

# The American Phytopathological Society

## POTOMAC DIVISION

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### ABSTRACTS

Alphabetized by first author's last name.

TOXIGENIC ASPERGILLUS FLAVUS AND AFLATOXINS IN SRI LANKAN MEDICINAL PLANT MATERIAL. Krishanthi Abeywickrama & G.A.Bean, Department of Botany, University of Maryland, College Park, MD 20742.

The fungal flora of 6 Asian medicinal plants, *Aerra lanata* (Linn.) Juss. *Alysicarpus vaginalis* D.C., *Tribulus terrestris* Linn. *Adhatoda vasica* Nees., *Centella asiatica* (L.) Urb., *Cardiospermum halicacabum* Linn. was determined. After surface disinfection *Aspergillus* spp. were most frequently observed. *Aspergillus flavus*, isolated from *Alysicarpus vaginalis* and *Aerra lanata* produced aflatoxins in culture. Aflatoxin B<sub>1</sub> was also detected in a sample of *Aerra lanata* at a level of 0.5 µg/g. Plant material destined for medicinal use should be stored carefully prior to its use to prevent growth of naturally occurring toxigenic mold fungi.

ISOLATION OF EPIPHYTIC MICROORGANISMS FROM NATIVE WEST VIRGINIA FLORA AND SCREENING FOR ANTAGONISTIC ACTIVITY AGAINST *ERWINIA AMYLOVORA*. E.W. Brown, J.C. Walter and T. van der Zwet. Biology Dept., Shepherd College, Shepherdstown, WV 25443 and USDA, ARS, Appalachian Fruit Research Station, Kearneysville, WV 25430.

Thirty three isolates were recovered on nutrient yeast dextrose agar (NYDA) from leaves of 6 plants in the woods on 3 sides near a blighted apple orchard. NYDA agar plugs were placed on plates seeded with a culture of *E. amylovora*. After 72 hr incubation at 25 C, ten isolates showed a growth inhibition zone of 1-12 mm. For *in vitro* tests, wells (7x7mm) made in immature pear fruit were seeded with 25 ul of each microorganism, followed by 25 ul of 10<sup>5</sup> CFU/ml of a 24-hr culture of *E. amylovora*. After 5 days incubation at 25 C, ten isolates exhibited a mild to strong inhibition of fruit infection, whereas all control fruit showed profuse ooze production. Three of these isolates demonstrated antagonism both *in vitro* and *in vivo*. All will be further examined as potential biological control agents in blossom infection tests.

Soybean mosaic virus strain G4 induces pathogenesis-related proteins in the SMV-resistant cultivar York. Choi, C.W. and Tolin, S.A. Plant Pathology, Physiology and Weed Science, Virginia Polytechnic Institute & State Univ., Blacksburg 24061.

Strains of soybean mosaic virus (SMV) overcoming resistance of soybean cultivars often induce local and systemic necrosis. 'York' trifoliolate leaves with necrotic lesions induced by SMV-G4 were subjected to acidic extraction 7 d after inoculation and analyzed for pathogenesis-related proteins

(SPR). Four groups of SPRs were identified and characterized. Groups with unknown functions are SPR1 (15 kD) and SPR4 (24 kD). The SPR2 (31-32 kD) group has three major isoforms and B-1,3-glucanase activity. The SPR3 (29-30 kD) group has six isoforms with strong chitinase activity. Increase in all SPRs and in these enzymatic activities occurred within 4 d after inoculation with SMV-G4, but not with non-infective SMV-G1 or mosaic-inducing SMV-G5 strains. Induction of PR proteins was thus correlated with necrosis but not specifically with resistance.

The Home and Garden Information Center - A New Program in Maryland for Educating the Public. David L. Clement, Mary K. Malinoski, Raymond V. Bosmans, and Denise D. Sharp, Regional Specialists, Cooperative Extension Service, University of Maryland System, 12005 Homewood Rd., Ellicott City, MD 21043.

In Maryland requests by telephone for gardening information and pest control advice have been steadily increasing at local county extension offices. In response, the Cooperative Extension Service has set up a centralized facility that features a staff available for telephone consultations and a self service phone tape system on topics related to home horticulture and pest control. In the first year of operation the Center received a total of 35,079 calls of which 19,668 were assisted by the Center staff. Of the assisted calls 36% dealt with ornamentals, 25% requested pest control information, 12% dealt with fruit and vegetable questions, 11% were on turf problems, and 16% were on miscellaneous topics. Plant diseases were 9% of the total assisted phone calls and 24% of the mailed in samples. Specific diseases such as Dogwood Anthracnose accounted for 15% of the assisted disease calls and 4% of the total publication requests. In its first year of operation the Center reduced the phone calls of the participating counties by approximately 30%.

DEVELOPMENT OF INTERACTIVE VIDEODISC TECHNOLOGY FOR TEACHING PLANT PATHOLOGY. T.A. Evans, G.L. Schumann, and F.L. Tainter, Dept. of Plant and Soil Sciences, University of Delaware, Newark, DE 19717, Dept. of Plant Pathology, University of Massachusetts, Amherst, MA 01003, and Dept. of Forestry, Clemson University, Clemson, SC 29634.

Videodisc technology is currently the cutting edge in the area of information delivery systems. Each side of a videodisc can store 54,000 still frames or slides or 30 minutes of video, or any combination of the two. Through the use of computer authoring systems, instructors can prepare teaching modules using text overlays with rapid and random access to a vast collection of visual images. It will allow teachers to develop teaching modules that can stimulate student learning through the discovery process. Students can be presented with real life scenarios requiring the use of their new skills and critical thinking to develop appropriate management strategies. Extension plant pathologists and diagnosticians can develop expert systems linking computer based decision making with high quality visual images.

BIOMASS AND TANNASE ACTIVITY ASSOCIATED WITH GROWTH OF *ENDOTHIA PARASITICA* ON ALLEPO-TANNIC ACID AND CHESTNUT EXTRACTS. G. Farias, J.R. Elkins and G.J. Griffin. V.P.I and S.U. Blacksburg, VA 24061

The activity of tannase (tannin acylhydrolase) produced by *E. parasitica* (Ep) was determined by estimating the amount of

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gallic acid produced during hydrolysis of methylgallate. When Ep was grown on allepo-tannic acid, mycelial extract had higher activity than culture filtrate and highest activity occurred 6 days after inoculation. The pH optimum of the tannase was 5.5 and maximum activity was observed at 28 C. Growth of Ep was higher on American chestnut total aqueous extracts than on Chinese chestnut total extracts during the first 3 days, with no significant differences in dry weight after 3 days. The fungus grew slightly more on lead-precipitated tannin extract from Chinese chestnut than on similar extract from American chestnut, but no significant differences were found. Tannase activity on American total and American tannin extracts was significantly higher than on Chinese total and tannin extracts. Non-tannin phenolics may be responsible, in part, for growth on Chinese lead-precipitated tannin extract.

RECOVERY OF *CLAVIBACTER XYLII* SUBSP. *CYNODONTIS* FROM OVERWINTERING PLANT PARTS OF BERMUDAGRASS. Rosemary H. Ford<sup>1</sup>, Stephen F. Tomasino<sup>2</sup>, and John S. Russin<sup>3</sup> <sup>1</sup>Department of Biology, Washington College, Chestertown MD 21620, <sup>2</sup>Crop Genetics International, Hanover MD 21076, and <sup>3</sup>Department of Plant Pathology and Crop Physiology, Louisiana State University, Baton Rouge LA 70803.

The association of *Clavibacter xyli* subsp. *cynodontis* (Cxc) to its host, *Cynodon dactylon*, bermudagrass, was examined in overwintering plant parts. The recovery of Cxc from stolons, rhizomes, and roots located at three different depths, 0 - 5, 5 - 10 and 10 - 15 cm was compared from samples collected monthly from February to May in 1989. Ten randomly selected samples containing stems and roots were collected monthly from a naturally colonized lawn of bermudagrass. Plant tissue samples were assayed qualitatively for Cxc by plating an aliquot of homogenized tissue on selective medium. The percentage recovery of Cxc from stems was increased from 12.5% to 100% for February and May, respectively. Recovery from root tissue was variable while recovery from rhizomes was highest during March (46.5%) and April (71.0%).

RESEARCH NEEDS FOR IMPROVED PESTICIDE RULEMAKING. Leonard Gianessi and Larry Elworth. Resources for the Future Inc., 1616 P Street, N.W., Washington, D.C., 20036 and Pennsylvania Apple Marketing Program, 2301 N Cameron, Harrisburg PA, 17110.

For pesticide regulatory decisions to reflect the real world complexities of crop production, the agricultural and scientific communities need to articulate how to accomplish this. The current assessment methodology falls short in evaluating a number of issues essential to the use of chemicals in disease management. A recent study of the impacts of the loss of EBDC fungicides for apple production illustrates many of these concerns. A regulatory policy that is narrowly focused on the EBDC's is likely to result in (1) an increase in fungicide use in apple production; (2) increased problems in resistance management; (3) disruption of established programs to minimize pesticide use (such as IPM); and (4) increased costs and lost revenues that are disproportionately borne by particular regions.

THE LA FRANCE DISEASE-SPECIFIC DOUBLE-STRANDED RNAs ARE ENCAPSIDATED IN ISOMETRIC VIRUS-LIKE PARTICLES. M. M. Goodin, B. Schlagnhauser, and C. P. Romaine, Department of Plant Pathology, The Pennsylvania State University, University Park PA 16802.

Three types of virus-like particles (Nature 196:962) and nine dsRNAs (Phytopathology 77:1321) are associated with La France disease of *Agaricus bisporus*. Using PEG precipitation, differential centrifugation, chloroform extraction, and isopycnic centrifugation in Cs<sub>2</sub>SO<sub>4</sub> gradients, we have obtained preparations from diseased mushrooms that were highly enriched in a 35 nm isometric particle and that contained minor amounts of both a 25 nm isometric particle and the ssRNA bacilliform virus. These particles were not detected in similar preparations from healthy tissues. Fractions of the Cs<sub>2</sub>SO<sub>4</sub> gradient that contained these particles (avg. density = 1.25 g/cc) also contained the nine dsRNAs as well as three previously unreported disease-specific polypeptides with MWs of 63 kd, 66 kd, and 129 kd. Neither the dsRNAs nor the polypeptides were present in fractions of the gradient that were devoid of the particles. Our data indicate that the nine dsRNAs implicated in the etiology of La France disease are encapsidated in the 35 nm and possibly the 25 nm isometric particles.

Effects of resistance and seed and/or foliar fungicide treatments on powdery mildew severity and yield in ten winter wheat cultivars. C.A. Griffey, E.L. Stromberg, and M.K. Das. Depts. of CSES and PPWS, VPI & SU, Blacksburg, VA 24061.

Ten winter wheat (*Triticum aestivum*) cultivars and three fungicide treatments were compared for their effects on yield and

development of powdery mildew (PM) (*Erysiphe graminis* f. sp. *tritici*) in a replicated split-plot experiment at Warsaw, Virginia in 1990. Treatments included (1) untreated, (2) triadimefon (140 g a.i./ha) foliar spray, (3) triadimenol (0.22 g a.i./kg) seed treatment, and (4) treatments 2 and 3. The PM severity was assessed on F-3, F-2, F-1, and flag leaves on three dates. Significant cultivar, treatment, and cultivar x treatment interaction effects for yield, PM severity on each date, mean mildew severity (MMS), and area under mildew progress curve (AUMPC) were observed. Significant yield advantage was obtained with fungicide treatments compared to untreated plots for susceptible cultivars Becker and Wakefield. Both MMS and/or AUMPC showed significant negative correlation with yield in susceptible cultivars, but were not significant for resistant cultivars.

THE QUESTION OF PHYSIOLOGICAL AGE OF CORN TISSUE AND SUSCEPTIBILITY TO *CERCOSPORA ZEAE-MAYDIS*. A. P. Grybauskas<sup>1</sup>, K. L. Smith<sup>2</sup>, C. Ringer<sup>1</sup>, and L. Carrera<sup>1</sup>. <sup>1</sup>Botany Department, University of Maryland, College Park, 20742, and <sup>2</sup>USDA ARS PAL, Beltsville, 20705.

Gray leaf spot (gls) of corn generally occurs late in the season. In four field trials in Western Maryland, examining a range of plant populations (17300 to 69200 plants/ha), the dew point duration increased but disease development decreased. Furthermore, delayed planting treatments did not delay disease development, but rather significantly reduced the rate of the epidemic. The physiological plant age hypothesis for late season disease development as opposed to increased periods of high humidity due to canopy closure appears to be supported. However, relatively younger leaf tissue (upper versus lower leaves) had faster epidemic rates at high plant populations, but not at low. Epidemic rates at upper canopy heights in inoculated studies were also faster. Differences in disease development relative to leaf position does not support a leaf age effect but may indicate the importance of light and cercosporin activation in gls epidemics.

INDUCED HATCHING AND JUVENILE MORTALITY CAUSED BY SOME MEDICINAL PLANTS TO SOYBEAN CYST NEMATODES. Sanaa Haroon, Robin N. Huettel, USDA ARS, Nematology Laboratory, Beltsville, MD 20705.

Field trials conducted in Egypt on the affect of medicinal plants on survival of soybean cyst nematode (SCN) indicated that several plants significantly reduced nematode populations under field conditions. These plants were *Alemites moluccama*, *Hyoscyamus niger*, *Calendula officinalis*, *Ambrosia trifida* and *Origanum vulgare*. Studies were continued under laboratory conditions to observe if the root exudates of these plants affected hatch rate and/or mortality of SCN. An increase in hatch rate of SCN was observed with exudates of *A. moluccama*, *H. niger*, *C. officinalis* and *O. vulgare* when compared to controls; whereas a lower hatch rate occurred with exudates of *A. trifida*. Survival of hatched juveniles was significantly reduced after 24 hr in exudates of *A. trifida*, *C. officinalis*, and *O. vulgare* when compared to controls. However, most juveniles survived up to 5 days in exudates of *H. niger* and *A. moluccama*. These results indicated that some medicinal plant may be effective as natural nematocides in control of SCN.

INFLUENCE OF TEMPERATURE AND OTHER FACTORS ON HATCH OF EGGS AND EMERGENCE OF SECOND STAGE JUVENILES FROM CYSTS OF HETERODERA ZEA. S. Hashmi & L. R. Krusberg, Dept. of Botany, University of Maryland, College Park, MD 20742.

The temperature at which the greatest number of second-stage juveniles (J2) of *H. zea* emerged from cysts incubated in tap water or in certain solutions was 30 C. At 30 C in tap water an average of 18% of the cyst contents emerged as J2 in 8 days and an average of 40% J2 in 28 days. No J2 emerged from cysts at either 10 C or 40 C. Freshly collected root leachate from 25-day-old corn plants growing in silty-loam soil or sand increased J2 emergence from cyst by 34% after 28 days incubation over emergence in tap water. However, leachate that had been stored at 4 C for 30 days, and fresh leachate of silt-loam soil or sand without growing corn plants inhibited J2 emergence. Emergence of J2 from cysts in aqueous 4mM zinc chloride was similar to that in tap water.

*PSEUDOMONAS SYRINGAE* PV. *SYRINGAE* HRP/HRM GENES ENCODE AVIRULENCE FUNCTIONS IN *P. SYRINGAE* PV. *GLYCINEA* RACE 4. S. Heu and S.W. Hutcheson, Department of Botany, University of Maryland, College Park, MD 20742 USA

Incompatibility of plant pathogenic bacteria has been linked to the presence of *avr* genes. A 30 kb gene cluster from *P. syringae* pv. *syringae* 61 (pHIR11), consisting of 13 *hrp* genes

and 1 *hrm* gene, enables nonpathogenic bacteria to elicit the hypersensitive response (HR) associated with incompatibility. *P. syringae* pv. *glycinea* race 4 (*Psgr4*) transconjugants carrying pHIR1 were found to elicit the HR in all indicator soybean cultivars. To localize the apparent *avr* gene associated with pHIR1, subclones and Tn mutants derived from pHIR1 were screened for *avr* activity in *Psgr4*. No pHIR1-derived subclones or pHIR1 derivative containing insertionally-inactivated *hrp* or *hrm* genes affected the phenotype of *Psgr4*. These results indicate that no single gene or subset of genes within the cluster have the qualities of an *avr* gene. The avirulence activity of pHIR1 is formed by the combined action of the *hrp/hrm* genes.

USE OF OIL AND WAX COMPOUNDS EXTRACTED FROM NEEM SEEDS TO CONTROL BEAN AND SNAPDRAGON RUSTS. J. C. Locke and J. R. Stavelly, USDA, ARS, Beltsville, Maryland 20705.

Two fractions, derived from a hydrophobic-solvent extracted neem seed oil, were evaluated as a foliar spray treatment to control bean and snapdragon rusts. Bean rust (*Uromyces appendiculatus*) and snapdragon rust (*Puccinia antirrhini*) were effectively controlled by 1.0% (w/w) aqueous emulsions of the oil or wax fraction when applied as a full coverage spray. Application rates of as low as 0.25% (w/w) gave significant reduction of bean rust pustule numbers. The effect is apparently preventative in mode of action since post infection applications gave inadequate control. The protective activity was demonstrated against eight of the most common bean rust races. Efficacy comparisons were made with commercially available petroleum-based horticultural oils using both diseases. In each case, the neem seed derived fractions were more efficacious than the horticultural oils.

REGULATION OF THE PRODUCTION OF THE METABOLITES GLIOTOXIN AND VIRIDIN BY THE BIOCONTROL FUNGUS, *Gliocladium virens*. R. D. Lumsden and C. J. Ridout, Biocontrol of Plant Diseases Laboratory, USDA, ARS, BARC-West, Beltsville, MD 20705.

*Gliocladium virens* controls damping-off caused by *Pythium* and *Rhizoctonia*. Antifungal metabolites implicated in the mechanism include gliotoxin, viridin, and gliovirin. Gliotoxin may be the most important metabolite in disease control and production of other metabolites may diminish its effectiveness. For this reason, we have attempted to regulate gliotoxin production. Gliotoxin was produced in Weindling solution culture containing low (14:1) and high (150:1) C:N ratios. At high ratios, gliotoxin (2.7 µg/mg mycelium) was favored at more acidic levels (< pH 6.0) and viridin (4.8 µg/mg mycelium) at more neutral levels (> pH 6.0). This information may be useful in regulating production of gliotoxin, diverting synthesis of other less effective metabolites.

FORMATION OF CLEISTOTHECIA IN *SPHAEROTHECA FULGINEA*, THE CAUSAL AGENT OF CUCURBIT POWDERY MILDEW. M. T. McGrath and M. S. Ghemawat, Dept. of Plant Pathology, Long Island Horticultural Research Laboratory, Cornell University, Riverhead, N.Y. 11901-1098

Following the rare observation of cleistothecia of *Sphaerotheca fuliginea* (only 3 reports from North America) on pumpkin in 1989, cleistothecial formation was studied in a controlled environment. The fungus was collected from 3 pumpkin fields with cleistothecia and maintained on cucurbit cotyledons or detached leaves growing in double petri dishes. Several single conidial-chain isolates were obtained. These were paired and incubated at 23 C/19 C (day/night) with a 12-hr photoperiod. Cleistothecia developed (in 9 out of 28 pairs tried) only when the pair included isolate L4-1-1; all other crosses were unsuccessful. Unpaired isolates never produced cleistothecia. *S. fuliginea* is therefore heterothallic and isolate L4-1-1 is a different mating type from all the other isolates tested. The earliest observation of cleistothecia was 8 days after inoculation; whereas, conidia were seen as soon as 4 days after inoculation. Cleistothecial formation did not require special stimulatory conditions such as low temperature and/or senescence of host tissue.

CYST DIMENSIONS OF *GLOBODERA TABACUM VIRGINIAE* AND *G. I. SOLANACEARUM* CULTURED ON *SOLANUM CAROLINENSE* AND *NICOTIANA TABACUM*. L. J. MILLER, Dept. of Plant Path., Phys. & Weed Sci., VPI&SU, Blacksburg, VA 24061.

Comparisons were made of the dimensions of young brown cysts, retained on a 250 µm sieve, of type locality isolates of *Globodera tabacum virginiae* (N1) and *G. I. solanacearum* (N2) when cultured on horsenettle (P1), *Solanum*

*carolinense*, and tobacco (P2), *Nicotiana tabacum* cv VA 312. P1 and P2 were efficient hosts for N1 and N2. Mean dimensions in µm of 125 specimens were as follows- length of neck(K): N1P1 145.8, N2P1 154.2, N1P2 102.7, N2P2 121.3; length excluding neck (L): N1P1 621.6, N2P1 563.4, N1P2 527.0, N2P2 585.4; maximum width (W): N1P1 527.0, N2P1 477.5, N1P2 496.4, N2P2 547.8. All comparisons between nematode subspecies cultured on P1 or P2 were significantly different (P=0.05). Dimensions of K for N1 and N2 were greater when cultured on P1 than on P2 (P=0.01). Dimensions of L and W for N1 were greater when cultured on P1 than on P2, but they were greater for N2 when cultured on P2 (P=0.01).

METHODS TO SCREEN MAIZE SEEDLINGS FOR RESISTANCE TO PHOMA TERRISTRIS. T. Miller, D. P. Whittington and R. B. Carroll, Department of Plant and Soil Sciences, Univ. of Delaware, Newark 19717-1303.

*Phoma terrestris* is associated with red root rot of maize, a disease of increasing importance in the Delmarva region. Previous field tests indicate sources of resistance among selected inbreds and hybrids. Studies were initiated to compare the efficacy of 3 techniques with the potential to rapidly identify resistance. Tests were replicated in a split-split design and included 3 isolates. Seedlings grown aseptically in test tubes and Magenta jars were inoculated after 7-9 days with *P. terrestris* and rated 1 and 2 wks later. Excised and tissue-cultured roots were screened in Petri dishes containing either water agar or modified Watson's straw medium and rated after 2, 4 and 6 days. Wounding prior to inoculation resulted in higher disease ratings and isolate response varied with the method. Resistance patterns observed in field studies were not consistent with those obtained via rapid screening.

EVIDENCE FOR PARTICIPATION OF TWO COMPONENTS DURING DAMAGE OF HYPHAE OF *RHIZOCTONIA SOLANI* BY GERMLING EXTRACTS OF BIOCONTROL FUNGI. Sue Mischke and C. F. Mischke, USDA, ARS, Biocontrol of Plant Disease Lab & Weed Science Lab, Beltsville, MD 20705.

A fluoresein efflux method was used to indicate damage to cells of *R. solani* by germling extracts of the biocontrol fungi *Trichoderma* spp. and *Gliocladium virens*. Aqueous extracts of only strain Gl-21 of *G. virens* injured hyphae of *R. solani*. An *in vitro* bioassay of these extracts demonstrated more inhibition of *R. solani* by Gl-21, less inhibition by strain TRI-4 of *T. hamatum* and absence of inhibition by a mutant of *T. viride*, strain T1-R4. Comparable relative efficacies had previously been observed in greenhouse trials. The chloroform extracts of only Gl-21 were inhibitory. Levels of protein secreted by Gl-21 and TRI-4 were double the amount secreted by T1-R4. Thus damage to *R. solani* appears to be caused by two gene products. One is only in the aqueous extract, is produced by Gl-21 and TRI-4 and is probably an enzyme which damages the cell wall. The second is chloroform-extractable and is produced only by Gl-21.

IDENTIFICATION OF INTRACELLULAR PROTEINS ASSOCIATED WITH GLIOTOXIN-PRODUCING STRAINS OF THE BIOCONTROL FUNGUS *Gliocladium virens*. By C. J. Ridout and R. D. Lumsden, Biocontrol of Plant Diseases Laboratory, USDA, ARS, BARC-West, Beltsville, MD 20705.

Intracellular proteins from gliotoxin-producing strains of *Gliocladium virens* were compared with the proteins from non-producing strains using 2-dimensional electrophoresis. Prominent proteins at 33.5 kDa and 22.5 kDa were always produced by *G. virens*. The isoelectric points of both proteins were consistently lower in the non-producing strains. In addition, there were several minor proteins that were always associated with the ability or inability to produce gliotoxin. The results suggest that the prominent 33.5 kDa and 22.5 kDa proteins may not be biosynthetic enzymes, but they may affect the regulation of gliotoxin biosynthesis and export from the cell. The significance of this result may be relevant to enhancing gliotoxin production for improved biocontrol performance.

PRODUCTION OF MONOCLONAL ANTIBODIES TO THE YELLOWING STRAIN OF SOYBEAN DWARF VIRUS. A. J. Russo<sup>1</sup>, C. Keller<sup>1</sup>, R. J. Beck<sup>2</sup>, O. P. Smith<sup>2</sup>, and V. D. Damsteegt<sup>2</sup>. <sup>1</sup>Mount St. Mary's College, Emmitsburg, MD 21717 and <sup>2</sup>USDA-ARS, Frederick, MD 21702

The yellowing (Y) and dwarfing (D) strains of soybean dwarf virus (SDV) differ in host symptomatology, aphid transmissibility, and dsRNA profiles but exhibit substantial cross-reactivity when polyclonal antibodies (rabbit) are used in an

enzyme-linked immunosorbent assay (ELISA). As a first step towards the development of a strain-specific ELISA for SDV, we have produced monoclonal antibodies to purified SDV-Y. Immunization of BALB/c mice was performed using fifty micrograms of SDV-Y three times over a one month period. SDV-Y monoclonals from cloned hybrids were identified by testing extracts of SDV-Y-infected leaves versus healthy controls via ELISA. Eight SDV-Y monoclonals have been identified. Additional testing of these monoclonals is underway, including assaying for cross-reactivity to SDV-D-infected leaf extracts.

BIOLOGICAL CONTROL OF RHIZOCTONIA STEM CANKER AND BLACK SCURF OF POTATO WITH A BASIDIOMYCETOUS FUNGUS. Jasmit Sidhu and R. J. Young. Dept. of Plant Pathology, West Virginia University, Morgantown, WV 26506-6057.

*Laetisaria arvalis*, a soil-inhabiting basidiomycete provided effective biological control of *Rhizoctonia solani* on potato cv. Elba in greenhouse and field experiments. Soil incorporation of *L. arvalis* grown on sterile oat kernels, reduced stem lesions by 68% in greenhouse experiments. In the field, when seed pieces infested with sclerotia of *R. solani* were coated with mycelial and sclerotial preparation of *L. arvalis* before planting, plant stand was 15% greater than from untreated seed; also, yield was significantly increased. Disease severity was reduced from both seed treatment and from soil incorporation of oat kernel and wheat bran preparations of *L. arvalis*; also, formation of sclerotia on progeny tubers was significantly reduced.

TOMATO SPOTTED WILT VIRUS AND POTATO WART - CURRENT SITUATIONS IN MARYLAND. A.B. Sindermann, M.L. Putnam, and T.A. Evans. Plant Prot. Sect., Maryland Dept. of Agric., Annapolis MD 21401, Plant & Pest Diag. Lab., Purdue Univ., W. Lafayette IN 47907, and Dept. of Plant and Soil Sci., Univ. of Delaware, Newark DE 19717.

In 1989, tomato spotted wilt virus was widespread in tomato producing counties of Maryland and Delaware. Only fields planted with southern transplants contained infected plants. TSWV-L was detected by ELISA and bioassay. Western flower thrips were identified from one field. There was no evidence of overwintering virus or vector in adjacent weeds, or in trap-plantings of tomato and *D. stramonium* in diseased fields. Potato wart, caused by *Synchytrium endobioticum*, was believed eradicated from Maryland. The original quarantine area was resurveyed in 1987. Spores of *S. endobioticum*, estimated inoculum density of 160 spores/kg-1 soil, were identified from one site. Greenhouse viability assays using potato plantlets were negative. Preliminary results from planting susceptible potato varieties, including Russet Rural and Rural New Yorker, and attempts to recover spores from soil using a density dependent separation technique indicate that potato wart is no longer a threat in Allegany County, Maryland.

EVALUATION OF FUNGICIDE ASC-66825 AGAINST *SCLEROTINIA MINOR* AND OTHER PATHOGENS OF PEANUT USING AGAR ASSAYS AND FIELD TRIALS. F.D. Smith, P.M. Phipps, and R.J. Stipes, Tidewater Agricultural Experiment Station, VPI & SU, Suffolk, VA 23437-0099.

The fungitoxicity of ASC-66825 was evaluated against three major pathogens of peanut: *Sclerotinia minor*, causal agent of Sclerotinia blight; *Sclerotium rolfsii*, causal agent of southern stem rot; and *Rhizoctonia solani*, causal agent of limb rot. The ED<sub>50</sub> values for ASC-66825 in glucose yeast-extract agar against these three pathogens were 0.0025, 0.035 and 0.19 µg/ml, respectively. Based on these assays, ASC-66825 was 48 times more active against *S. minor* than iprodione, the standard fungicide used for management of Sclerotinia blight in Virginia. ASC-66825 also showed a high level of *in vitro* activity against *S. rolfsii* and *R. solani*. In field trials using a randomized complete block design with four replications, two applications of ASC-66825 at 0.56 kg a.i./ha or iprodione at 1.12 kg/ha significantly (P=0.05) suppressed disease incidence of Sclerotinia blight by 79 and 52% and increased yields 2515 and 1179 kg/ha, respectively. ASC-66825 possesses a broad spectrum of fungitoxicity that may be useful in control of several peanut diseases.

RECENTLY IDENTIFIED NEW PATHOGENIC VARIABILITY IN *UROMYCES APPENDICULATUS*. J. R. Stavelly and L. R. Batra, Microbiology & Plant Pathology Laboratory, ARS, USDA, Beltsville, MD 20705.

From 76 field collections of bean rust obtained from Germany, Switzerland, Thailand, and six states since 1987, 13 previously unreported pathogenic races have been identified by the speci-

fic reactions of the 19 standard differential cultivars to pathogenically uniform, single uredinium isolates. From three to 13 of the 19 cultivars are susceptible to these 13 races. Among the differential cultivars that have previously been resistant to most races, Compuesto Negro Chimaltenango is susceptible to one, AXS37 and Mexico 309 to two, and Mexico 235 and Ecuador 299 to three of the new races. The only one of the 12 previously unobtained virulence combinations for pairs of differential cultivars (Stavelly et al. 1989. Plant Disease 73: 428-432) that occurred among these new races was for Mexico 235 Ecuador 299, and Golden Gate Wax in race 74. The Beltsville germplasm releases that have been resistant to all previous races are also resistant to these newly identified races.

MOLECULAR REGULATION OF THE PISATIN DEMETHYLASE GENE IN *NECTRIA HAEMATOCOCCA* (MP VI). David Straney<sup>1</sup>, Hans VanEtten<sup>2</sup>, Yijun Ruan<sup>1</sup>, Stephen Wilhite<sup>1</sup>. 1. Department of Botany, Univ. of Maryland, College Park, MD 20742. 2. Department of Plant Pathology, Univ. of Arizona, Tucson AZ 85721

The pisatin demethylase (PDA) gene of *N. haematococca* allows it to detoxify the pea phytoalexin pisatin. This gene is induced by pisatin and repressed by glucose and amino acids; the pisatin induction is at the level of mRNA accumulation. We have identified two proteins that bind to the PDA gene promoter and show behavior of a pisatin-responsive activator and an amino acid-responsive repressor. Both proteins bind to a 35 bp. region of the promoter approximately 450 bp upstream of the mRNA start site. The pisatin mRNA induction requires protein synthesis for full induction, suggesting that induction of the gene for the activator is required along with another mechanism for producing competent activator.

SUSCEPTIBILITY OF APPLE SHOOTS TO *E. amylovora* RELATIVE TO SHOOT AGE AND PHYSIOLOGICAL STATUS. P. Suleman, P.W. Steiner and G. Stutte. University of Maryland, College Park, MD 20742.

The age and physiological status of apple shoots were determined using plastochron index (PI), relative water content (RWC), osmotic potential (OP), and leaf photosynthate content (PN). Leaf susceptibility and shoot blight severity declined from PI 8 to 15 following inoculation with 10<sup>8</sup> cells/ml at 22°C. Regardless of shoot age the first 3 apical leaves were the most susceptible, the next 2 were moderately susceptible while leaves ≥6 were difficult to infect. Shoots infected at PI ≥20 seldom showed the characteristic "shepherd's crook" wilt symptom. Glucose was the predominant sugar in the 4 apical leaves, while sorbitol was greatest in fully mature leaves. The RWC was about the same in all leaves, but OP increased downward to the seventh leaf from the tip. The failure to infect mature leaves and the decline in blight severity with increasing shoot age appears to be related to the total soluble carbohydrate content, especially sorbitol. The high PN levels may be inhibitory or may affect osmotic relationships with the intercellular pathogen by denying it substances necessary for the production of bacterial ooze which is responsible for tissue damage.

FIELD ACTIVITY OF A *CLAVIBACTER XYLI* SUBSP. *CYNODONTIS*/BACILLUS THURINGIENSIS RECOMBINANT AGAINST EUROPEAN CORN BORER. Stephen F. Tomasino and R. Mark Beach. Crop Genetics International, Hanover, MD 21076.

*Clavibacter xyli* subsp. *cynodontis* (Cxc), genetically engineered to produce the delta-endotoxin of *Bacillus thuringiensis* subsp. *kurstaki* (Cxc/Bt), was field tested for activity against the European corn borer (ECB) (*Ostrinia nubilalis*) during 1990. The evaluation was performed at the Crop Genetics International research farm in Ingleside, MD and included both stab-and-seed inoculated studies with 6 corn hybrids and the following treatments: 1) phosphate-buffered saline (PBS), 2) wild-type Cxc, and 3) Cxc/Bt recombinant MDR1.586. ECB larvae were applied during the pollen shed stage of development. Cxc/Bt reduced damage by ECB larvae in inoculated plants compared to PBS and Cxc controls. This effect was greatest in one hybrid (PD3) and was apparent using either stab or seed inoculation. In PD3, significant reductions in numbers of surviving corn borer larvae, numbers of corn borer tunnels, and total lengths of the tunnels were observed. Lower levels of activity were demonstrated in 3 other hybrids.

DELETION OF ENGINEERED DNA SEQUENCES FROM A RECOMBINANT STRAIN OF *CLAVIBACTER XYLI* SUBSP. *CYNODONTIS*. John T. Turner, Jay S. Lampel, and George W. Sundin. Crop Genetics International, Hanover, Maryland 21076.

Segregation of chromosomally inserted DNA sequences from an engineered strain of *Clavibacter xyli* subsp. *cynodontis* was studied both *in vitro* and *in planta*. Populations of cells lacking the gene encoding the delta-endotoxin protein from *Bacillus thuringiensis* subsp. *kurstaki* and genes conferring antibiotic resistance were detected in bermudagrass and in shake flasks originally inoculated with pure cultures of a recombinant strain. In both cases the segregant population increased over time to result in a sigmoidal curve in which the segregant population approached 100 percent. Segregant cells were shown to have a higher growth rate than recombinant cells *in vitro*, thus resembling the wild-type. The environmental consequences of the extinction of engineered genes will be discussed.

EVALUATION OF CORN INBRED LINES FOR RESISTANCE TO *DIPLODIA* (*Stenocarpella maydis*) EAR ROT. T. Weldekidan and J.A. Hawk, Dept. of Plant and Soil Sciences, University of Delaware. Newark, DE 19717-1303

Corn inbred lines were evaluated for resistance to *Diplodia* (*Stenocarpella maydis* [Earle] Sutt) ear rot at the University of Delaware farm in 1989 and 1990. Plots were 5.3m single-row arranged in a randomized complete block with four replications. Plants were inoculated with a spore suspension derived from ten virulent isolates by spraying silks with a concentration of  $2.5 \times 10^5$  spores/ml ten days after mid silk. Significant differences in disease incidence and/or severity was obtained ( $p=0.05$ ). There was an interaction between inbreds and year indicating that inbreds performed differently over years. Results obtained indicate that inbreds VA22, DE811, CB59G, and H93 are resistant. These inbreds can be used as a source in breeding commercial hybrids for resistance to *Diplodia* ear rot. Elite inbreds B73, LH51, and LH132 were highly susceptible and should be avoided in *Diplodia* ear rot improvement.

OCCURRENCE OF CLOSTEROVIRUS-LIKE PARTICLES IN *MEGAKEPSAMA ERYTHROCHLAMYS* FROM SINGAPORE. S.M. Wong, C. G. Chng, and M. Tan\*. Botany Department, National University of Singapore, Lower Kent Ridge Road, Singapore 0511 and \*Singapore Zoological Gardens, Mandai Lake Road, Singapore 2572, Republic of Singapore.

A flowering shrub, *Megakepasama erythrochlamys* L., (Family Acanthaceae) was found showing mosaic and distorted leaves in the Singapore Zoological Gardens in May 1990. Flexuous particles, 2000 nm in length, were observed in a partially purified virus preparation. Similar particles and vesicles were observed in ultrathin sections also from these leaves. After three successive single lesion transfers were carried out on *Chenopodium quinoa* L., double-stranded RNA bands were observed in nucleic acid preparations from inoculated *C. quinoa*. Purification of the particles, the putative disease causal agent, is now underway.

ENVIRONMENTAL REGULATION OF *PSEUDOMONAS SYRINGAE* PV. *SYRINGAE* 61 HRP GENES. Y. Xiao and S.W. Hutcheson, Department of Botany, University of Maryland, College Park, MD 20742, USA

One or more of the genes that function in the bacterial elicitation of the hypersensitive response (HR) appear to be environmentally regulated. Our previous work has suggested that the bacterial elicitation of the HR is controlled by a clustered set of 13 *hrp* genes and one *hrm* gene. To determine whether these genes are environmentally regulated, random transcriptional fusions with *uidA* (encodes  $\beta$ -glucuronidase; GUS) were constructed by Tn5-*gusA1* mutagenesis and marker exchange mutagenesis. The transcriptional fusions with 12 of the complementation groups were poorly expressed in KB medium but exhibited detectable expression in planta. A representative *hrp-uidA* fusion could be rapidly induced independently of plant by growth in minimal salts medium. This apparent induction could be repressed by complex nitrogen sources but not by glutamine or ammonium. These results suggest the *hrp/hrm* genes are regulated by nutritional conditions.

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