

# **ABSTRACTS OF PAPERS**

**Presented at Meetings of the  
American Phytopathological Society**

Potomac Division

Annual Meeting

March 31-April 2, 1982

ABSTRACTS

INHIBITION OF MONILINIA FRUCTICOLA AND PENICILLIUM EXPANSUM BY CARBONYL SULFIDE. F. B. Abeles and P. L. Pusey, USDA, Appalachian Fruit Research Station, Kearneysville, WV 25430.

Both carbon disulfide (CS<sub>2</sub>) and carbon monoxide (CO) have been used as fumigants to control disease producing organisms in stored produce and grains. The purpose of work reported here was to test the mycotoxic activity of carbonyl sulfide (COS), a molecule which shares molecular features with both CS<sub>2</sub> and CO. COS at 1%, v/v, prevented germination of conidia of *M. fruticicola* and *P. expansum* on potato dextrose agar. Mycelium growth was inhibited by the presence of COS; however, it resumed growth after the gas was removed. Decay development on apples was delayed when fruit were fumigated after a spore challenge against wounded tissue. COS did not reduce development of lesions if fruit was fumigated prior to a spore challenge. Deterents to the use of COS as a fumigant include its toxicity and flammability. In addition, fruit exposed to COS produced off flavors. The mechanism of toxicity is not known and use of COS appears limited to experimental studies.

FECONDITY, PATHOGENICITY AND CHEMICAL INACTIVATION OF BURSAPHLENCHUS XYLOPHILUS. J. C. Adams and A. L. Morehart. Dept. of Plant Science, Univ. of Delaware, Newark, DE 19711.

Populations of *Bursaphelenchus xylophilus* extracted from *Pinus* spp. in Delaware, were investigated for reproduction, pathogenicity and reaction to systemic insecticides. Of the *xylophilus* fungi isolated from declining *P. thunbergii*, only *Gliocladium roseum*, *Pestalotia funerea*, *Botrytis cinerea* and an unidentified species (B47) supported nematode reproduction in axenic mycocultures. There was no significant difference (P=0.05) in pathogenicity to *P. thunbergii* of *B. xylophilus* reared on *P. funerea*, *B. cinerea* or B47. When identical concentrations were inoculated into two-yr-old *P. thunbergii* and *P. taeda*, *B. xylophilus* reproduction was significantly greater (P=0.001) on the former but induced more pronounced symptoms on the latter species. In greenhouse experiments, *P. thunbergii* seedlings treated individually with oxamyl (Oxamyl 10% granular and Vydate L 24% liquid) and carbofuran (Furadan 4 Flowable) yielded significantly lower (P=0.05) populations than untreated trees.

HISTOPATHOLOGY OF SUSCEPTIBLE SOYBEAN ROOTS INOCULATED WITH ZOOSPORES OF PHYTOPHTHORA MEGASPERMA F. SP. GLYCINEA. Jean E. Beagle and Jane F. Rissler, Dept. of Botany, Univ. of Maryland, College Park, MD 20742

Zoospores of *Phytophthora megasperma* f. sp. *glycinea* are effective inoculum for development of soybean root rot. Our objective was to observe certain pre- and postpenetration phenomena on 2-wk old 'Harosoy' roots inoculated with zoospores. Samples taken at intervals to 72 hr after inoculation were prepared for light and scanning electron microscopy. By 1 hr, zoospores had encysted, cysts had germinated to form germ tubes averaging 18µm in length, and penetration had occurred primarily from simple germ tubes between anticlinal walls of epidermal cells. Hyphae, growing inter- and intracellularly within cortical tissue and the stele, branched and reached lengths of 1 mm by 72 hr. Oogonia observed at 24 hr increased in number by 48 hr, then decreased by 72 hr as the number of oospores increased. Root necrosis was macroscopically evident at 72 hr.

SEVERITY OF PHYTOPHTHORA ROOT ROT ON NODULATED AND NON-NODULATED SOYBEANS. Jean E. Beagle and Jane F. Rissler, Dept. of Botany, Univ. of Maryland, College Park, MD 20742

*Phytophthora megasperma* f. sp. *glycinea* (Pmg) causes a serious

root rot of soybean. Experiments were conducted to compare the severity of disease on nodulated and non-nodulated soybeans and to determine whether root nodules were colonized by the fungus. Three-wk-old nodulated and non-nodulated plants of the susceptible cultivar Harosoy (H) and the resistant cultivar Harosoy 63 (H-63) were inoculated with Pmg. Two weeks after inoculation, shoot height, shoot and root dry weights, root rot scores and root nodule scores were measured. Data were statistically analyzed. Height and dry weight means of inoculated plants were significantly lower and root rot scores were significantly higher on nodulated than on non-nodulated H plants. No significant differences in disease severity were found on inoculated plants in the H-63 treatments. Pmg-inoculated H plants had significantly fewer nodules than uninoculated plants. Pmg colonized nodules of H but not H-63 plants.

LOCATION OF PRIMARY INFECTION SITES OF APPLE POWDERY MILDEW AND ITS SIGNIFICANCE IN MANAGEMENT STRATEGIES. L.P. Berkett and K.D. Hickey, Department of Plant Pathology, The Pennsylvania State University Fruit Research Lab, Biglerville, PA 17307.

The location of primary infection sites of *Podosphaera leucotricha* was determined for 60 apple trees. Data were collected on type of bud infected, position of infected bud on the shoot, and location of the shoot on the tree. Most primary infection sites developed in apical vegetative buds; only 2% involved blossoms. Of the shoots containing infection sites, 77% had originated from flowering buds. Sixty-seven percent of all primary infection sites developed from buds that had set at least twice during the growing season. The significance of the location in indicating time of initial bud infection is discussed in relation to management strategies.

HISTOCHEMICAL ASPECTS OF CYTOSPORA CANKER OF HYBRID POPLAR. A. R. Biggs, D. D. Davis, and W. Merrill, Dept. of Plant Pathology, University Park, PA 16802

Poplar hybrid NE-388 (*P. maximowiczii* X *trichocarpa*) was inoculated with mycelium of *C. chrysosperma*. Bark samples were taken from slowly enlarging cankers, rapidly killing "blackstem," control wounds, and uninoculated healthy plants. Rapid establishment of the pathogen via large diameter hyphae is not associated with noticeable pectin degradation. In colonized areas associated with small diameter hyphae, degradation of pectin, starch, and some loss of birefringence occurs. Vigorous host response to the pathogen or control wound is characterized by formation of non-suberized impervious tissue (NIT) which is impervious to water, autofluorescent, phloroglucinol-HCl-positive, and orcinol-positive. Tests for starch, suberin, and lignin are negative. Formation of NIT is prerequisite to initiation of a meristogenous layer which gives rise to suberized necrophylactic periderm. Weakened plants differ from vigorous hosts in the timing and extent of NIT formation. Plants with blackstem appear incapable of producing NIT.

PATHOLOGICAL ANATOMY OF CYTOSPORA CANKER OF HYBRID POPLAR. A. R. Biggs, D. D. Davis, and W. Merrill, Dept. of Plant Pathology, University Park, PA 16802

Poplar hybrid NE-388 (*P. maximowiczii* X *trichocarpa*) was inoculated with mycelium of *C. chrysosperma*. Bark samples were taken from slowly enlarging cankers, rapidly killing "blackstem," control wounds, and uninoculated healthy plants. Slowly enlarging cankers are characterized by necrosis of the cambium ahead of visible bark symptoms, large- and small-diameter hyphae between and within host cells, and hyphal aggregations associated with the leading edge of bark colonization. Blackstem is characterized by rapid colonization of the cortex and phloem by large diameter intercellular hyphae followed by the digestion of cell contents by small diameter

intracellular hyphae. Non-specific autonomous processes are prerequisite to compartmentalization of diseased tissue from healthy tissue. Formation of non-suberized impervious tissue, followed by formation of necrophylactic periderm, and redifferentiation of new phellogen and cambium, are observed in both inoculated and control-wounded stems.

HOST RANGE OF *PERONOSCLEROSPORA PHILIPPINENSIS*. M. R. Bonde and G. L. Peterson. USDA, ARS, Plant Disease Research Laboratory, Frederick, MD 21701.

Seventy-two gramineous species in 22 genera in 8 tribes were tested for their susceptibility to systemic colonization after conidial inoculation with two Philippine isolates of *P. philippinensis*. Twenty-two species in the tribes Andropogoneae and Maydeae were susceptible to at least one isolate, and usually both. The susceptible species were *Andropogon gerardii*, *Bothriochloa ambigua*, *B. babinodis*, *B. decipiens*, *B. edwardsiana*, *B. ischaemum*, *B. lagroides*, *B. perforata*, *B. springfieldii*, *B. woodrowii*, *Eulalia fulva*, *Saccharum officinarum*, *Schizachyrium hirtiflorum*, *S. microstachyum*, *S. scoparium*, *Sorghum bicolor*, *S. halepense*, *S. plumosum*, *Tripsacum dactyloides*, *Zea diploperennis*, *Z. mays* (subsp. *mays* and subsp. *mexicana*), and *Z. perennis*. According to our host range results presented here and previously with *P. sacchari* from Taiwan, the remarkable similarity of host range reactions indicates a very close phylogenetic relationship between isolates of the two species.

SOURCES OF SPECIFIC RESISTANCE TO SOYBEAN RUST. K. R. Bromfield and J. S. Melching. USDA, ARS, Plant Disease Research Laboratory, Frederick, MD 21701.

Each of the three soybean accessions PI 200492, PI 230970, and PI 462312 ('Ankur') carries a dominant gene governing specific resistance to *Phakopsora pachyrhizi*, the cause of soybean rust. These genes are at a different locus in each accession and not in an allelomorph series. Additional sources of specific resistance recently identified are soybean accessions PI 459024 and PI 459025, both from southern China, and *Glycine soja* accession PI 339871 from Korea. The genetic basis for rust resistance in these latter accessions is currently being investigated. The resistance of PI 459025 and PI 339871 is of special interest because it is effective against *P. pachyrhizi* isolate Taiwan-80-2, which is capable of attacking the genes for specific resistance in PI 200492, PI 230970, and PI 462312.

TOMATO RINGSPOT VIRUS ISOLATED FROM MARYLAND GROWN RED RASPBERRIES. W. G. Buchholz and M. K. Corbett, Botany Dept., Univ. Maryland, College Park, MD 20742.

Indexing Maryland grown *Rubus* sp. on herbaceous hosts indicated that at least three mechanically transmitted viruses are present. The most commonly occurring virus was identified as the *Rubus* isolate of tobacco streak virus. An isolate from Hilton red raspberry infected a wide range of herbaceous hosts, inducing symptoms characteristic of Nepoviruses. In crude sap the physical properties were: thermal inactivation 58 C; dilution end point  $10^{-2}$ - $10^{-3}$  and aging in vitro of 2-3 days. Density gradient centrifugation of partially purified preparations in 10-40% sucrose gave two light-scattering zones of 64S and 129S. Both zones contained icosahedral particles about 28 nm in diameter but infectivity was associated only with the 129S particle. In gel double-diffusion tests precipitin zones of serological relatedness occurred with the apple and apricot strains of tomato ringspot virus. No reaction occurred with antiserum to tobacco ringspot virus.

A MEDIUM SELECTIVE FOR *PSEUDOMONAS CEPACIA*. D. A. Burbage, M. Sasser, Univ. of Delaware, Newark, DE 19711, and R. D. Lumsden, USDA, ARS, Beltsville, MD 20705.

*Pseudomonas cepacia* (Burkholder) causes sour skin of onion, and a recent emendation of the description included two human pathogens, *Pseudomonas kingae* and *Pseudomonas multivorans*. We have developed a selective medium (PCAT) for *P. cepacia*. The medium consists of (g/L): azelaic acid, 2.0; tryptamine, 0.2;  $MgSO_4$ , 0.1;  $K_2HPO_4$ , 4.0;  $KH_2PO_4$ , 4.0; yeast extract, 0.02; agar, 15.0; and 1 ml of a 1:24 aqueous suspension of chlorothalanyl (Bravo 500). Following autoclaving for 10 min at 121 C, the medium is adjusted to pH 5.7. To prepare PCAT, the  $MgSO_4$  is dissolved in the distilled water, the azelaic acid is added, followed by heating and stirring until it dissolves. The remaining ingredients are then added. Sixteen isolates of *P. cepacia* were

quantitatively recovered by dilution plating, but 12 isolates of *Agrobacterium*, *Erwinia*, *Xanthomonas* and *Pseudomonas* failed to grow on PCAT. Isolations of *P. cepacia* from plant material and from soil were successfully performed.

PRECIPITATION CHEMISTRY IN THE SOUTHERN APPALACHIAN MOUNTAINS OF VIRGINIA. B. I. Chevone and Y. S. Yang, Dept. of Plant Pathology and Physiology, Va. Polytech. Inst. & State Univ., Blacksburg, VA 24061.

Precipitation chemistry was monitored at a remote mountain site (elev. 960m MSL) in Giles County, VA on a weekly basis since November 1978. Precipitation was generally acidic with mean pH (non-volume weighted) for 1978-79, 1980 and through September 1981 of 4.56, 4.30 and 4.18. Mean sulfate and nitrate ion concentrations (mg/l) during this period were 3.62, 4.73 and 8.46 and 1.27 and 2.41 and 4.41, respectively. Sulfate was most highly correlated with ammonium, with R values ranging from 0.89 to 0.99 during the three years. Correlation of sulfate or nitrate ions with hydrogen ions on a yearly basis was variable with R values ranging from 0.31 to 0.59 and 0.38 to 0.86, respectively. Concentrations of all ionic species (Ca, K, Mg, Na,  $NH_4$ , Cl,  $NO_3$ ,  $PO_4$  and  $SO_4$ ) varied similarly with pH. Maximum ion concentrations occurred at pHs < 4.0 and > 6.50 and minimum concentrations occurred between pH 4.50 and 5.50.

DOUBLE-STRANDED RNA IN *AGARICUS BITORQUIS*. K. L. Deahl, Veg. Lab., ARS, USDA, Beltsville, MD 20705

Five strains of *Agaricus bitorquis* were tested for susceptibility to the mushroom virus disease. Inoculum consisted of soil containing spores and mycelium from mushroom beds of *A. bisporus* previously shown to contain virus-like particles and double stranded RNA (dsRNA). To verify infectivity of the inoculum, trays of *A. bisporus* and *A. bitorquis* were simultaneously inoculated; uninoculated trays of all test strains served as controls. Although the yields of all the inoculated strains of *A. bitorquis* were 15% less than the yields of the uninoculated controls, only strain 26-K showed symptoms of virus disease. All inoculated strains of *A. bisporus* showed severe symptoms and 75% reduction in yield. The symptomatic 26-K strain and all the symptomatic *A. bisporus* strains contained dsRNA. Asymptomatic mushrooms from *A. bitorquis* strains as well as mushrooms from the inoculated trays contained no detectable dsRNA. Attempts by electron microscopy to detect virus-like particles in crude or semi-purified preparations from the 26-K strain have failed.

A MEDIUM HIGHLY SELECTIVE FOR *PSEUDOMONAS SYRINGAE* PV. *GLYCINEA*. D. J. Fieldhouse and M. Sasser, Department of Plant Science, University of Delaware, Newark, DE 19711.

A selective medium for *P. syringae* pv. *glycinea* has been devised and tested. It contains (g/L): D-quinic acid, 2.0; D-serine, 0.2;  $K_2HPO_4$ , 2.0;  $KH_2PO_4$ , 2.0;  $MgSO_4$ , 0.1; boric acid, 1.5; yeast extract, 0.03; agar, 15; 1 ml of a 1:25 aqueous suspension of chlorothalanyl; ampicillin, 16  $ug\ ml^{-1}$ ; and novobiocin, 24  $ug\ ml^{-1}$ . The  $MgSO_4$  is dissolved before adding the other ingredients, and the pH is adjusted to 6.8 before autoclaving for 10 minutes at 121°C. The ampicillin and the novobiocin are added after autoclaving and cooling to 50°C. When compared to M-71 and to nutrient agar, our medium (BANQ) allowed 100% recovery of 8 known isolates of *P. syringae* pv. *glycinea*. The bacterium was quantitatively recovered from a nonsterile soil. Ten isolates of *Erwinia*, *Agrobacterium*, *Pseudomonas* and *Xanthomonas* failed to grow on this medium. BANQ is inexpensive, simple to prepare, extremely selective, and very efficient in isolation of *P. syringae* pv. *glycinea*.

WINGED BEAN MOSAIC CAUSED BY A POTY VIRUS. Mark Fox and M.K. Corbett, Botany Dept., Univ. MD, College Park, MD 20742.

Winged beans (*Psophocarpus tetragonolobus*) growing in experimental plots in MD exhibited mosaic symptoms. Electron microscopy of leaf-dip preparations showed that the plants were infected with a 750 nm flexuous rod virus. The virus was mechanically transmissible and had a narrow host range. It induced a systemic mosaic in inoculated plants of winged bean and only local lesions in plants of *Chenopodium quinoa* which served as source and assay hosts. The physical properties of the virus were: thermal inactivation 30-40 C; dilution end point  $10^{-4}$ - $10^{-5}$  and aging in vitro 6-12 days. Density gradient centrifugation of partially purified preparations obtained by chloroform-butanol clarification and precipitation by 4% polyethylene glycol and 0.05% NaCl gave a light-scattering zone



of 150S. Electron microscopy of ultra-thin sections from infected winged bean tissue showed nuclear crystalline striated inclusions and cytoplasmic crystalline and pinwheel inclusions similar to those of the Potyvirus group.

A TECHNIQUE FOR MAINTAINING ISOLATES OF HELMINTHOSPORIUM SOLANI. Robert W. Geth. Vegetable Laboratory, Horticultural Science Institute, Beltsville Agricultural Research Center, Beltsville, Maryland 20705.

Isolates of *Helminthosporium solani*, the causal organism of potato silver scurf, were maintained on sterilized rye grains without transferring for 18 months. When these infested rye grains were placed on V-8 juice agar, water agar, corn meal agar and PDA, large numbers of conidiophores bearing conidia developed on the grain surface and on the surface of the supportive medium immediately adjacent to the grain. Inoculum consisting of conidia derived from both the grain surface and the supportive media has been successfully used as inoculum to study the development of silver scurf.

GREENHOUSE EVALUATION OF SEED TREATMENTS FOR CONTROL OF FUSARIUM DAMPING-OFF OF SOYBEAN. C. E. Grant, C. W. Roane and P. M. Phipps, Dept. of Plant Pathology and Physiology, VPI & SU, Blacksburg, VA 24061.

Fusarium damping-off of soybean caused by *Fusarium oxysporum* and *F. solani* has been a problem in King and Queen County and surrounding areas of Virginia since 1978. Management efforts to control damping-off in the problem fields have not been successful. Results from greenhouse studies on rates and combinations of chemicals for the control of the organisms were undertaken. Slurry treatment of seeds with combinations consisting of captan 25% + thiram 25% + PCNB 25%, captan 30% + botran 30%, and maneb 30% + captan 30% at rates of 170 and 255g a.i. of the combined fungicides per 45 kg seeds significantly ( $P < .05$ ) increased seedling emergence in naturally and artificially infested soil. Mild phytotoxicity symptoms on cotyledons resulted from all treatments, but this effect was not observed on the true leaves. Seedlings from chemically treated seeds grew as well as those from untreated controls in pasteurized uninfested soil.

THE EFFECTS OF POPULATION LEVELS OF PHYTOPHTHORA PARASITICA VAR. NICOTIANAE ON THE GROWTH AND DEVELOPMENT OF GREENHOUSE-GROWN FLUE-CURED TOBACCO. C. E. Grant and J. J. Reilly, Dept. of Plant Pathology and Physiology, VPI & SU, Blacksburg, VA 24061.

Six-week-old seedlings of 'McNair 944', 'Coker 319' and 'VA 81' tobacco, classified as highly and moderately resistant and susceptible, respectively, were evaluated under greenhouse conditions. Seedlings were inoculated with five zoospore populations (10, 20, 30, 40 and 50 per gram dry soil). Factors measured were plant height and leaf number at inoculation, and 21 days later, plant height, leaf number, shoot dry weight and root disease development. At inoculation, leaf numbers differed significantly ( $P < .05$ ) among cvs. Three weeks later plant height, shoot dry weight and the effects of populations on disease development differed significantly ( $P < .05$ ) among cvs., between treatments and cvs. and among treatments within cvs. All populations caused approximately 100% disease development in 'VA 81' while a linear increase in disease development was noted with 'Coker 319'. Leaf number differed significantly ( $P < .05$ ) among treatments within cvs., but with inconsistent patterns.

HYBRIDOMAS PRODUCING MONOCLONAL ANTIBODIES AGAINST PRUNUS NECROTIC RINGSPOT (NRSV) AND APPLE MOSAIC (ApMV) VIRUSES. E. L. Halk, H. T. HSU, J. AEBIG, AND K. CHANG. American Type Culture Collection, 12301 Parklawn Dr., Rockville, MD 20852.

Hybridomas secreting monoclonal antibodies were produced by fusing spleen cells from BALB/c mice immunized with a mixture of NRSV and ApMV to cells of mouse myeloma lines NS1/1 or P3X63Ag8.643. Five hybridoma clones secreted antibodies against NRSV, three secreted antibodies against ApMV and one clone secreted antibody that reacted with both NRSV and ApMV. Antibody titers of cell culture media, measured by indirect ELISA, ranged from 1/25 to 1/4,000 whereas antibody titer of mouse ascites fluid ranged from 1/62,500 to 1/39,000,000. Antibodies from clone 70-C9 had ELISA titers  $>1/310,000$  against NRSV-G, Danish plum line pattern virus and ApMV-P and an ELISA titer of

$<1/2,500$  against two ApMV isolates from rose and one isolate from plum. Antibodies from clone 63-F10 reacted only with NRSV-G, and high titered antibodies from clones 74-F11 and 63-E10 reacted with all four ApMV isolates.

DIFFERENTIAL RESPONSE OF PEACH CELLS FROM BACTERIAL LEAF SPOT-RESISTANT AND -SUSCEPTIBLE VARIETIES TO CULTURE FILTRATES OF XANTHOMONAS CAMPESTRIS PV. PRUNI. F. Hammerschlag, USDA, ARS, Beltsville, MD 20705

A bioassay system using either peach mesophyll or leaf callus cells stained with merocyanine 540 (a probe of transmembrane electrical activity) was used to compare the response of cells from bacterial leaf spot-resistant and -susceptible cultivars to culture filtrates (CF) of *Xanthomonas campestris* pv. *pruni*. Fluorescence data revealed that significantly greater transmembrane electrical potential changes occurred when either leaf mesophyll or leaf callus cells from susceptible varieties were exposed to CF compared with cells from resistant varieties. This bioassay system may be useful in screening peach germplasm for resistance to *X. c.* pv. *pruni*.

WEEDS AS A SOURCE OF FUSARIUM OXYSPORUM PATHOGENIC ON SOYBEAN. J. B. Helbig and R. B. Carroll, USDA-APHIS, PPQ and Plant Science Dept., University of Delaware, Newark, DE 19711.

Fusarium blight of soybean has increased in the Delmarva region in recent years. A study was initiated in 1981 to evaluate weed hosts in soybean fields for their role in Fusarium blight. Weeds were collected monthly from planting to harvest at 20 locations. Isolations were made using V-8 and acidified potato-dextrose agar to assay for *Fusarium* sp. *Fusarium oxysporum* was isolated from 3 perennials including dogbane, horsenettle, milkweed; and from 11 annuals including, cocklebur, giant ragweed, ivyleaf morningglory, jimsonweed, lambsquarters, marehail, pigweed (redroot), ragweed, smartweed, tall morningglory, and velvetleaf. Pathogenicity tests using 21 isolates of *F. oxysporum* from these weeds were made in the greenhouse on a susceptible soybean cultivar. Disease ratings were highest for isolates from horsenettle, ivyleaf morningglory, ragweed and smartweed.

PENETRATION OF SCLEROTIA OF SCLEROTIUM ROLFII BY TRICHODERMA SPP. Y. Henis, P. B. Adams, G. C. Papavizas, and J. A. Lewis. University of Maryland, College Park, MD 20742 and USDA, Beltsville, MD 20705.

Penetration of sclerotia of *S. rolfsii* (Strain SR-3) by *Trichoderma* isolates Th-20, TR-40, and WT-6 was followed by a direct and an indirect approach. Sclerotia were immersed in an aqueous suspension of *Trichoderma* conidia ( $1.5 \times 10^7$  conidia/ml) and incubated on water agar. Periodically, sclerotia were removed from the agar, treated with 1% sodium hypochlorite solution for 2 min, washed and plated on a *Trichoderma* isolation medium. The three *Trichoderma* strains differed in their ability to penetrate the sclerotia, isolates Th-20 and WT-6 being more effective than TR-40. Hyphae of *Trichoderma* penetrated the rind and cortex and ramified throughout the medulla, followed by softening of the sclerotia and by lysis of both the medulla cells and the penetrating *Trichoderma* mycelium. Conidia of *Trichoderma* were produced only on the sclerotial surface, whereas chlamydospores of *Trichoderma* were produced inside the infected sclerotia. The implications of these observations to biological control of *S. rolfsii* will be discussed.

SUBCELLULAR MECHANISM OF ACTION OF STEROL BIOSYNTHESIS INHIBITING FUNGICIDES. Matthew J. Henry and Hugh D. Sisler, Dept. of Botany, Univ. of Maryland, College Park, MD 20742.

The effect of sterol biosynthesis inhibiting (SBI) fungicides on cytochrome P-450 type oxygenases in fungi was investigated. Demethylation of p-chloromethylalanine by *Ustilago maydis* sporidia was relatively insensitive to inhibition by SBI fungicides or metopirone. This demethylase system was shown genetically to differ from the sterol 14 $\alpha$ -demethylase system. The 14 $\alpha$ -hydroxylation of progesterone by *Curvularia lunata* was strongly inhibited by SBI fungicides, metopirone or isopropylphenylimidazole all of which inhibit sterol 14 $\alpha$ -demethylation. Progesterone 14 $\alpha$ -hydroxylation in cell free systems from *C. lunata* was inhibited by carbon monoxide and by low concentrations of miconazole which exhibited competitive inhibition. These data suggest that the primary action of SBI fungicides is competitive inhibition of sterol/steroid type cytochrome P-450 enzymes rather than interfering with the function of sterol carrier proteins or enzyme modulating phospholipids.

MYCELIUM PRODUCTION IN LIQUID MEDIA BY NORMAL AND HYPOVIRULENT ISOLATES OF *ENDOTHIA PARASITICA*. D. F. Hindal and T. G. Hagen, West Virginia University, Morgantown, WV 26506.

Culture morphology on agar media is one character used to distinguish normal (N) isolates of *Endothia parasitica* from hypovirulent (HV) ones. Tests were conducted to determine if mycelium production (dry weight) in any of 20 liquid media could be used to discriminate between four N and nine HV isolates. Generally, potato dextrose broth and a glucose and yeast extract medium supported best mycelium production among these isolates. Of the amino acids and inorganic nitrogen sources tested in a defined medium, arginine supported optimal mycelium formation. Cellobiose, glucose, fructose and pectin were the best carbon sources in the arginine medium. Mycelium production, which varied among isolates in each medium, did not discriminate between the N and HV isolates. Usually, however, those HV isolates that grow faster and are white or only slightly pigmented on a potato dextrose agar medium produce more mycelium than those HV isolates that grow slower and pigment.

IMPROVING RESISTANCE OF ALFALFA TO *PHYTOPHTHORA MEGASPERMA*. B. A. Hohrein, Univ. of Md., Botany Dept., College Park, Md. 20742/W-L Research, Inc., 7625 Brown Bridge Rd., Highland, Md. 20777; G. A. Bean, Univ. of Md., Botany Dept.; and J. H. Graham, W-L Research, Inc.

A rapid, inexpensive greenhouse technique was developed for screening alfalfa seedlings for resistance to *Phytophthora megasperma*. The technique involves growing seedlings in plastic tubs, inoculating after 12 days with *P. megasperma*, and selecting resistant plants 14 days after inoculation. In the winter of 1980-81, two experimental varieties, T22 and T27, were screened for resistance to *P. megasperma*. Resistant seedlings were selected, planted in separate isolation cages, and cross-pollinated with honey bees. In the fall of 1981, the resulting progenies were evaluated in comparison with the original populations of T22 and T27. Improvement in the level of resistance to *P. megasperma* was significant in both experimental varieties following one cycle of selection. Using this technique, an alfalfa variety can be developed at reduced cost and in a shorter period of time.

CYTOPATHOLOGICAL STRUCTURES ASSOCIATED WITH EARLY INFECTION OF SOYBEAN WITH SOYBEAN MOSAIC VIRUS. P. L. Hunst and S. A. Tolin. Dept. Plant Path. & Physiol., VPI & SU, Blacksburg, VA 24061.

Soybean mosaic virus (SMV) particles were first detected at 6 days after inoculation in both inoculated and adjacent noninoculated areas of soybean primary leaves, using serologically specific electron microscopy. The relative concentration of virions within the two areas increased in a sinusoidal pattern with time, which may be due either to an alteration of viral replication rate or to translocation relationships within the leaf. Pinwheels were present in all cell types of the inoculated areas at 6 days, and were frequently attached to the plasmalemma and associated with plasmodesmata of the cell wall. Endoplasmic reticulum and small vesicles occurred near and within the pinwheels. Initial pinwheel formation appeared to occur at the plasmalemma upon movement of SMV into the cells. The presence of pinwheels within paraveinal mesophyll cells suggested that these cells were involved in viral translocation.

ENZYME-LINKED IMMUNOSORBENT ASSAY (ELISA) OF PLANT VIRUSES USING PROTEIN A-ENZYME CONJUGATE AND F(ab')<sub>2</sub> OF IMMUNOGLOBULIN G (IgG). H. T. Hsu and Joan Aebig, American Type Culture Collection, 12301 Parklawn Drive, Rockville, Md. 20852

In ELISA tests protein A-enzyme conjugate and F(ab')<sub>2</sub> fragments of IgG were successfully employed in detecting tobacco ringspot, tomato ringspot, apple mosaic, prune dwarf, *Prunus* necrotic ringspot, tobacco etch, potato A and potato X viruses. The F(ab')<sub>2</sub> was obtained by incubating 5 mg protein A-sepharose purified rabbit IgG with 50 µg pepsin in 0.1 M Na-acetate (pH4.5) at 37C for 20 hr. After adjusting to pH8.0, the mixture was passed through protein A-sepharose column. The F(ab')<sub>2</sub> was eluted with 0.02 M Na-phosphate (pH7.3) and concentrated by (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> precipitation. For virus detection, polystyrene or polyvinyl chloride microtiter plates were coated with F(ab')<sub>2</sub> in 0.05 M bicarbonate (pH9.8). Subsequently, virus samples, diluted rabbit antisera, protein A-enzyme conjugate and substrate were added. The method employs a universal protein A-enzyme conjugate in detecting different viruses. This eliminates the need for preparing individual antibody-enzyme conjugates for every virus to be assayed.

NEMATOCIDES TO CONTROL A TOBACCO CYST NEMATODE (*GLOBODERA SOLANACEARUM*) IN FLUE-CURED TOBACCO. D. A. Komm and J. J. Reilly, So. Piedmont Center, VPI & SU, Blackstone, VA 23824

Carbofuran, ethoprop, ethylene dibromide, ethylene dibromide + phenamiphos, phenamiphos, phenamiphos + carbofuran, and phenamiphos + fensulfothian were applied preplant at 3 locations at 6.7, 9.0, 4.5, 4.5 + 6.7, 6.7, 4.5 + 4.5, 3.4 + 6.7 kg a.i./ha, respectively. The effect of these nematocides on the control of a tobacco cyst nematode (TCN) (*Globodera solanacearum*) in flue-cured tobacco was determined by measuring plant and root vigor, yields, plant height, and stalk diameter. Treatments containing phenamiphos increased yields and certain other plant characteristics at locations 1 and 2. However, no yield increases occurred with single treatments of carbofuran and ethoprop. A yield increase occurred with ethylene dibromide at location 1. The effects of the nematocides on the control of TCN was related to the initial TCN egg population.

INHERITANCE OF THE PIONNOTAL VARIANTS IN *FUSARIUM LATERITIUM*. E.B. Lawrence, P.E. Nelson, and T.A. Toussoun, Department of Plant Pathology, The Pennsylvania State University, University Park, PA 16802.

Variation in colony morphology is common in the genus *Fusarium*. The most commonly encountered cultural variants in *Fusarium lateritium* are pionnotal, where conidia are produced in pionnotes rather than sporodochia. Sporodochial and pionnotal isolates of *F. lateritium* were crossed in all possible combinations and the progeny rated for colony morphology. Both random ascospores and unordered tetrads were studied. Production of pionnotes vs. sporodochia is controlled by two linked genes. Only one arrangement of alleles results in sporodochial formation. Genes controlling growth rate, color, and degree of mycelium are on the same linkage group as those controlling pionnote formation. Epistasis is evident between genes controlling pionnote formation and aerial mycelium production; pionnotal isolates have reduced amounts of aerial mycelium. A leaf spot assay run in the greenhouse on *Amaranthus tricolor* showed both sporodochial and pionnotal isolates to be pathogenic.

QUANTIFICATION OF CARNATION ETCHED RING VIRUS BY IMMUNOSORBENT ELECTRON MICROSCOPY. R. H. LAWSON, USDA, Beltsville, MD 20705

Immunosorbent electron microscopy (ISEM) is a sensitive procedure that can be used to quantitatively detect purified carnation etched ring virus (CERV). CERV antiserum prepared to purified virus with a microprecipitin titer endpoint of 1:256 was diluted 1:100 and incubated for 30 min on carbon evaporated, Formvar-coated 400 mesh grids. Grids were also coated with antiserum prepared against CERV inclusion bodies. Purified CERV was incubated on the grids for 1.5 hr, washed with 0.01 M PO<sub>4</sub> buffer, pH 7.0, and stained with neutral 2% PTA or 2% aqueous uranyl acetate. The number of CERV particles immobilized on the antiserum coated grids was directly proportional to the concentration of virus in the range of 2-10 µg/ml. Incubation of protein A (0.1 mg/ml for 10 min), followed by antiserum treatment for 30 min increased virion attachment more than 5-fold at each antigen concentration over a dilution range of 2-10 µg/ml. ISEM can be used to measure the release of CERV particles from partially purified inclusion bodies and may be used to detect CERV in commercial carnations.

FACTORS AFFECTING CHLAMYDOSPORE FORMATION BY *TRICHODERMA* SPP. ON LIQUID AND SOLID MEDIA. J. A. Lewis and G. C. Papavizas. USDA, SE-ARS, Beltsville, Maryland 20705.

Chlamydospores of *Trichoderma* spp. were formed in various amounts during growth of the antagonist in liquid and on solid media of different compositions. In submerged liquid cultures, the greatest number of chlamydospores of *T. hamatum* and *T. viride* (10.4 and 5.9 x 10<sup>8</sup>/g of mycelium, respectively) was formed in molasses-corn steep liquor medium and the smallest in glucose-tartrate medium. Production of chlamydospores in two other media was intermediate. Chlamydospore production by *T. harzianum* was low (0.1-1.1 x 10<sup>8</sup>/g) in all four media. Chlamydospore production was not affected by the initial and final pH of media or whether cultures were shaken or stationary. On a wide variety of solid media, chlamydospore development depended on the liquid used in the system. Corn cobs or bran moistened with water or sucrose-nitrate provided the best substrate for chlamydospore production. Chlamydospores from all preparations were viable and germinated (70%) on V-8 juice agar + antibiotics. The ecological aspects of chlamydospore production are discussed.

BIOCONTROL OF FUSARIUM WILT OF GREENHOUSE GROWN CHRYSANTHEMUMS. J. C. Locke, J. J. Marois, and G. C. Papavizas, USDA, Beltsville, Maryland 20705.

Twenty-two isolates of soil fungi, mainly strains of *Trichoderma* spp., were screened for their ability to control Fusarium wilt of florists' chrysanthemum (*Chrysanthemum morifolium*) caused by *Fusarium oxysporum* f. sp. *chrysanthemi*. Evaluation was done in raised bench plots in the greenhouse on vegetative stock plants. Three isolates of *T. viride* reduced disease more effectively than the other 19 isolates (<15% compared to 76% in the control). These isolates were retested alone and in combination with each other in replicated bench plots. Isolates T-1, T-1R4, and T-1R9 gave comparable protection. T-1R4 and T-1R9 are UV light induced biotypes of T-1 with tolerance to benomyl, a critical fungicide in greenhouse management. Because of the tolerance of T-1R9 to benomyl, and its greater capacity for growth and sporulation than T-1R4, it was utilized in another test to evaluate its efficacy compared to a benomyl, high-lime, all-nitrate-nitrogen system. Initial results showed that use of the T-1R9 resulted in control equal to the integrated system

#### DEVELOPMENT OF SEPTORIA CANCKER ON HYBRID POPLAR.

R. Long, T.W. Bowersox, and W. Merrill. School of Forest Resources and Dept. of Plant Pathology, The Pennsylvania State University, University Park, PA. 16802

*Populus* hybrids in a densely spaced, coppiced plantation developed cankers caused by *Septoria musiva* Pk. during 1981. Four plots, each containing three different hybrid clones (NE-388= *P. maximowiczii* X *P. trichocarpa*; NE-50= *P. maximowiczii* X *P. 'Berolinensis'*; NE-252= *P. 'Angulata'* X *P. trichocarpa*), were treated with captafol (difolatan 4F) at the rate of 5.6 kg. a.i. in 935.1 liters of water per hectare. Sequential sprays were applied to provide protection for 4 months, 3 months, 2 months, and 1 month. A fifth plot served as a control. *Septoria* cankers developed throughout the growing season, and usually were initiated at the bases of petioles or buds, or in lenticels. Captafol was ineffective in reducing canker incidence at severe inoculum pressures. Clone NE-50 developed significantly fewer cankers than NE-388 and NE-252 in severely infected plots.

ECOLOGICAL STUDY OF PSEUDOMONAS CEPACIA IN SOIL USING A NEW SELECTIVE MEDIUM. R. D. Lumsden, USDA, ARS, Beltsville, MD 20705; G. Friás-T., CSAT, Cardenas, Mexico; M. Sasser, Univ. of Delaware, Newark, 19711.

*Pseudomonas cepacia* is antagonistic to *Pythium* in vitro and protects cucumber seedlings from infection by *P. aphanidermatum* in natural soil. The population in soil of *P. cepacia*, assayed with a selective medium, increased from 0.7 to 55.4 x 10<sup>4</sup> colony-forming units (CFU)/g soil one week after adding in nutrient solution to a sandy loam soil, but then declined. Drying the soil prevented detection of *P. cepacia*. Survival in soil was better at pH 5.6 than 6.6, at 20 C than 30 C, and at -0.3 bars (high moisture) than at 5.0 bars (low moisture). *P. cepacia* numbers increased rapidly in soil on non-sterile, dried organic matter (1% w/w), increasing the most on alfalfa tissues (from 0.23 to 3780 x 10<sup>4</sup> CFU/g), followed by corn, cotton and bean tissues, but not on composted sludge or cacao hulls. The numbers of *P. cepacia* were high the longest (1 mo.) on alfalfa and bean tissues. Native populations of *P. cepacia* in the soil increased from non-detectable to 4.5 x 10<sup>4</sup>, with addition of corn, bean, and cotton to soil. These results show the dynamic behavior of *P. cepacia* in soil, which may reflect on its survival and biocontrol abilities.

BIOLOGICAL CONTROL OF VERTICILLIUM WILT OF EGGPLANT. J. J. Marois, S. A. Johnston, M. T. Dunn, and G. C. Papavizas. USDA, Beltsville, MD 20705; Rutgers R & D Center, Bridgeton, NJ 08302; and University of Maryland, College Park, MD 20472.

Thirty-four soil fungi were screened in the greenhouse for their ability to control Verticillium wilt of eggplant (*Verticillium dahliae*). Eggplants (cv. Classic and Special Highbush) were seeded in 10-cm-diam pots containing peat and vermiculite (1:1, w/w). The fungi were grown for 2 wk on potato-dextrose agar, conidia were dislodged from the cultures with a cotton-tipped applicator, and 10 ml of suspensions (10<sup>5</sup> conidia/ml) were added to the surface of the pots at 0, 3, 6, and 8 wk after planting. The seedlings together with the potting mixtures were placed in 20-cm-diam pots containing pathogen-infested soil (pH 5.8). Six fungi (*Aspergillus lutaceus*, *Gliocladium virens*, *Paecilomyces lilacinus*, *Talaromyces flavus*, *Trichoderma viride*), which reduced wilt in the greenhouse, were tested in two fields by applying the antagonists to seedlings in pots as before. *Talaromyces flavus* reduced disease by 76 and 67% and increased yield by 19 and 54% (weight) and 22 and 71% (number) in fields at Beltsville, MD and Bridgeton, NJ, respectively. The other five antagonists were less effective.

THE USE OF 2-(4-THIAZOYL)BENZIMIDAZOLE HYPOPHOSPHITE FOR THE DIFFERENTIATION OF *ENDOTHIA GYROSA* FROM *E. PARASITICA*. J.A. Micales and R.J. Stipes. Dept. Plant Pathol. and Physiol., Virginia Tech, Blacksburg, VA 24061.

The systemic fungicide 2-(4-thiazoyl)benzimidazole hypophosphite (Arbotect<sup>®</sup>-20,S) was used successfully as an agar-medium amendment to differentiate *Endothia gyrosa* from *E. parasitica*. The compound was incorporated into 0.5% glucose - 0.1% yeast extract - 1.5% agar, and the radial growth of ten specimen-vouchered isolates of each species was measured after 15 days. At 0.5 µg/ml, the percent growth inhibition of *E. gyrosa* varied between 18-50%, while the growth of *E. parasitica* was not affected. At 1.0 µg/ml, the percent growth inhibition of *E. gyrosa* ranged between 55-80%, while the growth of *E. parasitica* was totally suppressed. The differential sensitivities to this compound can be used as a simple and effective means of separating the two species.

RECOVERY OF RHIZOBIUM AND VESICULAR-ARBUSCULAR MYCORRHIZAL FUNGI FROM RED CLOVER IN ACID MINE SOILS. J. B. Morton, S. E. Wright, West Virginia University, Morgantown, W.V. 26506, and R. J. Wright, USDA/ARS at Beckley, W.V. 25801.

Red clover may be a useful forage legume for initial re-vegetation of strip mine sites if inoculated with *Rhizobium* and VA mycorrhizal fungi adapted to acid soil environments. Abandoned or partially reclaimed strip mine sites near Elkins, W.V. were sporadically colonized by red clover, the roots of which were nodulated and strongly mycorrhizal (as high as 91% infection). Rhizosphere soils from these plants with pH levels between 3.5-4.5 and extractable P levels between 2-42 ppm were mixed 1:1 with sand, potted, and re-seeded with Kenstar red clover. Initial seedling mortality ranged from 50-83%. After three months, surviving plants were effectively nodulated and mycorrhizal. Wide variations in incidence and intensity of mycorrhizal infection were observed among soils, which may be attributed either to endophyte efficiency or to inoculum density. Further characterization of interactions between symbionts, soil chemical properties, and red clover genotypes are in progress.

EFFECTS OF OZONE ON FOLIAR SYMPTOM EXPRESSION AND CUMULATIVE HEIGHT GROWTH OF SEVEN FOREST TREE SPECIES NATIVE TO THE SHENANDOAH NATIONAL PARK, VIRGINIA. James E. Nellesen and John M. Skelly, Dept. of Plant Path. & Phys., VPI & SU, Blacksburg, VA 24061

Virginia pine, eastern white pine, table mountain pine, eastern hemlock, black locust, tulip poplar, green ash, and sweetgum were rated for ozone effects on foliage (1980-81) and three-year cumulative height growth (1979-81) in an open top chamber study at Big Meadows, Shenandoah National Park. Tulip poplar, green ash, and black locust exhibited ozone stipple on the upper leaf surface in 1981 in non-filtered chambers and open plots. Based on foliar symptom expression, sweetgum, green ash, and tulip poplar were rated as most sensitive, black locust as intermediate, and other species as tolerant. The difference in average cumulative height growth between filtered chambers and open plots indicated that in both 1980 and 1981, black locust, green ash, and Virginia pine were most sensitive.

ISOLATION AND ENUMERATION OF PROPAGULES OF LAETISARIA ARVALIS FROM SOIL. G. C. Papavizas, B. B. Morris, and J. J. Marois. Soilborne Diseases Laboratory, USDA, Beltsville, MD 20705.

A selective medium was developed for the direct isolation and enumeration of the antagonist *Laetisaria arvalis* (Corticium) from soil. The medium contained per L 15 g agar, 25 mg chlorotetracycline HCl, and 100 mg streptomycin S04. The antibiotics were added to the medium after autoclaving. The best combination of other antifungal ingredients added to the basal medium were 25 mg active ingredient/L of thiabendazole (TBZ) and 5.0 ml of Hunt and Cobb's solution (Can. J. Bot. 49:2064-2065, 1971) used for isolation of wood-rotting basidiomycetes. This solution contained 50 ml ethanol, 50 ml water, 1 g phenol, 0.32 g benomyl (50 W), and 0.21 g Botran (75 W). Benomyl and TBZ inhibited growth of *Rhizoctonia solani*, but not that of *L. arvalis*. Recovery of the antagonist from artificially infested soil with the tableteb colonization method and with a multiple pellet soil sampler was between 80 and 90% of the inoculum added. Numbers of propagules in naturally infested soils ranged from zero to more than 20/g of soil. No significant difference was observed on the medium from soils that had the pathogen and the antagonist.

SAMPLING FOR APPLE POWDERY MILDEW IN EXPERIMENTAL PLOTS. Priscilla J. Parish and K. D. Hickey, Department of Plant



Pathology, The Pennsylvania State University, Fruit Research Laboratory, Biglerville, PA 17307.

Accurate and efficient assessment of apple powdery mildew (*Podosphaera leucotricha*) on leaves is necessary for evaluating fungicide efficacy and pathogen spread. In a field study on Rome Beauty cultivar, under uniform inoculum pressure no significant difference in mildew incidence was found between the upper and lower sections or among quadrants of the trees. The minimum mean number of terminals per tree required to assess disease incidence was four when less than 15% of the leaves were infected and eight when 15% to 40% of the leaves were infected. Several combinations of terminal number per tree and number of replicate trees produced the same sample estimates.

AN ASSESSMENT OF PEANUT CROP LOSS IN VIRGINIA DUE TO CYLINDROCLADIUM BLACK ROT IN 1979. P. M. Phipps, D. M. Porter, and N. L. Powell. Tidewater Research Center, VPI&SU, and USDA-ARS, Suffolk, Virginia. 23437.

Aerial infrared photographs were taken of the peanut acreage in Virginia just prior to harvest in 1979. Thirteen flight lines (5.7 x 103 km/line) composed of about 24 frames each were flown at an altitude of 3658 m. Photo-interpretations, based on the characteristic spectral/spatial appearance of *Cylindrocladium black rot* (CBR), were made on alternate flight lines. Frames were uniformly divided with a transparent grid and fields within a randomly selected square assessed for CBR symptoms. A total of 1587 ha or 3.8% of the total acreage in Virginia was measured with a planimeter, and ca 11 ha or 0.68% of the area exhibited CBR symptoms. CBR was detected in 18.9% of the 338 fields examined. Photo-interpretations and previous assessments of yield loss in diseased fields indicated that CBR affected 278 ha of the 1979 crop, caused pod losses of 381,699 kg, and reduced farm income ca \$180,000 in Virginia.

TOMATO RINGSPOT VIRUS ASSOCIATED WITH LITTLE GRAPE DISEASE OF VIDAL-256 GRAPEVINES. E. V. Podleckis and M. K. Corbett, Botany Dept., Univ. Maryland, College Park, MD 20742.

French hybrid, Vidal-256, grapevines in southern Maryland had fruit clusters of normal set but individual berries were about one-third normal size. Vines and foliage exhibited no symptoms of viral infection. A wide range of herbaceous hosts were infected by mechanical inoculation with extracts from young grape leaves in 2.5% aqueous nicotine. Plants of *Gomphrena globosa* served as virus source and assay hosts. Density gradient centrifugation of partially purified preparations gave two light-scattering zones of 60 and 125S. Both zones contained icosahedral particles, but infectivity was associated only with the 125S zone. In gel double-diffusion tests, precipitin zones of serological identity occurred with the apple and apricot strains of tomato ringspot virus. Electron microscopy of infected grapevine tissue indicated the presence of icosahedral particles in vesicles and plasmodesmata. In addition, the tissue also contained flexuous rod viral-like particles.

EFFECT OF BACTERIAL ANTAGONISTS ON FUNGAL ROTS OF DECIDUOUS FRUITS. P. L. Pusey and C. L. Wilson, USDA Appalachian Fruit Research Station, Kearneysville, WV 25430.

Spore-forming bacteria shown to inhibit fungal growth in culture were studied for potential biocontrol of fruit rots. Harvested fruits were wounded, sprayed with bacterial suspensions, dried, and then spray-inoculated with spore suspensions of rot fungi. Peaches, nectarines, apricots, plums, and cherries were treated with bacteria and inoculated with *Monilinia fructicola*. Three of four *Bacillus* isolates tested significantly reduced rot on fruits with a waxy surface but not on pubescent ones. Apples and pears were inoculated with *M. fructicola*, *Physalospora obtusa*, or *Penicillium expansum*. Test bacteria included the HD-1 strain of *Bacillus thuringiensis* (Bt), which is used commercially for insect control. Commercial formulations of Bt varied in ability to reduce rot. It was also found that all bacterial suspensions in nutrient broth were much more effective in reducing rot than suspensions in water. Apparently alterations in the chemical or nutritional milieu of the bacteria can enhance their biocontrol capability.

THE EFFECT OF PEACH TWIG EXTRACTS ON GERMINATION OF *CYTOSPORA LEUCOSTOMA*. Ralph Scorza and P. L. Pusey, USDA Appalachian Fruit Research Station, Kearneysville, WV 25430.

Dormant twigs from 10 peach cultivars were chopped and frozen in water. The water-sap mixture was filter-sterilized and tested for effect on germination of *Cytospora leucostoma* pycnidiospores. Significant differences in % germination were recorded. The least germination occurred in extracts from a cultivar rated in the field as relatively resistant and greater germination was observed in extracts from cultivars rated as more susceptible. Resistance to *Cytospora* infection in peach may involve, among other mechanisms, the presence of substances that inhibit or the absence of substances that support spore germination. Twig extract tests may possibly be used as an indicator of this mode of resistance.

CUMULATIVE INFLUENCE OF OZONE DOSE ON BIOMASS DEVELOPMENT OF NATIVE VEGETATION IN THE SHENANDOAH NATIONAL PARK. J. M. Skelly and J. E. Nellesen, Dept. of Plant Pathology and Physiology, VPI & SU, Blacksburg, VA 24061.

The effect of cumulative seasonal ozone dose was measured on native vegetation grown in charcoal filtered air and non-filtered air open top chambers and open plots in the Shenandoah National Park from April 1979 through September 1981. Foliar biomass was harvested twice each year, at approximately mid-season and in the fall. In each year, biomass was greatest in the filtered air chambers. The total 3 year cumulative weight for the treatments was significantly different ( $P < 0.01$ ) with 40.3, 31.9, and 25.9 kg for the filtered, non-filtered, and open plots, respectively. However, a significant interaction ( $P < 0.01$ ) was observed between harvest date and year. Significant differences between treatments occurred for both harvests in 1979, the first harvest of 1980, and for neither harvest of 1981. The lack of reduction in the 1981 biomass may have been related to the lower dose of ozone which was only 55% of that monitored in 1979 or 1980.

PATHOGENICITY OF A PIERCE'S DISEASE (PD)-LIKE BACTERIUM CULTURED FROM LEAF SCORCH AFFECTED SYCAMORES. J. Sherald, S. Hearon, S. Kostka, D. Morgan. Nat. Park Serv., Wash., DC 20242; USDA, Beltsville, MD 20705; Dept. Plant Path., U. of Mass., Amherst, MA 01003; Dept. Hort., Texas A & M, Dallas, TX 75252

Bacteria resembling the PD organism occurred in sycamores with leaf scorching in Wash., DC, Dallas, TX, and New Orleans, LA. Bacteria were cultured from 24/25 diseased and 1/17 symptomless trees by incubating wood chips in S-8 or modified PD-2 media at 28 C. Subcultures, maintained on PD-4, fluoresced in IFAS tests using anti-PD and elm scorch organism sera. Xylem-limited, rippled-walled bacteria were in ultrathin sections of leaf veins from diseased, but not symptomless trees from Washington and Dallas. Pipettes containing 10 ml aliquots of one isolate ( $10^8$  cells/ml buffer) or buffer only were attached to the main roots of 20 sycamore seedlings in June 1981. In October bacteria were observed by phase contrast or electron microscopy in concentrated stem extracts of 7 seedlings with scorch-like symptoms and in 4 bacteria-inoculated, symptomless seedlings. Bacteria were cultured from the 7 symptomatic and 2 of 4 bacteria-inoculated, symptomless plants, but not from the 6 controls examined.

DISTRIBUTION OF *PSEUDOMONAS CEPACIA*, A BROAD-SPECTRUM ANTAGONIST TO PLANT PATHOGENS, IN NORTH CAROLINA. Harvey W. Spurr, Jr. and Myron Sasser. North Carolina State University, Oxford USDA-ARS Laboratory, Oxford, NC 27565 and Dept. Plant Science, University of Delaware, Newark, DE 19711.

A medium selective for *P. cepacia* (Pc) developed by M. Sasser makes it possible to identify and count Pc in mixed microbial populations from soil extracts or plants. Soil and mature tobacco roots were sampled in coastal plain, piedmont and mountain areas of North Carolina. Water extracts were prepared and aliquots spread on agar media. After incubation, bacterial colonies were counted. Total bacteria extracted from soil were  $10^6$ - $10^7$  colony forming units (CFU)/gm dry wt; Pc were  $10^3$ - $10^5$  CFU, .4-2.6% of the population. Total bacteria on tobacco roots were  $10^3$ - $10^7$  CFU/cm<sup>2</sup> root surface area; Pc was  $10^2$ - $10^4$  CFU or .1-1% of the population. All soil and root samples contained Pc. Pure Pc cultures were obtained from selective medium and several were antagonists to one or more tobacco pathogens.

REACTIONS OF SNAP BEAN CULTIVARS TO UNITED STATES COLLECTIONS OF *UROMYCES PHASEOLI*. J. R. Stavely, USDA, ARS, Applied Plant Pathology Laboratory, Beltsville, MD 20705.

At Beltsville at least 20 pathogenic races of *U. phaseoli* are

identifiable from collections and cultures of rust from snap and dry beans from six states. Rust reactions of 75 snap bean cultivars have been determined using these collections and cultures. Based upon pustule size, most of these cultivars, such as BBL 47, BBL 274, Blue Crop, Early Gallatin, Eagle, Gator Green, Tendercrop, and Tenderette are moderately susceptible to most races. Breeders developing new cultivars should avoid losing this slight resistance. These and most of the other popular cultivars are very susceptible (pustules larger than 800  $\mu$ m) only to rust collection 16. Collection 16 is similar to race 32 and to other collections commonly obtained from snap beans in the Eastern states. The Kentucky Wonder cultivars, some newer or minor cultivars, and most dry beans are immune or highly resistant to 16. Resistance to 16 should be given high priority in breeding rust resistant snap beans.

CULTURAL AND MORPHOLOGICAL STUDIES OF *FABRAEA MACULATA* IN VITRO. H. F. Stroo and T. van der Zwet, USDA, Appalachian Fruit Research Station, Kearneysville, WV 25430.

Studies of growth and conidial production by *F. maculata* colonies were initiated to understand host-pathogen interactions in *Fabraea* leaf spot of pear and to increase spore production for use in a seedling screening program. Using a basal glucose-casein hydrolysate medium, a growth requirement for thiamine was discovered. When thiamine was added to potato dextrose agar, growth rate doubled and conidial production increased ten-fold. Casein hydrolysate further increased sporulation but only when thiamine was also present. High light intensity (5000 lux) inhibited growth and germination; maximum spore production occurred at 100 lux. Conidia from colonies maintained in culture for 3 months were less virulent than conidia kept on diseased leaves at 2 C. The loss in virulence was associated with an increase in germination and growth in culture. Germination in culture was stimulated by glucose and minerals, but was inhibited by ammonium nitrate and by pear leaf extracts.

A QUALITATIVE AND QUANTITATIVE EVALUATION OF MAIZE LINES FOR REACTION TO COLLETOTRICHUM GRAMINICOLA. Kelly L. Tomer and James A. Hawk, Plant Science Department, Univ. of Delaware, Newark, Delaware 19711.

Maize inbred lines were evaluated under greenhouse conditions for reaction to *Colletotrichum graminicola*. A disease rating based on lesion type and percent lesion coverage was performed nine days after inoculation. Data were collected on fresh weight, total chlorophyll content, chlorophyll a content, and protein content. No significant differences in protein content were detected between inoculated and uninoculated seedlings. However, analysis of inbred lines showed significant differences in protein content. The interaction between inbred and inoculation was found to have significant effects on fresh weight, total chlorophyll content, and chlorophyll a content.

ENVIRONMENTAL EFFECTS ON THE LENGTH OF LATENT PERIOD FOR APPLE SCAB. J. R. Tomerlin and A. L. Jones. USDA, ARS, PGGI, Field Crops Lab, BARC-W, Beltsville, MD 20705, and Dept. of Botany and Plant Pathology, Michigan State University, East Lansing, MI 58824.

The apple scab fungus had a longer latent period on McIntosh apple seedlings maintained at 10 C than at 20 C. In temperature alternating experiments, seedlings kept at 10 C for 5 days before being transferred to 20 C and seedlings kept at 20 C for 5 days before being transferred to 10 C developed symptoms at the same time as seedlings kept continuously at 20 C. Symptoms did not develop on seedlings maintained at a temperature of 26 C from 6 days after inoculation onward. Symptoms developed on inoculated seedlings maintained at low relative humidity (RH) for up to 24 days before transfer to high RH. Seedlings transferred to low RH after maintenance at high RH for 6 days or less did not develop symptoms. Temperature and relative humidity data were used to develop a regression model to predict length of latent period.

HAUSTORIA OF PERONOSPORA TABACINA IN TOBACCO. R. N. Trigiano, C. G. Van Dyke and H. W. Spurr, Jr. Department of Plant Pathology and ARS, USDA, North Carolina State University, Raleigh, NC 27650.

Light microscopy, SEM and TEM were used to examine the devel-

opment of haustoria in tobacco by the blue-mold fungus *P. tabacina* Adam. Electron-lucent, callose-like appositions formed between the host plasmalemma and the host wall, prior to haustorial penetration. An electron-opaque penetration matrix developed between the apposition and the host cell wall. The haustorial mother cell (HMC) wall consisted of two layers. The haustorial wall was also two-layered, but primarily composed of and continuous with the inner wall layer of the HMC. All haustoria were encased. All encasements were thickened at the proximal regions of haustoria but were thinner along the distal portions. Vesicles occasionally were observed attached to the host side of the host plasmalemma and might contribute to the deposition of the encasement material. Haustorial morphology was variable.

STIMULATION OF *Puccinia obtegens* TELIOSPORE GERMINATION. S. K. Turner, A. Kwiatkowski and P. K. Fay. Department of Plant Pathology, and Plant and Soil Science, Montana State University, Bozeman, MT 59717.

A brachycyclic autoecious rust, *Puccinia obtegens*, is a potential biological control agent of Canada thistle (*Cirsium arvense*). Since successful infection and spread by urediospores can be limited by unfavorable environment, the use of teliospores to initiate root infection was studied. However, teliospore germination percentage is low and erratic, so an investigation was conducted to determine possible ways of enhancing spore germination. Canada thistle root extracts stimulated germination when flooded over teliospores placed on water agar. The optimum incubation conditions consisted of complete darkness at 21 C for at least 96 hours. Teliospore germination also increased after several months of cold storage. Infection of Canada thistle plants has been attained by treating teliospores in host plant root extracts and subjecting inoculated plants to favorable environmental conditions.

LONG DISTANCE DISSEMINATION OF ERWINIA AMYLOVORA AS RESIDENT BACTERIA IN APPARENTLY HEALTHY PEAR BUDWOOD. T. van der Zwet, R. L. Bell, and H. F. Stroo, USDA, Appalachian Fruit Research Station, Kearneysville, WV 25430.

Standard pear (*Pyrus communis*) rootstocks in the nursery of this station were budded in August 1980 with scionwood from numerous pear varieties collected at Beltsville, Maryland. In July 1981, shoot tips of 2 'Starkrimson' trees showed fire blight (*Erwinia amylovora*). These were the very first trees with blight symptoms. The nearest commercial orchard trees with blight in previous years were at least 2 Km away. Bud chips, collected in August 1981 from the original source trees and plated on Miller-Schroth selective medium, revealed the presence of *E. amylovora* inside the apparently healthy shoots. Of 18 positive identifications in culture, 6 isolates proved virulent in pathogenicity tests on immature pear slices. These were recovered from bud primordia and from associated woody tissue. Such internal resident bacteria of *E. amylovora* may play a small but highly significant role in the dissemination of the fire blight pathogen.

A STEROL C-14 DEMETHYLASE DEFICIENT MUTANT OF *USTILAGO MAYDIS*. R. C. Walsh and H. D. Sisler, University of Maryland, College Park, Maryland 20742.

An ergosterol deficient mutant of *Ustilago maydis* was compared to the wild type in regard to morphology, growth rate, lipid content, and response to the ergosterol biosynthesis inhibitors fenarimol, miconazole, etaconazole, and azasterol A25822B. Morphology of mutant sporidia was abnormal and resembled that of fenarimol treated wild type sporidia. Doubling time of the mutant was 6.3 hr compared to 2.5 hr for the wild type. The sterol and lipid patterns of the mutant were similar to those of wild type treated with sterol C-14 demethylation inhibitors. Growth of mutant sporidia was uninhibited by low concentrations of fenarimol, etaconazole, miconazole, and azasterol that inhibit growth of wild type sporidia. These findings suggest that the mutant is deficient in sterol C-14 demethylase activity and support the contention that inhibition of sterol C-14 demethylation in *U. maydis* is the primary mode of toxicity of fenarimol, miconazole, and etaconazole.

EFFECT OF METALAXYL ON PHYTOPHTHORA ROOT ROT, AND YIELD OF ALFALFA. R. E. WELTY and C. Lee Campbell, U. S. Dept., Agri., ARS, Oxford, NC 27565 and Dept. Plant Pathology, North Carolina State Univ., Raleigh 27650.



Alfalfa (*Medicago sativa*) was treated with metalaxyl at 0, 0.58, 1.13, and 2.28 kg (a. i.)/ha in 3 experiments in Wake and Rowan Counties, NC. Plants in one experiment were inoculated with mycelium of *Phytophthora megasperma* f. sp. *medicaginis*; natural inoculum was also present in all plots. Five destructive samples were taken from each plot between 11/80 and 7/81 and evaluated for plant number and weight per 0.3 m of row and for root rot. In Rowan Co., metalaxyl treatment had no significant effect ( $P < 0.05$ ) on seedling number and plant weight. Root rot in metalaxyl-treated plots was significantly lower in noninoculated plots in May and June and in inoculated plots in May. Yields were significantly greater in inoculated and non-inoculated metalaxyl-treated plots than in controls in June, but were similar to controls in May and July. In Wake Co., metalaxyl treatment had no significant effect on seedling number, plant weight, root rot or dry matter yields.

**SUSCEPTIBILITY OF SPIROPLASMAS TO HEAVY-METAL SALTS IN VITRO.** S. C. Whitmore, J. F. Rissler, Dept. of Botany, Univ. of Maryland, College Park, MD 20742, and R. E. Davis, Plant Virology Lab, PPI, SEA-USDA, Beltsville, MD 20705

Selection of heavy-metal salt (HMS) resistant mutants of spiroplasmas requires knowledge of HMS susceptibility. We determined this susceptibility for 6 spiroplasma strains from 4 serogroups. Minimal inhibitory concentration ranges and minimal biocidal concentration ranges were determined for 8 heavy-metal salts in a broth, tube-dilution test. The strains were more susceptible to mercuric chloride and silver nitrate than to cadmium sulfate, copper sulfate, cobalt chloride, lead nitrate, nickel chloride, or zinc sulfate. Differences in susceptibility among the strains were generally slight. For example, *Spiroplasma citri* strains Maroc R8A2 and C189 were only slightly more susceptible to the HMS than flower spiroplasma strains. Mercuric chloride and silver nitrate will be used in attempts to select resistant mutants.

**INTERACTION OF ENDOMYCORRHIZAE AND THIELAVIOPSIS BASICOLA BLACK ROOT DISEASE OF JAPANESE HOLLY.** R. L. Wick and L. D. Moore, Dept. of Plant Path. & Phys., VPI & SU, Blacksburg, VA 24061.

An histological study was undertaken to determine if *Glomus mosseae* or a mixed population (MP) of holly rhizosphere endomycorrhizal fungi altered the host-parasite interactions of *T. basicola* black root disease of Japanese holly. Mycorrhizal and non-mycorrhizal plants were inoculated with *T. basicola* and examined over time. Periderm formation in response to *T. basicola* colonization was considered a measure of resistance. *Thielaviopsis basicola* was not excluded from host cells previously colonized by *G. mosseae* or MP fungi. No correlation was found between incidence of mycorrhizae and incidence of periderm formation in individual roots. However, when incidence of periderm formation in roots was averaged for individual plants, the *G. mosseae*, but not the MP treatment, was found to compartmentalize *T. basicola* at a faster rate than the non-mycorrhizal control. This suggested that the *G. mosseae*-induced increased resistance was due to enhanced host vigor as opposed to a direct effect by the mycorrhizal fungi.

**SPREAD OF PHYTOPHTHORA CINNAMOMI AND MORTIERELLA ALPINA IN MODIFIED PINE BARK MEDIA.** W. H. Wills and R. C. Lambe, Dept. of Plant Pathology and Physiology, VPI & SU, Blacksburg, VA 24061.

*Mortierella alpina* (Ma) has been shown earlier to inhibit growth of *Phytophthora cinnamomi* (Pc) *in vitro* and to prevent development of root-rot of azaleas under some conditions. In this study it was attempted to modify pine bark potting media to favor Ma and not Pc. Autoclaving bark for periods longer than 15 min made bark unfavorable for both. Simple steaming did not. Pc spread quickly throughout bark, pH 4.2-4.5, whereas Ma remained near point of infestation. In a mixture of pine bark, expanded shale and peat (pH 5.9-6.3), Ma spread throughout while Pc did not. Lime-amended bark (pH 6.3) allowed spread of Pc, but bark amended to pH 5.3 and up to 6.5 also allowed spread of Ma. However, Pc was relatively immobile in the potting mixture with lime (pH 7.0) or without (pH 5.3). When bark was amended with as little as 10% peat plus lime (pH 6.0), Ma spread throughout. Under similar conditions Pc did not spread.

**CRITERIA FOR EVALUATING EXOTIC PATHOGENS.** Charles L. Wilson, USDA Appalachian Fruit Research Station, Kearneysville, WV 25430.

Presently we can detect and examine only a small fraction of the exotic pathogens threatening U.S. agriculture. Also, most potentially destructive exotics, if introduced, do not "flag" themselves in their native habitat. Criteria, therefore, are needed to narrow the field of potentially destructive exotic pathogens. The following ecological principles can be used: (1) Continuous and perennial ecosystems (forests, orchards) are more threatened by exotics than discontinuous, annual agroecosystems (annual crops); (2) Organisms from large land mass areas (e.g. Eurasia) are more apt to replace native ones in smaller land mass areas if introduced; (3) Organisms are limited in their distribution and spread by the climatic conditions in regions where they evolved. From these criteria the greatest threat to our agriculture from exotic pests should come from pests of forests, rangelands, and orchards in the Eurasia land mass with climates comparable to ours.

**COMPOUNDS WHICH SPECIFICALLY BLOCK EPIDERMAL PENETRATION BY COLLETOTRICHUM LINDEMUTHIANUM.** P. M. Wolkow and H. D. Sisler, Dept. of Botany, Univ. of MD, College Park, MD 20742.

Tricyclazole, pyroquilon, and PP 389 (4,5-dihydro-4-methyltetrazolo (1,5-a) quinazolin-5-one) at nonfungitoxic concentrations block melanin biosynthesis in *C. lindemuthianum*. Excised hypocotyls from bean plants treated with these compounds by root application were protected from infection by *C. lindemuthianum*. Protection did not occur when the epidermis was punctured. Control and treated conidia germinated and formed appressoria on detached strips of bean or *Bryophyllum* epidermis or bean cuticle. Penetration was accomplished by melanized control appressoria, but not by treated, unmelanized appressoria. Control appressoria were more narrowly focused at the appressorial-cuticle interface than treated appressoria. This suggests that walls of the latter lack the design and rigidity required for mechanical penetration.

**EFFECT OF TRICYCLAZOLE ON APPRESSORIAL DEVELOPMENT AND FUNCTION IN PYRICULARIA ORYZAE.** C. P. Woloshuk, H. D. Sisler and E. L. Vigil, Dept. of Botany, Univ. of Maryland, College Park, MD. 20742

Tricyclazole applied to roots protected intact rice plants from infection by *Pyricularia oryzae* but failed to protect when the plants were wounded. The compound, at concentrations as low as 0.1 µg/ml, prevented penetration of detached *Bryophyllum* epidermal sections by *P. oryzae*. Tricyclazole had no effect on spore germination or appressorial production on the epidermis; however, it inhibited melanization of the appressoria. Transmission electron microscopy of mature untreated appressoria revealed a distinct electron dense layer in the wall which thickened at the periphery of the base in contact with the plant cuticle. The base was thin-walled and lacked the electron dense layer. Tricyclazole treated appressoria failed to produce the electron dense layer. These data indicate that the unmelanized wall of the treated appressoria lack the rigidity necessary for shearing the cuticle by mechanical force.

**CHARACTERIZATION OF AMBIENT OZONE IN THE BLUE RIDGE MOUNTAINS OF VIRGINIA.** Yaw-Shing Yang and Boris I. Chevone, Department of Plant Pathology and Physiology, VPI & SU, Blacksburg, VA 24061.

Ambient oxidant concentrations (primary ozone ( $O_3$ )) in the Shenandoah National Park, Blue Ridge Mts., and Southern Appalachian Mts. of Virginia were monitored continuously from Jan. 1979 to Dec. 1981. Hourly average ambient  $O_3$  conc. at all 3 monitoring sites exhibited a typical mountain<sup>3</sup> diurnal cycle during the summer oxidant season. Usually, the oxidant season occurred each year beginning in mid-April and ending in early October with monthly  $O_3$  average conc. between 0.040 to 0.065 parts per million (ppm). Ozone episodes occurred several times each year during which peak  $O_3$  hourly conc. often ranged from 0.080 to 0.129 ppm and lasted for 1 to 3 days. These  $O_3$  episodes were primarily regional in nature as evidenced by the high  $O_3$  conc. at each of the monitoring sites. The accumulative  $O_3$  dosage during the plant growing season (April 1 - Sept. 30) at all sites ranged from 73.4 to 87.6, 59.0 to 74.2, and 50.5 to 77.3 ppm-hr for 1979, 1980, and 1981, respectively.