

Abstracts of Presentations at the 1988 Annual Meeting

of the American Phytopathological Society Potomac Division

March 30–April 1, 1988
Frederick, MO

Alphabetized by first author's last name.

STIMULATION OF ATPASE ACTIVITY IN PLASMA MEMBRANES ISOLATED FROM HYPERSENSITIVE TOBACCO LEAVES. Merelee M. Atkinson, Elizabeth Wietor-Orlandi, L. Dale Keppler and C. Jacyn Baker, USDA, ARS, Microbiol. and Plant Path. Lab., Beltsville, MD, 20705.

Pseudomonas syringae pv. *syringae*, a bean pathogen, induces a hypersensitive response on tobacco, a non-host. This response is associated with induction of a K^+ efflux: net H^+ uptake exchange within 4 h after inoculation. *Pseudomonas syringae* pv. *syringae* strain B7, a Tn5 insertion mutant, induces neither the hypersensitive response nor the K^+/H^+ exchange. Plasma membranes were isolated from tobacco leaves at hourly intervals after inoculation with wild-type (WT) or B7 bacterial strains. Membranes from WT-inoculated leaves exhibited increased vanadate-sensitive ATPase activities relative to controls (B7) at 4 or more h after inoculation. These results suggest that plasmalemma ATPase stimulation is associated with K^+/H^+ exchange. It is hypothesized that stimulation may be triggered by cytoplasmic acidification associated with the exchange.

PURIFICATION OF SOIL-BORNE WHEAT MOSAIC VIRUS BY IMMUNO-AFFINITY CHROMATOGRAPHY. D. C. Bays and S. A. Tolin, Dept. of Plant Pathology, Physiology and Weed Science, VPI & SU, Blacksburg, VA 24061.

Soil-borne wheat mosaic virus (SBWMV) was purified from naturally infected wheat by a method modified from Diaco et al. (J. Gen. Virol. 67:345, 1986). SBWMV-specific rabbit serum IgG was coupled with an active ester agarose immunoaffinity support matrix (Bio-Rad Affi-Gel 10) in a 1.5 X 10 cm column by overnight incubation at 4C, with agitation, of 1.5 mg IgG per ml gel matrix in 0.05 M HEPES, pH 8.0. Frozen leaves were ground in 0.05M sodium borate, pH 8.0 (1 g/5 ml). After low speed centrifugation, the supernatant was added to the gel and incubated overnight at room temperature. The column was washed with borate, then eluted with distilled water adjusted to pH 3.0 with 0.1M HCl. Resulting virus was strongly antigenic in ELISA and, when viewed by ISEM, consisted of rigid rods characteristic of SBWMV. The immunoaffinity column is reusable and adaptable to processing small or large samples.

CALCIUM OXALATE PRODUCTION BY dsRNA-FREE AND dsRNA-CONTAINING STRAINS OF ENDOTHIA PARASITICA. A. R. Bennett, USDA-FDWSRU, Fort Detrick, Bldg. 1301, Frederick, MD 21701 and D. F. Hindal, Dept. of Plant Pathology & Agr. Micro., 401 Brooks Hall, West Virginia University, Morgantown, WV 26506.

In vitro oxalate production by three dsRNA-containing and two dsRNA-free strains of *Endothia parasitica* was assayed by gas chromatography after 10 days incubation on an agar medium containing 0.025M, 0.010M or no calcium acetate. Oxalate, which accumulated on the mycelium of four strains, was greatest when the fungus was incubated on the 0.025M calcium acetate medium, despite poor growth. In contrast, more oxalate accumulated in the agar when the strains were incubated on the 0.010M calcium acetate medium. Oxalate accumulation in the agar containing 0.025M calcium acetate did not differ from that without added calcium acetate. Formation of oxalate by *E. parasitica* may function to regulate calcium uptake and oxalic acid production.

Camera-ready abstracts are published as they were submitted by the Division. The abstracts are not edited or typed in the APS headquarters offices.

BAMBARRA GROUNDNUT MOSAIC CAUSED BY A SEED-TRANSMITTED POTYVIRUS. A.E. Bird and M.K. Corbett, Botany Dept., University of Maryland, College Park, MD 20742

Bambarra groundnut, *Voandzeia subterranea*, is a forage and grain legume of current interest in arid tropical Africa. Seeds collected in West Africa and grown in a screened greenhouse gave plants of which 2% showed mosaic symptoms. Electron microscopy of chromium shadowed or negatively stained leaf extracts detected 767 nm flexuous rod virus-like particles. The virus was transmitted mechanically and by aphids, as a stylet-borne virus, to healthy Bambarra plants with the induction of the original mosaic symptoms. The virus has a narrow host range, limited to 3 genera of leguminosae. Density-gradient centrifugation of partially purified preparations obtained by n-butanol clarification and precipitation by 7% polyethylene glycol gave a light-scattering zone of 153S. Electron microscopy of ultra-thin sections from infected Bambarra and *Phaseolus vulgaris* plants showed cytoplasmic pinwheel inclusions similar to those of the potyvirus group.

CHARACTERIZATION OF TERBINAFINE-RESISTANT MUTANTS OF *USTILAGO MAYDIS*. Ann M. Buchman and Hugh D. Sisler, Department of Botany, University of Maryland, College Park, MD 20742.

Twelve isolates of *Ustilago maydis* resistant to the antimycotic allylamine terbinafine were obtained by treatment with N-methyl-N'-nitro-n-nitrosoguanidine and selection on terbinafine-containing medium. The ED₅₀ of terbinafine for the wild type was 0.5 µg/ml, whereas for mutant AR217 it was 4.3 µg/ml. The ED₅₀ for other mutants ranged from 0.7 to 2.9 µg/ml. Only mutant AR217 had a growth rate as rapid as the wild type. Mutant AR217 showed a low level of cross resistance to etaconazole, ketaconazole, propiconazole, imazalil, triadimefon, fenarimol and tridemorph, whereas AR212 showed hypersensitivity to etaconazole, ketaconazole and propiconazole. Treatment with terbinafine (2 µg/ml) suppressed net sterol production in the wild type by 97%, compared to only 47% in AR217. This phenomenon supports the conclusion that the primary site of toxic action of terbinafine is in the sterol biosynthetic pathway.

EFFECT OF FUNGICIDE SPRAY SCHEDULES CONTROLLING EARLY BLIGHT ON THREE POTATO CULTIVARS. B. J. Christ, Department of Plant Pathology, The Penna. State Univ., University Park, PA 16802.

A field experiment was conducted to evaluate two fungicide application schedules on three potato cultivars. Norchip, Katahdin and Chiefain represented a susceptible cultivar, moderately resistant and resistant cultivar, respectively. The fungicide mancozeb (1.8 kg/ha a.i.) was applied at weekly intervals, initiated at flowering or at first observations of early blight lesions (*Alternaria solani*). Severity was assessed throughout the growing season. There were significant differences among cultivars and among fungicide schedules. There was also a cultivar by fungicide schedule interaction. Norchip had the highest severity over all fungicide schedules and required more fungicide applications for control of early blight. Early blight severity on Chiefain with no fungicide treatment was similar to that on Norchip with 7 sprays. A reduction in the number of sprays and a later date for initiating sprays may be recommended for cultivars that are moderately resistant to early blight.

EFFECT OF FOLIAR FUNGICIDES AND SEED TREATMENTS ON FOLIAR DISEASES OF SOFT RED WINTER WHEAT. B. J. Christ and J. A.

Frank. Department of Plant Pathology, The Penna. State Univ., University Park, PA 16802.

Field plots were established in Rock Springs and Landisville, PA. A split plot design was used with three cultivars Tyler, Roland and Scotty representing main plots each replicated four times. The subplots were a foliar fungicide treatment (triadimefon and mancozeb), a fungicide seed treatment (triadimenol), a combination of the foliar and seed treatment and a nontreated control plot. There were differences among cultivars and there was a cultivar by treatment interaction. Seed treatment and foliar sprays reduced the severity of powdery mildew. Foliar sprays reduced Septoria leaf blotch and leaf rust severity. A reduction in severity of these two diseases by seed treatment was not consistent. Foliar sprays contributed to significant yield increases in all trials while the seed treatment only contributed to yield increases in 42% of the trials.

EFFECT OF SOYBEAN OIL ON CONTROL OF EARLY LEAFSPOT OF PEANUT WITH CHLOROTHALONIL. R. M. Cu, P. M. Phipps and R. J. Stipes, Tidewater Exp. Sta., VPI & SU, Suffolk, VA 23437.

Soybean oil (SoyOil 937) at rates of 0, 0.7, 1.4, 2.8, and 5.6 L/ha was tested as an adjuvant for chlorothalonil (Bravo 720) at rates of 0, 0.3, 0.6 and 1.2 kg/ha. Sprays (140 L/ha) were applied to acetate film on a spray table and to field plots of Florigiant peanut with a CO₂-pressurized (344 kPa) sprayer with D₂13 nozzles. Percent spray coverage on film increased linearly ($r = .94$) with soybean oil rates. SoyOil alone did not suppress incidence of early leafspot, caused by *Cercospora arachidicola*. The effect of SoyOil on disease control by chlorothalonil fitted a quadratic polynomial curve with maximum disease control at 0.7 to 1.4 L/ha of soybean oil. Soybean oil at 5.6 L/ha (4% of spray volume) significantly reduced the efficacy of chlorothalonil. Yields of plots treated at 0.6 or 1.2 kg of chlorothalonil per ha tank mixed with Soybean oil at 0.7 to 1.4 L/ha (0.5 to 1% of spray volume) were significantly higher than the rest of the treatments.

COMPARISON OF METHODS FOR DETECTING TELIOSPORES OF *TILLETIA INDICA* IN CONTAMINATED WHEAT SEED. L. E. Datnoff, M. R. Bonde, USDA-ARS, Frederick, MD, T. T. Matsumoto, J. McCarty, CDFR, Sacramento, CA, and R. E. Yekema, AZ Comm. Agr.-Hort., Phoenix.

Karnal bunt of wheat caused by *T. indica* is established in India, Mexico, Nepal, and Pakistan. Because of the potential economic damage to export markets through quarantine, a reliable method is needed for detecting teliospores (TS) of *T. indica* on contaminated or infected wheat seed. Three methods of extraction were compared for TS recovery: (1) bubbling in 50% glycerol for 10 min in a 20 x 50-cm glass column using compressed air; (2) shaking in 50% glycerol in a water bath at 300 rpm for 10 min; and (3) the centrifugation-seed wash technique. The liquid containing extracted TS was filtered through a 20-um mesh nylon screen and the screen viewed at 100x. The mean number of TS determined and percent recovery for the bubbling, shaker, and centrifugation-seed wash methods were 101.8 (83.5%), 82.0 (68.0%), and 71.8 (59.8%), respectively. The bubbling glycerol method was the most sensitive means for detecting TS of *T. indica*.

CONTROL OF SOILBORNE DISEASES OF PEANUTS WITH FOLIAR APPLICATIONS OF HEXACONAZOLE. J. A. FRANK, G. I. FORNECKER, M. C. BROWN, S. P. HEANEY, AND J. J. BATES. ICI Americas and ICI PPD, Goldsboro, NC 25733-0208.

Hexaconazole provided excellent control of foliar diseases of peanuts in field trials in southeastern U. S. in 1986-87. In these trials, it was evident that as hexaconazole rates increased, yields increased, even beyond the optimum rate for foliar disease control. Soilborne disease ratings indicated that both *Rhizoctonia* peg rot and southern blight (*Sclerotium rolfsii*) were reduced with foliar applications of hexaconazole. Based on these experiments, the critical time of application for soilborne disease control is at pegging or post pegging. Applications of 134 g ai/ha applied at pegging and two weeks later provided a reduction of 82-93% in peg rot and 65-79% in southern blight. Under heavy soilborne disease pressure, peanut yields were increased 55-81% when compared to controls.

INDUCTION OF VACUOLES BY NICOTINE IN TELIOSPORES OF *UROMYCES VIGNAE* AND *PUCCINIA PUNCTIFORMIS*. R. C. French and A. R. Lightfield. USDA-ARS, Ft. Detrick, Bldg. 1301, Frederick, MD 21701.

Nicotine is a prominent flavor compound in tobacco and is a widely used insecticide. Previously we reported induction of

vacuoles in urediniospores by nicotine at 1000 ul/L and a moderate stimulation of germination at lower levels. We now report that nicotine at 1000-3000 ul/L induced vacuolation in teliospores of *Uromyces vignae* and *Puccinia punctiformis* at 24-48 hr at 24 C. Levels of nicotine from 25 to 1000 ul/L inhibited germination of teliospores of *U. vignae* at 3 days. Nicotine was most effective in inducing vacuolation at pH 9 or above. Teliospores in 0.05 M phosphate or borate buffer solutions above pH 9 accumulated the vital stain, neutral red, in the induced vacuoles after 72 hr. This indicated that the vacuoles were normal, at least in their ability to take up the dye. We know of no other research reports of chemically induced vacuolation.

DISTRIBUTION OF VERTICILLIUM WILT OF ALFALFA IN MARYLAND. A. P. Grybauskas, E. M. Dutky, and M. Seipel. Department of Botany, The University of Maryland, College Park 20742.

Prompted by the 1986 discovery in Maryland of *Verticillium* wilt (*Verticillium albo-atrum* Reinke and Berth.) of alfalfa (*Medicago sativa* L.), a survey was conducted in 1987 to establish the extent of the infestation. We processed 181 samples that scouts, scout supervisors, and Extension personnel collected in spring from 10 alfalfa-producing counties in Maryland. Seventeen samples from 5 counties contained *V. albo-atrum*. These counties were: Baltimore, Carroll, Cecil, Harford and Montgomery. Nine additional samples, including one from Frederick county, were suspected of being infected with *V. albo-atrum* but this could not be confirmed. Isolates from the 17 *V. albo-atrum* samples were tested for pathogenicity on 6-week-old 'Saranac AR' alfalfa seedlings. Pathogenicity scores, on a scale of 0 to 5, ranged from 0.5 to 4.4 after one month incubation at 21 C. Koch's postulates were completed on all but one isolate.

INVESTIGATIONS ON ROSE ROSETTE AND ITS VECTOR ON MULTIFLORA ROSE IN SOUTHERN INDIANA. D. F. Hindal, J. W. Amrine, and T. A. Stasny, Div. of Plant and Soil Sci., West Virginia University, Morgantown, WV 26506, and J. A. Appel, Dept. of Nat. Res., Div. of Entomol., State Office Bldg., Indianapolis, IN 46204.

Multiflora rose is a serious weed pest in West Virginia (WV). Interest is developing in a disease, rose rosette, that is vectored by an eriophyid mite, *Phyllocoptes fructiphilus*, for biocontrol. Since the disease is not present in WV, disease and mite development on multiflora rose were monitored in 1987 at Clifty Falls State Park near Madison, IN. This is one of the eastern most areas where there is heavy infestation of rose rosette on multiflora rose. The percentage of symptomatic plants in six transects increased from 31% to 53% during a six-month period and the number of dead plants from 2 to 10. Mites were present on most 3 to 5 inch long stem tips collected, but symptomatic tips averaged 53 mites per tip whereas there were only three (3) per asymptomatic one.

AN ALFALFA LEAF ASSAY FOR SCREENING BACTERIAL ANTAGONISTS OF *PHOMA MEDICAGINIS*. M. E. Jones, F. L. Lukezic, Department of Plant Pathology, The Pennsylvania State University, K. T. Leath and R. R. Hill, Jr., ARS-USDA, University Park, PA 16802

We previously reported that *in vitro* screening for bacterial antagonists of foliar pathogens of alfalfa was not indicative of efficacy on the intact plant surface. However, intact plants do not facilitate screening large numbers of bacterial strains. An assay was developed that uses excised alfalfa leaflets from greenhouse grown plants. Leaflets were placed in Petri plates containing 0.6% agar made with 1/2 strength Hoagland's solution and 0.02g/L kinetin, and inoculated with a bacterial strain, the pathogen, or both; leaflets were rated for disease severity 5 days later. Control leaflets retained their color and shape for at least 7 days. A *Flavobacterium* species that had not exhibited *in vitro* antagonism but significantly reduced severity of Phoma leafspot in greenhouse trials was effective in this leaf assay. *Bacillus* species that exhibited antagonism *in vitro* but were not effective in the greenhouse were ineffective in the leaf assay.

EFFECT OF WATER STRESS ON DISEASE PROGRESS OF SUMMER PATCH OF KENTUCKY BLUEGRASS CAUSED BY *MAGNAPORTHE* SP. K.E. Kackley and A.P. Grybauskas, Dept. of Botany, University of Maryland, College Park, MD 20742

Field plots of two cultivars ('S-21' and 'Aspen') of Kentucky bluegrass (*Poa pratensis* L.) were inoculated with either live or autoclaved inoculum of *Magnaporthe* sp. (ATCC 60239), the causal agent of summer patch. These cultivars had exhibited poor ('S-21') and good ('Aspen') resistance to

natural infections of summer patch in cultivar evaluation trials. Plots were either water stressed or not water stressed by irrigation treatments. Diameters of individual patches were measured over time. Disease developed only in plots receiving live inoculum and was greatest in non-stressed plots. Maximum patch diameter was reached at 45 days with mean patch diameters of 25.2 cm in stressed plots and 31.1 cm in non-stressed plots. Apparent recovery after 45 days was also greatest in non-stressed plots. There was no significant difference in disease injury or recovery between cultivars.

ROLE OF PEROXIDASE-MEDIATED NAD(P)H OXIDATION IN A BACTERIA-INDUCED HYPERSENSITIVE REACTION. L. Dale Keppler, Merelee M. Atkinson & C. Jacyn Baker, USDA, ARS, Microbiol. and Plant Path. Lab., Beltsville, MD, 20705.

This study assesses the possible role of peroxidase-mediated NAD(P)H oxidation in the increased extracellular pH observed during a bacteria-induced hypersensitive reaction (HR). The increased extracellular pH has been explained as due to an influx of H^+ 's into the plant cell. An alternate possibility is removal of extracellular H^+ 's during peroxidase-mediated oxidation of NAD(P)H. Peroxidase activity was monitored spectrophotometrically. Changes in H_2O_2/O_2^- levels (required for NAD(P)H oxidation by peroxidase) were monitored using chemiluminescence. Also, the effect of adding NAD(P)H/NAD(P)⁺ on extracellular pH during the HR was monitored. The results support involvement of peroxidase-mediated NAD(P)H oxidation in the increased extracellular pH observed during bacteria-induced HR.

COMPARATIVE STUDIES WITH TWO PHYTOPATHOGENIC ISOLATES OF MYROTHECIUM RORIDUM. Joseph O. Kuti and George A. Bean, Dept. of Botany, University of Maryland, College Park, MD 20742.

Growth rates of two *Myrothecium roridum* isolates from diseased muskmelon and tomato fruits were compared on potato dextrose agar at temperatures ranging from 0-35°C and pH levels 3.9-9.0. Studies were also done on the influence of carbon and nitrogen sources on spore germinability and growth of the isolates. The muskmelon isolate grew most rapidly at temperatures between 20 and 25°C while the tomato isolate grew best at temperatures between 25-30°C. The least favorable temperature-pH combination for growth was temperatures below 20°C and pH levels below 4.5. Glucose, mannitol and sucrose were generally the best carbon sources for growth of the tomato isolate while glucose and fructose were the best for the muskmelon isolate. Both fungi readily utilized NH_4NO_3 for growth. Benomyl and cupric hydroxide were effective *in vivo* in controlling *M. roridum* on tomato while chlorothalonil and thiophanate methyl controlled *M. roridum* on muskmelon. Results of this study suggest that these two *M. roridum* isolates are distinct physiologic strains.

GLUCOSE OXIDASE IS THE ACTIVE ANTIMICROBIAL AGENT IN THE ANTIBIOTIC TALARON FROM TALAROMYCES FLAVUS. K. Kwang-Ae Kim, USDA, ARS, Beltsville, MD 20705.

Talaromyces flavus is a promising biocontrol agent against *Verticillium dahliae*. An authentic sample of talaron from this fungus displayed varying degrees of antimicrobial properties towards bacteria and fungi only in the presence of glucose in the bioassay media. When the talaron sample was analyzed by HPLC, the elution profile of the antimicrobial peak coeluted with that of glucose oxidase. Approximately 30-40% of the solid talaron sample was glucose oxidase. Fluorescence emission and excitation wavelength maxima for the talaron sample were similar to that of glucose oxidase from *Aspergillus niger*. Molecular weight of the glucose oxidase from the talaron was 152,000 with the subunit molecular weight being 80,000. The pI of the enzyme was determined to be 4.2. Therefore, glucose oxidase is the active ingredient of the previously reported antifungal-antibiotic talaron produced by *T. flavus*.

BIOCONTROL OF RHIZOCTONIA SOLANI (RS) BY SOME NOVEL SOIL FUNGI. J. A. Lewis and G. C. Papavizas, USDA, ARS, Beltsville, MD 20705.

Survival of Rs in infested beet seed was reduced (>60%) in soil by bran preparations (BP) of *Laetisaria arvalis*, but not by BP of the fungi, *Coniothyrium minitans*, *Dendrostilbella* sp., *Cladorrhinum* sp., and *Trichurus* sp. Growth of Rs in soil was inhibited (50-70%) by BP of all fungi except *Trichurus* sp. When biocontrol inoculum was added to soil in alginate pellets, only pellets of *L. arvalis* inhibited growth (40%) of Rs in soil. BP of the fungi, except *Trichurus* sp.,

prevented damping-off of cotton caused by Rs. The plant stands in uninfested soil, Rs-infested soil, and in infested soils amended with BP of *Cladorrhinum* sp., *C. minitans*, *L. arvalis*, and *Dendrostilbella* sp. were 85,8,56,44,68, and 40%, respectively. Damping-off was also prevented using BP of various ages (3,7,14-day old) of *L. arvalis*. The results suggest that several soil fungi may be effective in biocontrol under proper conditions for growth and delivery.

EFFECT OF PLANTING DATE AND GENOTYPE ON SOUTHERN CORN STALK BORER AND STALK ROT OF CORN IN DELAWARE. J. M. Marshall, R. B. Carroll, and D. B. Dempsey, Department of Plant Science, University of Delaware, Newark, DE 19717-1303.

Populations of Southern corn stalk borer (SCB) are increasing in Southern Delaware. Field studies were initiated to determine the effect of planting date and genotype on occurrence of SCB and its relationship to stalk rot fungi. Four plantings of a commercial hybrid were made at two-week intervals starting 27 April 1987. Control plots were treated four times with *Bacillus thuringiensis* (BT) at 12-14 day intervals. Presence of stalk-rotting fungi was determined via isolation from insects and plants at various growth stages. At maturity stalks were split and rated for extent and severity of rot. For all planting dates BT resulted in a decrease of SCB and stalk rot. In addition, 24 genotypes were screened in the field and found to be significantly different with regard to tunnel length and stalk rot severity. Choice of planting date and genotype selection was useful in reducing SCB infestation and stalk rot.

EXTRACHROMOSOMAL ELEMENTS IN TRICHODERMA VIRIDE. Robert J. Meyer, USDA-ARS, SBMNL, BARC-West, Bldg. 011A, Rm. 313, Beltsville, MD 20705.

Extrachromosomal elements (ECEs) have been discovered in mitochondrial DNA (mtDNA) preparations from strains of *Trichoderma viride*. Eight of twelve strains had ECEs, which appeared as bands that migrated more rapidly than the high molecular weight DNA in agarose gels. The ECEs appear to be double stranded DNAs because they were digested by restriction enzymes and were not digested by RNase or Proteinase K. Each strain had a unique ECE pattern. ECEs in four of the strains had simple patterns with one to several bands. The four other strains had a complex pattern, with at least two major bands and many minor bands between ca. 2.1 kb and 4 kb in size. These many bands reduced to a single band following digestion with an appropriate restriction enzyme. The results suggest that the complex ECE patterns were produced by covalently closed circular molecules that were in various degrees of supercoiling. Studies are under way to determine if these ECEs are plasmids, amplified mtDNA sequences, or viruses.

PRELIMINARY ANALYSIS OF TWENTY FUNGI AS BIOLOGICAL CONTROL AGENTS OF THE SOYBEAN CYST NEMATODE, HETERODERA GLYCINES. S. L. F. Meyer, R. M. Sayre, and R. N. Huettel. Nematology Laboratory, USDA ARS, Beltsville, MD 20705.

The deregistration of many chemicals that were once available for control of plant-parasitic nematodes has made the development of biocontrol agents for these organisms increasingly important. Twenty fungi were tested in culture for pathogenicity to eggs of the soybean cyst nematode. Two *Verticillium* spp. and one *Phoma* sp. caused a statistically measurable decrease in the number of viable eggs. These three species, along with seven other fungi that exhibited some antagonism to nematode eggs, were inoculated onto agar containing Benomyl (E. I. duPont de Nemours & Co., Wilmington, DE). Benomyl is currently registered for use on soybean, and ability to grow in its presence may increase the competitive advantage of a fungus used as a biocontrol agent for a soybean pest. All ten fungi tolerated some level of Benomyl, although several fungi only grew in the presence of very low concentrations of the fungicide.

THE EFFECT OF TUNICAMYCIN ON THE PRODUCTION AND STABILITY OF EXTRACELLULAR CARBOHYDRATE-DEGRADING ENZYMES OF POSTIA PLACENTA. J. A. Micales and T. L. Highley, U.S. Forest Products Laboratory, Madison, WI, 53705

The extracellular carbohydrate-degrading enzymes of wood-decay fungi are usually heavily glycosylated and stable under most denaturing conditions. Tunicamycin prevents the glycosylation of glycoproteins. The effect of tunicamycin on the production of extracellular carbohydrate-degrading enzymes of *Postia placenta* was determined in liquid culture. Enzyme production was inhibited at concentrations of 2.5 - 5 µg/ml; glycosidases

were more sensitive than glycanases. The thermostabilities of xylanase and α -galactosidase, and the pH stability of xylanase, decreased when formed in the presence of 2.5 μ g/ml tunicamycin. This suggests that the enzymes are produced in an active but nonglycosylated (or underglycosylated) form. The deglycosylation of glycoproteins may be a physiologically specific means of controlling wood-decay fungi.

COMPARISON OF VEGETATIVE COMPATIBILITY OF *ENDOTHIA PARASITICA* FROM MICHIGAN AND WEST VIRGINIA SITES. A. Michna and D. Hindal, Dept. of Pl. Path., West Virginia Univ., Morgantown 26506

The success of hypovirulence as a biological control of *E. parasitica* may depend on the transfer of dsRNA between strains by hyphal anastomosis. When isolates are vegetatively incompatible transfer is restricted. The frequency of vegetative compatibility (VC) groups were determined in 4 sites not recovering from chestnut blight, 3 in WV and 1 in MI, and 2 recovering MI sites. Single conidial isolates from 123 cankers were paired with 36 WV test isolates of known compatibility. Three VC groups were identified in each of the recovering MI sites; all but one were compatible with the WV testers. Fourteen VC groups were found at the nonrecovering MI site; only 4 were compatible with the test isolates. Fifteen, 16, and 17 VC groups were found at the 3 WV sites; 22 were compatible with the test isolates. The lower number of VC groups in the recovering MI sites may allow more rapid transmission of dsRNA and partly be responsible for the recovery of chestnut.

MORPHOLOGICAL COMPARISONS OF SECOND-STAGE JUVENILES OF ONE ISOLATE EACH OF *HETERODERA SCHACHTII*, *H. GLYCINES* AND ONE OF THEIR HYBRIDS. L. I. Miller. Dept. Plant Path., Phys., & Weed Sci., VPI & SU, Blacksburg, VA 24061.

Comparisons were made of 2nd-stage juvenile characters of 31 specimens each of one isolate of *Heterodera schachtii* (C) cultured on 'US75' sugarbeet and one isolate of *H. glycines* (M) and one of their hybrids (CM) cultured on 'Lee' soybean. Dimensions in μ m were as follows: stylet length: C 22.8-27.0 (mean 25.3, s.d. + 1.2), M 22.0-24.0 (23.4 + 0.6), CM 22.0-25.0 (24.0 + 0.7), breadth of stylet knobs in lateral view: C 4.9 - 5.7 (5.1 + 0.2), M 4.0 - 5.0 (4.5 + 0.3), CM 4.1 - 5.0 (4.7 + 0.3); head tip to center of median bulb: C 68.0-83.0 (74.3 + 4.0), M 61.5-74.5 (67.6 + 3.4), CM 67.0 - 79.0 (71.2 + 3.3). C, M, and CM measurements were significantly different ($P > 0.01$) for all characters compared. The CM hybrid was able to reproduce on both sugarbeet and soybean, whereas C was able to reproduce on sugarbeet but not on soybean and M was able to reproduce on soybean but not on sugarbeet.

THE PRESENCE OF PLASMIDS IN THE BIOCONTROL FUNGUS *GLOIOCLADIUM*. Sue Mischke, USDA, ARS, Beltsville, MD 20705.

For the first time, plasmids have been found in strains of antagonists belonging to the *Gliocladium virens* complex. These plasmids are associated with the mitochondria of the fungi and appear to be composed of DNA. The majority of strains examined possess one or more plasmids which range in size from less than 1 kb to more than 4 kb. Based on size differences, these plasmids presumably differ from strain to strain. At least one strain (GL-3) exhibits genetic instability, with a high mutation frequency to senescence. It is not yet known whether there is a relationship between this phenotype and plasmids. The characteristics and function of these plasmids are under study.

SURVEY OF POTATO VIRUSES IN PENNSYLVANIA. D. M. Petrunak, F. E. Gildow, and B. J. Christ. Department of Plant Pathology, The Penna. State University, University Park, PA 16802.

Second generation tubers of three cultivars of potato derived from virus-indexed meristem cultured plantlets were planted in plots adjacent to commercial potato fields in six Pennsylvania counties representing the Northwest, Southeast, and Southwest areas of the state. Leaf tissue was randomly sampled from three cultivars in "virus-free" plots and adjacent commercial fields, and tested for six viruses (PLRV, PVS, PVX, PVY, PVM, PVA) by ELISA. All six viruses were identified in Pennsylvania. PVS and PVY were the most commonly occurring viruses, with averages of 41% and 22%, respectively. The highest frequency of PLRV (9%), PVS (55%), PVM (11%), and PVA (17%) occurred in Southwestern Pennsylvania. PVX (5%) and PVY (32%) occurred with highest frequency in the Northwest. All viruses occurred in higher proportion in commercially grown cultivars than in the second generation "virus-free" plants.

EVALUATION OF RESISTANCE TO SCLEROTINIA CROWN AND STEM ROT CAUSED BY *SCLEROTINIA TRIFOLIORUM* IN SELECTED ALFALFA CULTIVARS IN THE GREENHOUSE. C.D. Pooranampillai, E.L. Stromberg, Dept. of Plant Pathology, Physiology and Weed Science, and S.W. Van Scoyoc, Dept. of Agronomy, VPI&SU, Blacksburg, VA 24061.

Sclerotinia trifoliorum-induced crown and stem rot causes severe losses in some fall-seeded, no-tillage alfalfa plantings in Virginia. To initiate a breeding program, a method of evaluating resistance of germplasm lines is needed. Resistance of cvs Arc, Vertus, Cimarron, Anstar, Armor, Maxim, WL 320, Endure, Euver, Pioneer Brand 526, Raidor, and Saranac AR was evaluated: two-month-old plants were inoculated near the crown with mycelium plugs (6 mm dia) from the margin of 5-day-old cultures of *S. trifoliorum*, incubated in a dew chamber for 96 hr at 18 C and 100% RH and moved to the greenhouse; disease severity ratings (DSR) of individual plants were taken on a scale of 1 (healthy)-6 (dead). All cvs were susceptible but Anstar consistently had a low mean DSR while Pioneer Brand 526 and Raidor had a high mean DSR, 20 days after inoculation. This technique appears promising for evaluating resistance.

EFFECT OF HYDROLYTIC ENZYMES OF *GLOIOCLADIUM VIRENS* ON *PYTHIUM ULTIMUM*. D. P. Roberts and R. D. Lumsden, USDA, ARS, Beltsville, MD 20705.

Gliocladium virens (GL-21) is an antagonist of *Pythium ultimum* (Pu). Culture supernatants from *G. virens* grown in 5% bran extract inhibited germination of sporangia of Pu. The supernatants had laminarinase, carboxymethylcellulase, chitinase, protease, and amylase activities. However, these enzymes did not play a role in inhibiting sporangial germination or mycelial growth. Incubation of culture supernatants at 80 C for 20 min inactivated these enzymes, but did not affect the factor(s) which inhibited sporangial germination and mycelial growth. Also, four *G. virens* isolates, which varied in their ability to reduce disease in vivo, had similar levels of the above enzymes. Ultrafiltration of culture supernatants indicated that the inhibitory factor(s) was a low molecular weight metabolite (<5 kDa). Enzymes, however, may play a secondary role in biological control of damping-off caused by Pu by releasing essential nutrients from food bases.

THE OCCURRENCE OF DSRNA-CONTAINING STRAINS OF *ENDOTHIA PARASITICA* IN DIFFERENT AGE CHESTNUT STANDS. J. M. Sillick and W. L. MacDonald. Department of Plant Pathology and Agricultural Microbiology, West Virginia University, Morgantown, WV. 26506.

Biological control of chestnut blight, presumably mediated by dsRNA, has been observed in Michigan and European groves where chestnut populations have remained high for many years. Similar control has not occurred among sprouts in the Appalachians, even though dsRNA is present. This study was undertaken to determine if chestnut sprout populations in Appalachian stands of different age influence the occurrence of dsRNA. Six cankers were collected from 38 sprout stands of varying age. Isolates obtained from these infections were grown for 10 days in liquid media, harvested, and analyzed for dsRNA content by agarose-gel electrophoresis. Of 226 isolates tested, 75% contained dsRNA. The frequency of dsRNA recovery varied among sites, but no relationship existed between stand age and the number of isolates containing dsRNA.

EFFECT OF RH-3486 ON DICARBOXIMIDE-RESISTANT ISOLATES OF *SCLEROTINIA MINOR*. F. D. Smith, P. M. Phipps and R. J. Stipes. Tidewater Agr. Exp. Sta., VPI&SU, Suffolk, VA 23437.

The sensitivity of *Sclerotinia minor* to RH-3486 and three dicarboximides (iprodione, vinclozolin, chlozolinate) was tested on fungicide-amended, glucose-yeast extract agar (GYEA). ED50 values with a dicarboximide-sensitive isolate were 0.004, 0.08, 0.18, and 0.38 μ g/ml for RH-3486, vinclozolin, iprodione, and chlozolinate, respectively. A dicarboximide-resistant field isolate (B-83-T2) showed enhanced growth on GYEA containing iprodione or vinclozolin at 1.0 μ g/ml, or chlozolinate at 5.0 μ g/ml. RH-3486 strongly inhibited growth of B-83-T2 and showed an ED50 of 0.01 μ g/ml. Three applications of chlozolinate at 1.12 kg/ha, iprodione at 1.12 kg/ha, or vinclozolin at 0.84 kg/ha to field plots of Florigiant peanut suppressed the incidence of *Sclerotinia* blight by 19, 29, and 46%, respectively. RH-3486 applied once at 1.12 and 2.24 kg/ha suppressed disease incidence by 39 and 74%, respectively. No fungicide resistant isolates of *S. minor* were found in field plots.

EFFECT OF BENOMYL AND MANCOZEB ON THE GROWTH OF FIELD CORN. K.L. Smith, A.P. Grybauskas, and P.R. Thomson. USDA-ARS-ECL, BARC-West, Beltsville, MD 20705, and Depts. of Botany and Agronomy, The University of Maryland, College Park, MD 20742.

A field experiment was conducted in 1986 to investigate the effects of benomyl, mancozeb, and the combination of benomyl and mancozeb on the growth and yield of field corn in the absence of disease. Pioneer Brand 3184 was sprayed six times on a biweekly schedule with benomyl or benomyl plus mancozeb, or 11 times on a weekly schedule with mancozeb. Measurements were made of leaf area, leaf dry weight, stalk dry weight, and ear dry weight periodically, and of ear moisture, plant height, node number, and grain yield at harvest. There were no significant differences among the three fungicide treatments and untreated control for 15 of the 17 growth components measured. For only one component, node number, was there a significant difference between the control treatment and a fungicide treatment. This difference was a reduction of less than one node per plant in the benomyl plus mancozeb treatment below that of the untreated control.

A STANDARDIZED AGAR PLATE - SPORE GERMINATION BIOCONTROL BIOASSAY. H. W. Spurr, Jr., ARS-USDA Laboratory, P. O. Box 1555, Oxford, NC 27565 and North Carolina State University.

A rapid, reliable bioassay was required to facilitate research with bacteria antagonistic to *Cercospora arachidicola*, a fungus causing leafspot on peanut. Petri plates were prepared using commercial PDA (DIFCO) adjusted to a final pH of 4.5 with 85% lactic acid. One ml of spore suspension (60000 spores) was swirled per plate. Plates were held 24 hrs at 5C to equilibrate moisture content. Bacterial suspensions or chemicals for assay were added directly to media or to 32 mm² sterile filter paper discs (1-6/plate). Discs cut from treated peanut leaves were also used successfully. Plates were incubated at 25C with an 8 hr photoperiod for five days. Inhibition zones were measured precisely using an ARTEK Systems Corp. counter. The assay proved reliable for measuring efficacy and toxin production when all environmental variables were standardized.

REDUCED INTENSITY REACTIONS OF CERTAIN PHASEOLUS VULGARIS CULTIVARS TO UROMYCES APPENDICULATUS IN THE FIELD. J. R. Stavelly and J. P. Zhang, USDA, ARS, MPPL, Beltsville, MD 20705 and Inner Mongolia Acad. Agr., Huhhot, Inner Mongolia, Peoples Rep. China.

Field development of a rust epidemic, caused by five races of *U. appendiculatus*, was recorded on ten bean cvs. Uredinium intensity was recorded at five day intervals from the appearance of uredinia until maximum severity occurred on the cv. having the highest intensity, Pinto 111. Races 40 and 41 were primarily responsible for the epidemic. Fewer uredinia occurred on cvs. KW 780 and Nodak, that have specific resistance to races 40 and 41, respectively. On cvs. Earlybird, Jamaica Red, PI 171772, Resisto, Royal Red, Tidal Wave, and TMO 75, races 40 and 41 produced moderate to large uredinia and intensity was lower than on Pinto 111. Presence or absence of uredinia on the youngest expanded leaf correlated with specific resistance.

MATING TYPE, NUCLEAR DNA CONTENT, RACE COMPOSITION, AND ISOZYME ANALYSIS OF PERUVIAN ISOLATES OF PHYTOPHTHORA INFESTANS. P. W. Tooley, C. D. Therrien, and D. L. Ritch, USDA-ARS, Frederick, MD 21701 and Dept. of Biology, The Pennsylvania State University, University Park, PA 16802.

Thirty-four isolates of *P. infestans* from potatoes growing in the Central Highlands of Peru were compared with isolates from the U.S., Europe, and Mexico. All Peruvian isolates were of A1 mating type. Feulgen cytophotometry was used to measure the DNA content of individual zoospore nuclei. The distribution of DNA content values observed for Peruvian isolates was nearly identical to that previously observed for isolates from the U.S. and Europe (Exp. Mycol. 11:19-26). Races 0, 1, and 1.5 were most common among Peruvian isolates, representing 33%, 39%, and 15% of the population, respectively. Isozyme banding patterns observed for Peruvian isolates at 10 enzyme loci were identical to those of U.S. and European isolates. These results strongly suggest a common ancestry for Peruvian, U.S., and European populations of *P. infestans*.

OBSERVATIONS OF AN UNKNOWN VIRUS-LIKE DISEASE AFFECTING DELAWARE SOYBEANS. T. Weldekidan, T. A. Evans, R. B. Carroll and R. P. Mulrooney, Dept. of Plant Science, Univ. of Delaware, Newark, DE 19717-1303.

Studies were initiated to determine the occurrence, etiology, transmission, and varietal response to a new disease of soybean. Symptoms occur on the first true leaves and infected plants are stunted, have superficial stem cankers, bunched thickened dark green leaves, and reduced number of flowers and pods. The causal agent was determined to be virus-like and is sap-transmitted to soybean with greenhouse symptoms identical to those in the field. Host range included all legumes tested. TIP is 55°C, LIV 24hr, and DEP 10⁻³. Transmission is through soil but not via seed. The disease was confirmed in 19 of 29 commercial fields surveyed. *Xiphinema* spp. and *Trichodorus* spp. were recovered from a number of infested fields. Of 16 soybean varieties field tested Asgrow brand A5149, Stanford brand HT5203, and Sparks had a high level of resistance whereas Essex, Dolphin, and Verde were most susceptible.

EFFECT OF LOWER RAIN-FALL AND SOIL MOISTURE ON THE ACCUMULATION OF METALAXYL IN POTATO TUBERS. Robert J. Young, 401 Brooks Hall, P.O. Box 6057, West Virginia University, Morgantown, WV 26506-6057.

In 1987, rainfall in July and August at the Experiment Station Farm, Reedsville, WV was 43 and 76 percent of normal, respectively. During this period, metalaxyl at 2.2 Kg MZ58/HA, was applied as eight foliar treatments between 7/21 and 8/19. Treatments consisted of single and double applications. Tubers were harvested at 24 hr intervals for six days after each treatment and assayed for metalaxyl activity by tuber slice bioassay. Tubers from treated plants and control plants failed to inhibit growth of *Phytophthora infestans*. With no accumulation of metalaxyl, tubers were unprotected during the latter stages of growth when tuber infection is most likely to occur. Normal rainfall for July and August is about 14 cm/mo and in prior years metalaxyl activity was detected. Uptake and translocation of metalaxyl into tubers appears to be related to rain events.

RELATION OF LEAF SURFACE CHARACTERS TO RECEPTIVITY OF PHASEOLUS VULGARIS TO UROMYCES APPENDICULATUS. J. P. Zhang and J. R. Stavelly, Inner Mongolia Acad. Agr., Huhhot, Inner Mongolia, Peoples Rep. China and USDA, ARS, MPPL, Beltsville, MD 20705.

Bean cvs. Jamaica Red, PI 171772, and Earlybird had fewer uredinia than Pinto 111 when infected by several mixed or single races of *U. appendiculatus* in the field and greenhouse. This partial resistance has been called low receptivity. The optimal stomatal lip or ridge height recently suggested for appressorium induction was 0.05 µm. Scanning electron microscopy showed that the above four cvs. differ in percentages of stomata having ridges 0.4-0.6 µm high. The mean percentages ranged from 38.4 in Pinto 111 to 15.0 in Jamaica Red. Lower receptivity was partially correlated with lower percentages of ridges 0.4-0.6 µm high. Density and length of leaf hairs and stomatal density showed no such correlation in these cvs.

DEGREE OF BLOSSOM BLIGHT SEVERITY IN APPLE AND PEAR FOLLOWING QUANTITATIVE INOCULATION WITH THREE ISOLATES OF ERWINIA AMYLOVORA. T. van der Zwet, United States Department of Agriculture, Agricultural Research Service, Appalachian Fruit Research Station, Kearneysville, West Virginia, 25430

Individual blossoms of 'Bartlett' (B) pear and 'Jonathan' (J) apple were inoculated with *Erwinia amylovora* in the field during bloom 1987. The inoculum (50 ul of phosphate buffer suspension with 10², 10⁵ or 10⁸ cfu/ml) of three isolates (NY 266,173 and Harrow, Ont. 2002A) was applied with a micro pipet dispenser on several dates. Significantly more pear and apple blossoms blighted following inoculation with 10⁸ than with 10⁵ or 10² cfu/ml, when isolates were applied individually or as a mixture. This difference was also reflected in the number of blighted blossom clusters, counted 10 days after initial symptoms were observed. When blossoms were inoculated April 24 (B) and May 1 (J), first symptoms (ooze droplets at base of blossom receptacle) were observed after 10 days. Blossoms inoculated with buffer solution only resulted in 3% (B) and 1% (J) of blossoms infected, presumably due to the presence of natural epiphytic *E. amylovora* bacteria.