants were observed for symptom development. False positive reactions, as indicated by turbidity, occurred in greater than 50% of plants inoculated with sterile buffer. For all Xcp treatments, the number of plants developing symptoms was greater than the number of verified Xcp positive obtained through CI. The experiment was repeated and similar results were obtained. Incorporation of ELISA and a dot-blot immunoassay with CI reduced false positive reactions and shortened the time needed to verify the presence of Xcp.