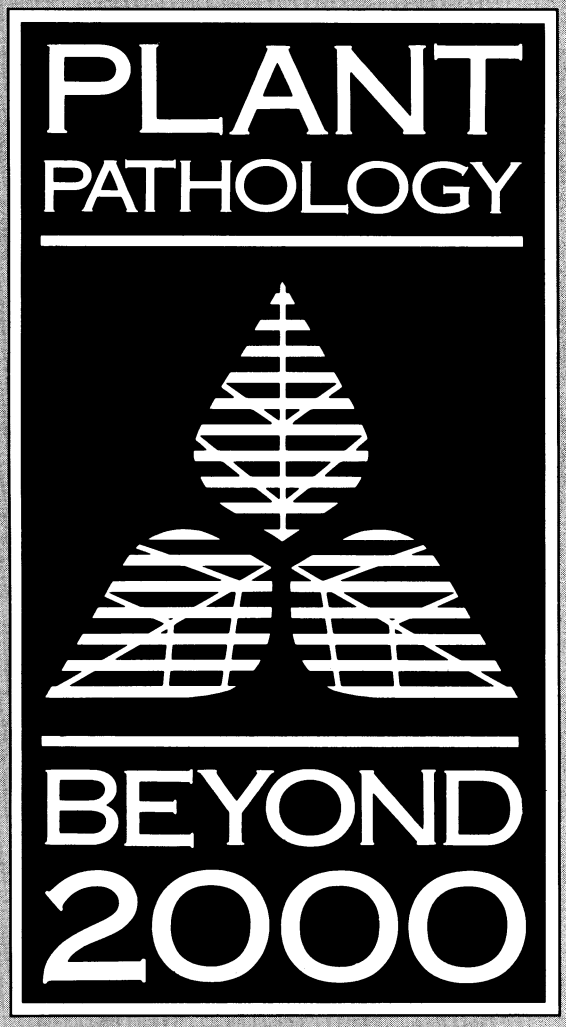
THE AMERICAN PHYTOPATHOLOGICAL SOCIETY

ABSTRACTS OF PRESENTATIONS



APS/SON JOINT MEETING

NOVEMBER 6-10, 1993

OPRYLAND HOTEL

NASHVILLE, TENNESSEE

THE SOCIETY OF NEMATOLOGISTS

Abstracts of Presentations

■ ■ ■ ■ ■ The American Phytopathological Society ■ ■ ■ ■ ■

The number above an abstract corresponds to its designation in the program of the 1993 APS/SON Annual Meeting in Nashville, TN, November 6–10. If a presentation was not given at the meeting or was published in the Society of Nematology's *Journal of Nematology*, the abstract is not printed among the following pages.

A3

The effect of tomato cultivar and nematicide application on root-knot nematode infection of double-cropped cucumbers. P.D. Colyer[1], T. Kirkpatrick[2], and H.Y. Hanna[1]. [1]Louisiana Agricultural Experiment Station and [2]University of Arkansas.

Root-knot infection of direct-seeded and transplanted cucumbers following root-knot susceptible and resistant tomatoes was evaluated in a field heavily infested with root-knot nematodes. At planting, plots were split with one-half receiving a nematicide and the other half not receiving a nematicide. Soil populations of root-knot nematodes at planting and root galling were higher in cucumber plots following susceptible tomatoes than in plots following resistant tomatoes. Application of the nematicide reduced galling. Soil populations of root-knot nematode at harvest were variable. Transplanted cucumbers produced higher yields than direct-seeded cucumbers. Tomato variety and nematicide application also affected yield.

A16

USE OF LUCIFERASE LABELED *PSEUDOMONAS SOLANACEARUM* TO MONITOR BACTERIAL WILT DEVELOPMENT IN POTATO AND TOBACCO AND THE SUPPRESSION OF BACTERIAL MOVEMENT IN TRANSGENIC TOBACCO EXPRESSING AVRD GENE. Y. Huang, M. Di, N. T. Keen*, and J. H. McBeath. Dept. of Plant and Animal Science, UAF, AK 99775; *: Dept. of Plant Pathology, UCR, CA 92521.

Plasmid pUCD620 carrying promoterless *Lux* genes was used to deliver Tn4431 to *P. solanacearum* strain K60 by electroporation. Transformants expressing the *Lux* genes directed by a heterologous promoter of *P. solanacearum* were selected and used to inoculate potato and tobacco plants. For both stem and leaf inoculation of potato and tobacco plants, bacteria rapidly moved toward the stem and roots through vascular bundles as detected by x-ray film. However, in *avr*D-transformed tobacco plants, bacterial multiplication was dramatically reduced and their movement was severely suppressed in the vascular tissue. The detailed patterns of bacterial movement in potato and tobacco as well as in *avr*D-transformed tobacco plants will be discussed.

A17

SUSCEPTIBILITY OF WOUNDED PEPPER ROOTS TO PHYTOPHTHORA CAPSICI. D.L.Adorada, C.L.Biles, and C.M.Liddell. New Mexico State University, Department of Entomology, Plant Pathology and Weed Science, Box 3BE, Las Cruces, New Mexico 88003.

Greenhouse experiments were conducted to determine disease progression of Phytophthora root rot on non-wounded and wounded pepper roots and whether susceptibility to *Phytophthora capsici* decreased with wound age. Plants treated with an aggressive strain (NM6011) were 3 to 4 times more severely diseased than those inoculated with a less aggressive strain (NM6040). Trimming roots prior to inoculation with either strain increased susceptibility compared to plants with untrimmed roots. Resistance to *P. capsici* increased as wounds aged resulting in significantly lower disease severity on 3- and 5-day-old wounds as compared to 0-day-old wound and not-trimmed,not disturbed controls. Increase in resistance correlated with increase in total peroxidase activity. IEF-PAGE indicated increased band intensity of three acidic and one basic peroxidase isozyme as wounds aged. These data suggest that wound repair plays a role in increasing resistance of pepper to *P. capsici* after wounding.

A18

CHEMOTAXIS AND ATTACHMENT OF *PHYTOPHTHORA CAPSICI* ZOOSPORES TO HEALTHY AND WOUNDED ROOTS OF PEPPER, TOMATO, AND CUCUMBER. M. E. Waugh, K. Onsurez, C. L. Biles, and C. M. Liddell. New Mexico State University, Department of Entomology, Plant Pathology, and Weed Science, Box 3BE, Las Cruces, NM 88003, U.S.A.

New Mexico strains of *Phytophthora capsici* Leonian isolated from pepper are virulent to pepper (*Capsicum annuum*), and tomato (*Lycopersicum esculentum*) and avirulent to cucumber (*Cucumis sativus*). A micrographic survey of inoculated healthy and wounded roots from each of the three species was conducted to quantify levels of zoospore attachment by root zone. The elongation and root hair zones showed significantly higher levels of attachment relative to any other point on unwounded roots in all three plant species. Attachment to healthy pepper roots was 5 times higher than either tomato or cucumber, which were not significantly different, suggesting a host mediated response. Wound sites acted as foci for zoospore attachment. Observed attachment decreased as wounds aged suggesting an association with wound repair. These data indicate that host-specific root exudates play an important role in chemotaxis and attachment of *P. capsici* zoospores to healthy roots.

A19

THE ROLE OF THE INDOLE-3-ACETIC ACID BIOSYNTHETIC OPERON IN THE INTERACTION OF *P. SYRINGAE* PV. SYRINGAE AND *P. VULGARIS*. Frank F. White and Mark M. Mazzola. Department of Plant Pathology, Kansas State University, Manhattan, KS 66506.

The apparent homologues of the genes for indole-3-acetic acid (IAA) biosynthesis from *Pseudomonas syringae* pv. syringae were isolated from *P. syringae* pv. syringae, the causal agent of brown spot of bean, and the nucleotide sequence was determined. Two ORFs that corresponded to *iaaM* and *iaaH*, the genes for tryptophan monooxygenase and indoleacetamide, respectively, were present, and the derived amino acid sequences had greater than 90% sequence identity with the sequences from *P. syringae* pv. savastanoi. An additional ORF (ORF3) was located downstream from *iaaH*. A database search using the putative 221 amino acid residue protein revealed a possible relatedness to a group of proteins that are involved in carboxylation/decarboxylation reactions. The function of the putative ORF3 product in *P. syringae* pv. syringae is unknown. A mutation in the IAA operon of *P. syringae* pv. syringae was associated with altered growth dynamics in beans plants. In addition, the ability to synthesize syringomycin appeared to be altered.

A20

A VITRONECTIN-LIKE PROTEIN IS IMPORTANT FOR SPORE ADHESION AND APPRESSORIUM FORMATION IN *MAGNAPORTHE*

Camera-ready abstracts are published as submitted. The abstracts are not edited or retyped in the APS headquarters office.

GRISEA. D.S. Whitehead[1], V.T. Wagner[2], Y.-H. Lee[1], R.S. Quatrano[2] and R.A. Dean[1]. [1] Dept. of Plant Pathology, Clemson Univ., Clemson, SC 29634, [2] Dept. of Biology, Univ. of NC at Chapel Hill, NC 27599.

Adhesion is an important part of the life cycle of *Magnaporthe grisea*, the causal organism of rice blast. Upon hydration, spores release a tip mucilage in order to adhere to the plant surface. Following germination, infection proceeds by the formation of an appressorium from the tip of the germ tube. Vitronectin (Vn) is a substrate adhesion molecule found in the extracellular matrix of mammalian cells. We show that a protein similar to Vn is present in *M. grisea*. Polyclonal antibodies to human Vn recognise a protein of approximately 100kDa in Western blots of fungal protein. Addition of purified IgG to a spore suspension reduces spore adhesion and appressorium formation *in vitro*. Addition of IgG to previously adhered spores also reduces appressorium formation. Immuno-localization studies demonstrate that the antibody binds to the spore tip mucilage, but only infrequently to appressoria.

A21

cAMP RESTORES APPRESSORIUM-DEFICIENT MUTANTS OF *MAGNAPORTHE GRISEA*. Y.H. Lee, D.S. Whitehead, and R.A. Dean, Department of Plant Pathology, Clemson University, Clemson, SC 29634

Magnaporthe grisea, the rice blast pathogen, requires the formation of a darkly pigmented, dome-shaped structure, an appressorium, to infect host plants. The germ-tube tip differentiates into an appressorium on leaves or hydrophobic artifical surfaces, but not on growth media or hydrophilic surfaces. We have shown recently that appressorium formation is regulated by cAMP. To begin to dissect the signalling mechanism(s) involved in this important developmental process, mutants blocked in appressorium formation were obtained following UV mutagenesis. With the exception of appressorium formation, the physiological and morphological characteristics of these mutants including growth rate, sporulation, number of cells per conidia, and conidial size are similar to the parental wild type. We report here that cAMP, its analogs, and IBMX (phosphodiesterase inhibitor) were able to restore appressorium formation in several of these mutants. Furthermore, appressorium formation on rice leaves and pathogenecity tests of these mutants suggest that factors in addition to hydrophobicity stimulate appressorium formation.

A22

PHYSIOLOGICAL RACES OF *Bipolaris zeicola* PATHOGENIC TO MAIZE. E. J. Traut and H. L. Warren. Dept. of Plant Pathology, Physiology and Weed Science, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061-0331.

Forty-nine isolates of *Bipolaris zeicola* from different geographical areas including all the previously described races and pathotype were characterized by their disease reaction, severity and symptoms incited on 14 maize inbred lines. Eleven physiological races were distinguished based on their differential reaction on the 14 inbred lines; however, 6 inbred lines were adequate to differentiate the races. A system based on binary notation is proposed to designate races of *B. zeicola* on maize. Two races induced typical symptoms of *Helminthosporium carbonum*-toxin (HC-toxin) production on the inbred line Pr. Seven races produced oval to irregular lesions on susceptible hosts and did not produce typical lesions of HC-toxin production on Pr. One race induced predominantly long, linear lesions, and another was avirulent on all 14 maize inbred lines.

A23

HOST SPECIALIZATION IN *PHYTOPHTHORA INFESTANS*: FITNESS DIFFERENCES ON POTATO AND TOMATO. Legard, D.E., Goodwin, S.B., Sujkowski, L.S., and Fry, W.E. Dept. Plant Pathology, Cornell Univ., Ithaca, NY 14853.

Fitness of *P. infestans* isolates from potato and tomato lesions was assessed in growth chamber tests. Forty-nine isolates representing nine genotypes collected from potato and tomato during the 1992 epidemics in Canada, the United States, and Los Mochis, Mexico, were inoculated onto detached leaflets of greenhouse grown potato (cv. Norchip) and tomato (cv. Vendor). Fitness was determined after four to six days by measuring lesion area and sporangial production. Three groups of isolates emerged from the bioassay: non-specialized strains which infected potato and tomato equally well; specialized potato strains which produce larger lesions on potato than tomato; and strains specific for potato which did not infect tomato. *Phytophthora infestans* isolates from potato were either specialized or specific for potato in this study, and isolates from tomato were non-specialized. In general, isolates with the same allozyme genotype had the same pathogenicity profile. However, two isolates of the 'old genotype' (*Gpi:86/100, Pep:92/100*) from tomato were non-specialized, whereas 19 old genotype isolates from potato were specific to potato and caused hypersensitive reactions on tomato.

A24

Genetic analyses of avirulence/virulence of a U.S. field isolate of *Magnaporthe grisea* on rice cultivar Katy. C. T. Chao and A. H. Ellingboe. University of Wisconsin, Madison. WI. 53706. USA.

An isolate of *Magnaporthe grisea*, Tm4, from Texas was crossed with a laboratory isolate 70-6 (cross 85). Progenies from cross 85 were backcrossed for 2-3 generations to 70-6 to produce isolates that were avirulent on Katy but virulent on other U.S. cultivars. Six isolates were obtained which, when crossed to virulent siblings, gave a 1 avirulent : 1 virulent segregation ratio on Katy. These results suggested that each of these six isolates had one gene for avirulence on Katy. Intercrosses among the six populations were made to determine whether they had the same gene or different genes for avirulence on Katy. All progenies were expected to be avirulent from crosses between two isolates that had the same aviurlence gene. A 3 avirulent : 1 virulent ratio was expected in progenies from crosses between two isolates if they each contained different, unlinked avirulence genes. Five crosses gave either very few or no virulent progenies. Other crosses between avirulent isolates gave unexpected avirulence : virulence segregation ratios of 4:11, 16:11, 15:12, 16:19, 16:27, etc.. These observations and further testcrosses suggest that the interactions of two or more genes are necessary for the expression of avirulence on Katy.

A25

DEFENSE RESPONSES OF SUSCEPTIBLE AND RESISTANT PEPPERS TO *PHYTOPHTHORA CAPSICI*. Sylvia Fernandez-Pavia, C. M. Liddell, and C. L. Biles. New Mexico State University, Department of Entomology, Plant Pathology, and Weed Science, Box 3BE, Las Cruces, NM 88003, U.S.A

We hypothesize that the resistant serrano type pepper 'Criollo de Morelos' (CM-334) restricts the colonization of *Phytophthora capsici* in the root system by either biochemical or mechanical inhibitors. Six-week old CM-334 plants showed a high level of resistance to a virulent strain of *Phytophthora capsici* from New Mexico. Five days after inoculation the susceptible commercial pepper cultivar NM 6-4 showed stem necrosis and wilting. CM-334 did not show visible symptoms during the length of the trial (23 d). Visualization of the susceptible root system indicated severe necrosis of lateral and tap roots. CM-334 had necrosis of the lateral root tips, but no necrosis was present in the tap root. *P. capsici* was isolated from CM-334 lateral necrotic root tips 23 d after inoculation. Microscopic examination indicated that infection was blocked within the lateral roots. Preliminary experiments detected similar patterns of peroxidase activity in the susceptible and resistant plants during 7 days after inoculation .

A26

INFECTION OF *ARABIDOPSIS THALIANA* BY THE GEMINIVIRUS, BEET CURLY TOP VIRUS Keith R. Davis[1,2], Sukchan Lee[2], Drake C. Stenger[4] and David M. Bisaro[1,3] The Biotechnology Center[1], Depts. of Plant Biology[2] and Molecular Genetics[3], The Ohio State University, Columbus, OH and Dept. of Biological Sciences[4], Northern Illinois University, DeKalb, IL.

We have shown that specific isolates of the geminivirus, beet curly top virus (BCTV), are capable of infecting some land races of *Arabidopsis thaliana* but not others. Symptoms appear on susceptible plants approximately 2-3 weeks after inoculation with BCTV-Logan and 10-15 days with BCTV-CFH and are characterized by leaf curling and stunted, deformed inflorescence structures. BCTV-CFH causes more severe symptoms than BCTV Logan. Analysis of viral DNA accumulation indicate that symptom development and severity is correlated with the accumulation of viral DNA in the plants. In Columbia, viral DNA began to accumulate between 10 and 15 days after inoculation, and continued to accumulate over the 4 week period after inoculation. Levels of BCTV-CFH DNA were approximately 5 times higher than that observed for BCTV Logan 28 days after inoculation. Viral DNA was undetectable in phenotypically resistant land races, indicating that BCTV replication and/or movement is blocked in these plants.

A27

EARLY INDUCTION AND ACCUMULATION OF CHITINASES AND β-1,3-GLUCANASES IN TOBACCO LINES RESISTANT TO *PERONOSPORA TABACINA*. T. L. Robertson[1], A. S. Johnson[2] and S. Tuzun[1], [1]Department of Plant Pathology and [2]Department of Agronomy, Auburn University, AL 36849-5409.

Differential accumulation of two antifungal proteins, chitinases (CHL) and β-1,3-glucanases (BGL), was studied in tobacco lines resistant (NC-BMR 42, NC-BMR 90, Ovens 62, DH113, and a chemical mutant) and susceptible (Kentucky 14) to *P. tabacina*, the causal agent of blue mold. Greenhouse grown plants were inoculated at four- to five-leaf stage with a sporangiosporal suspension ($5x10^4$ sporangiospores/ml) of *P. tabacina*. Foliar samples were taken at zero, two, four and seven days after inoculation (DAI). Samples were extracted with acidic and neutral buffers. SDS-PAGE and Western blot analysis indicated the presence of at least two CHL and three BGL isozymes. Early induction and accumulation of CHL and BGL isozymes were seen in the tobacco lines resistant to *P. tabacina*. In addition, both CHL isozymes were constitutively present at low levels in extracts of tobacco lines resistant to *P. tabacina*, and these levels increased with time. Accumulation of BGL isozymes was found at two and four DAI in tobacco lines resistant to *P. tabacina*. Neither CHL nor BGL isozymes were accumulated until seven DAI in the susceptible variety. Induction and accumulation patterns of CHL and BGL isozymes correlated with resistance to *P. tabacina*.

A28

PURIFICATION OF A CHITINASE/LYSOZYME ISOZYME (CHL2) THAT IS CONSTITUTIVELY EXPRESSED IN CABBAGE VARIETIES RESISTANT TO BLACK ROT. K. M. Dodson[1], J. J. Shaw[2] and S. Tuzun[1], Departments of [1]Plant Pathology and [2]Botany and Microbiology, Auburn University, AL 36849-5409.

Resistant (Hancock, Cheers, Strukton and Green Cup) and susceptible (Perfect Ball) cabbage varieties were inoculated by petiole injections with the causal agent of black rot, *Xanthomonas campestris* pv. *campestris* (XCC). A strongly pathogenic XCC strain, genetically modified to bioluminesce (*Vibrio fischeri lux* cassette), was employed. The *in planta* location of bacteria was precisely monitored by means of a computer-assisted charge-coupled-device camera, and samples were taken from different stages of pathogenesis. SDS-PAGE and Western blot analyses demonstrated the presence of a constitutively expressed chitinase/lysozyme isozyme (CHL2) in acidic extracts of resistant varieties, which was upregulated in tissue after bacterial colonization. In contrast, CHL2 accumulated in the susceptible variety only after symptom development. Lysozyme activity assay results paralleled the Western blot data. The levels of CHL2 were strongly correlated with the degree of resistance in these varieties and is thought to be a mechanism for black rot resistance. This isozyme was purified from Hancock utilizing 2D protein purification procedure (IEF and Native PAGE) and appears to be a 32 kd protein with a pI of 4.0.

A29

COMPARATIVE ULTRASTRUCTURE IN THE RESISTANT RESPONSE OF SOYBEAN CULTIVARS TO SOYBEAN CYST NEMATODE. **Y. H. Kim, K. S. Kim and R. D. Riggs.** Department of Plant Pathology, University of Arkansas, Fayetteville, AR 72701.

Subcellular resistant responses of two soybean cultivars (PI 88788 and PI 437654) to two races (race 3 and race 14) of soybean cyst nematode (SCN), *Heterodera glycines*, were compared. Syncytia formed in each cultivar by race 3 were usually smaller than those formed by race 14. Syncytia of PI 437654 infected with race 3 were least developed among all cultivar-race combinations, consisting of only a few syncytium-component cells. Resistant responses to race 3 were similar in both cultivars, characterized by extremely dense cytoplasm of syncytium-component cells surrounded often by an electron dense necrotic layer (hypersensitive reaction). In syncytia caused by race 14, on the other hand, cytoplasm of syncytium-component cells was loose and degenerated without an obvious necrotic response. Prominent secondary wall thickenings were formed in syncytium-component cells in PI 437654 with race 14, but not in PI 88788 with either race. These structural differences may be a result of the genetics of the host and/or the nematode.

A29a

A PATHOGEN-INDUCED PEROXIDASE ISOZYME FROM BARLEY. J.S. Scott-Craig, K. Kerby and S.C. Somerville, MSU-DOE Plant Research Lab, Michigan State University, E. Lansing, MI 48823

Infection of barley by Erysiphe graminis, causal agent of powdery mildew, produces an increase in extracellular peroxidase activity. One induced peroxidase isozyme, with an apparent isoelectric point of 8.5 (P8.5), was purified from intercellular wash fluids and used to generate a highly specific antiserum [Kerby K, Somerville SC (1992) Plant Physiol 100, 397-402]. A barley line containing the Mla resistance gene was inoculated with E. graminis strain CR-3 (avirulent) and a cDNA expression library was constructed using mRNA extracted 12 hours post-infection. This library was screened with the anti-P8.5 antiserum and two immunopositive clones were identified. One cDNA is 1.3 kb in length and has better than 99% similarity with a peroxidase cDNA identified previously by differential hybridization [Thordal-Christensen H et al (1992) Physiol Mol Plant Pathol 40, 395-409]. Northern analysis indicates that a single 1.3 kb mRNA species is induced starting at about seven hours post-infection and that message levels increase steadily up to 24 hours. The message induction precedes actual penetration of the epidermis by the fungus and continues despite the fact that greater than 99% of the spores fail to form elongating secondary hyphae.

A31

BARLEY STRIPE RUST-RACE 24 CONTROL WITH TRIAZOLE FUNGICIDES. V.R. Velasco, J.P. Hill, and W.M. Brown, Jr. Dept. of Plant Pathology and Weed Science, Colorado State University, Fort Collins, CO 80523.

Barley stripe rust (Puccinia striiformis West) race 24 was introduced into the Western Hemisphere in 1974 in Columbia. The fungus spread rapidly through South America and into Texas (1991), Colorado (1992), Arizona and California (1993). Commercially acceptable stripe rust resistant malting varieties are not available. Fungicide trials were done in Bolivia in 1991, 1992 and 1993. Mancozeb (Manzate 200 DF), chlorothalonil (Bravo), fusilazol (Punch), propiconazole (Tilt) and triadimefon (Bayleton) were applied at label rates. Fusilazol, propiconazole and triadimefon gave significant stripe rust control when applied at first sign of rust pustules and again 20 days later. Triadimefon (Baytan) gave early season control up to 36 days after barley emergence.

A32

SPORE PRODUCTION OF *PYRENOPHORA TRITICI-REPENTIS*. J.M. Krupinsky. USDA, Agricultural Research Service, Northern Great Plains Research Laboratory, P.O. Box 459, Mandan, ND 58554-0459.

Rotorod spore samplers were established from 1986 through 1992 in spring and winter wheat fields with various field management treatments to study the spore production of *P. tritici-repentis*, cause of tan spot disease. The fields sampled included spring wheat after fallow, spring wheat after sunflowers, winter wheat after spring wheat, and fallow fields with only the previous year's spring wheat residue. Annual fluctuations of precipitation during the growing season was a dominant factor influencing the number of spores recovered. Low spore numbers were associated with below average precipitation. The average number of conidia recovered each year was higher than the number of ascospores, indicating the importance of conidia in the epidemiology of tan spot. Overall, spore numbers were generally higher within the winter wheat fields. In the fallow fields, spore numbers were associated with the quantity of spring wheat residue present.

A33

M.A. El-Meleigi, A.A. El-Rokibah, G.H. Ibrahim, S.M. El-Shair and M.E. Al-Omir. Survey of wheat diseases in Central Saudi Arabia in 1987, 88 & 89. Plant Protection Dept. College of Agriculture, KSU, Gassim, P.O. Box 1482, Burydah, Saudi Arabia.

The survey was conducted in 51 farms scattered in 40, 000 K^2 in Gassim region that were cultivated mostly with Yecora rojo wheat (Triticum aestivum) cutlivar. The following diseases were found: common (dry land) root rot (Fusaium graminearum) brown root rot (Pythium spp.), eye spot (Rhizoctonia spp.), take-all (Gaeumannomyces spp.), septoria leaf spots (Septoria spp.), Phoma spots (Phoma spp.), dowdeny mildew (Erysiphe sp.), Sclerophthora sp), loose smut (Ustilago sp), covered smut (Tilletia sp), Stem rust (Puccinia graminis var. tritici), leaf rust (P. recondita), stripe rust (P. striiformis), kernel black-end point, white blotch (Bacillus spp.), Black chaff (Xanthomonas spp), basal glume rot (Pseudomonas spp.) cereal cyst nematode (Hetrodera sp) and seed gaal nematode (Anguina sp.). The common root rot occurred in all the fields and in 20 to 83% of the plants. The leaf spots were found in all fields in 39% of the plants. The basal glume blotch was found in 53% of the fields and in 9 % of the plants.

A34

VIRULENCE OF *BLUMERIA GRAMINIS* F. SP. *TRITICI* IN OHIO. R. Persaud and P. E. Lipps. Department of Plant Pathology, OARDC/The Ohio State University, Wooster, OH 44691

Isolates of *Blumeria graminis* f. sp. *tritici* were collected from 17 counties in Ohio in 1992. Single colony progenies from these isolates were tested for virulence against 11 major powdery mildew resistance genes using 8 day-old seedlings of monogenic differential host lines and cultivars. Virulence to all but two resistance genes, Pm1 and Pm17, were found. All isolates tested showed virulence to Pm7 and Pm8, while virulence to Pm3b was rare relative to virulence on the other resistance genes (Pm2, Pm3a, Pm3c, Pm4a, Pm5 and Pm6). More than 60% of the isolates tested showed virulence to Pm2, Pm3c, Pm5, Pm6, Pm7 or Pm8. Results indicated that complex races of the pathogen are fairly common in the pathogen population sampled. Presently, isolates collected in 1993 are being tested for virulence against the above mentioned 11 powdery mildew resistance genes.

A35

VIRULENCE PATTERN AND DISTRIBUTION OF THE 1992 LEAF RUST POPULATION IN NEBRASKA. S.S. Rutledge, J.E. Watkins, and P.S. Baenziger. University of Nebraska, Lincoln. 68583

Puccinia recondita f. sp. tritici isolates were collected in 1992 throughout Nebraska to monitor the virulence pattern of the leaf rust population. Of the 155 isolates collected and then tested for virulence on 16 single gene differentials, 42 percent were virulent on 10 or more single gene lines. Differential lines Lr1, Lr3, Lr10 and Lr30 were not resistent to any of the rust isolates collected, while differential lines Lr9, Lr16, Lr3ka, and Lr19 showed resistance to all the isolates. There was a high frequency of isolates(>80%) that were virulent against differential host genes Lr2a, Lr2c, Lr24 Lr11, Lr10 and Lr18. Forty-seven percent of the isolates collected were virulent to Lr1, Lr3, Lr10, Lr24 and Lr26, genes commonly found in breeding lines. This emphasizes the threat of the leaf rust population to cultivars with one or more of these genes as the primary source of resistance.

A36

Virulence of *Tilletia barclayana* on Selected Rice Cultivars. F.N. Lee, G.E. Templeton, R.H. Dilday, K.A.K. Moldenhauer and K.A. Gravois. University of Arkansas RREC. P.O. Box 351. Stuttgart, AR 72160.

T. barclayana was tested for virulence to rice cultivars by injecting spore suspensions into the boot of rice plants 24 h prior to panicle exsertion. Little or no kernel smut resulted in tests using individual isolates or paired combinations of isolates from the same teliospore. Depending on the particular isolate combination, a smut index varied from zero to 409 regardless of whether paired isolates originated from the same or different infected rice kernels. Compatible pairs of filiform secondary sporidia resulted in high smut levels while allantoid secondary sporidia resulted in low smut incidence. Mycelial combinations did not result in detectable infections. Differential host responses were observed when 10 rice cultivars were tested. The most resistant, CI 9633, was rated tolerant to three and immune to six isolate pairs tested.

A37

RESISTANCE TO CROWN RUST IN BARLEY AND ITS INHERITANCE IN CI 1243. Y. Jin[1], B. J. Steffenson[1], and J. D. Franckowiak[2]. [1]Department of Plant Pathology, and [2]Department of Crop & Weed Sciences, North Dakota State University, Fargo, ND 58105.

Recent outbreaks of barley crown rust, caused by a variety of *Puccinia coronata*, occurred in the Upper Midwest region. Thirty barley cultivars from the USA and Canada were evaluated for their reaction to the barley crown rust pathogen at the seedling stage, and resistance was not found in the adapted germplasm. From the evaluation of 600 barley accessions of diverse origin, nine exhibited low infection types (0; to 1). One of these resistant accessions was CI 1243 which also possesses the *Rph9* gene for leaf rust resistance. CI 1243 was crossed with the susceptible cultivar Bowman (infection types 3 to 4). Segregation in F_2 for low and high infection types indicated that one partially dominant gene conferred resistance to the crown rust pathogen. The relationships between the crown rust resistance gene, the leaf rust resistance gene (*Rph9*), and several genetic markers are being investigated.

A38

ADHESION OF COLLETOTRICHUM GRAMINICOLA CONIDIA. B. Leite[1], E. Mercure[2], and R. L. Nicholson[2]. [1]Department of Biochemistry, Universidade Federal do Paraná, Curitiba 81531-970 - Brazil and [2]Botany and Plant Pathology Dept., Purdue University, West Lafayette, IN 47907 - USA.

The adhesion of conidia of Colletotrichum graminicola to maize and sorghum leaves or to a hydrophobic polystyrene surface is prevented by a pre-treatment of conidia with Concanavalin A, a lectin which binds specifically to D-mannose and D-glucose residues. Two types of adhesion events are apparent in C. graminicola conidia and germlings. One type (phase I adhesion) is activated immediately after the conidium makes contact with a substratum. In this stage of adhesion conidia release an adhesive material. The second adhesive stage (phase II) occurs after the development of appressorium has begun. In this investigation we have addressed phase I of adhesion which occurs before the onset of conidial germination. Extracts from the surface of conidia demonstrated the presence of several sugars, most notably mannose. The findings are in agreement with the concept of a carbohydrate-mediated adhesion of the conidium to the substratum surface. We demonstrate that the number of adhering conidia increases over time, indicating a progressive release of adhesive material. The importance of adhesion to fungal infection is discussed.

A39

STENOCARPELLA MAYDIS (Syn. *Diplodia maydis*) PRODUCES CHLAMYDOSPORES IN CULTURE. A.E. Dorrance, H.L. Warren, and O.K. Miller. Dept. of Plant Pathology, Physiology, and Weed Science and Dept. of Biology, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061-0331

Stenocarpella maydis causes several maize (*Zea mays*) diseases worldwide including stalk, ear, and crown rot. Asexually produced pycnidiospores are the only means of reproduction known for this fungus. We observed large numbers of terminal and intercalary chlamydospores on hyphae growing on sucrose basal salts minimal liquid medium (SBSM) and autoclaved kernels of maize or oats. The chlamydospores are irregular in shape and size ranging from 3 to 12 µm in length and 3 to 8 µm in width, form single or branched chains consisting of 3 to 20 beads, have a thick (0.5 to 2.0 µm) double cell wall, and are multinucleate when stained with DAPI (4',6-diamidino-2-phenylindole). They developed within 2 wk on the SBSM media at 25 C under continuous light. Following storage at 4 C for 8 mo on sterile maize kernels, germination occurred within 12 h on acidified water agar. We have begun experiments to determine their function and if they are formed in host tissue.

A40

EFFECT OF LIGHT ON SPORULATION OF *CERCOSPORA ZEAE-MAYDIS* . C.Y. Tucker, H.L. Warren, E.J. Traut, and A.W. Way. Dept. of Biology, Hampton University, Hampton, VA 23668 and Dept. of Plant Pathology, Physiology and Weed Science, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061-0331.

Sporulation and vegetative growth of *Cercospora zeae-maydis* were evaluated by growing the organism on V-8 Juice (VJA), potato dextrose (PDA) and water agar (WA) at 23 C under diurnal and constant illumination. Conidia were harvested 7, 10 and 14 days after light treatment. VJA yielded the largest quantity of conidia under both light regimes. More conidia were produced under diurnal than under continuous light. Among the 3 harvests periods spore production was greatest on day 10 (118, 46 and 0 conidia/ml, diurnal; 27, 140 and 38 conidia/ml, continuous lighting). *C. zeae-maydis* sporulated on VJA at all 3 harvest periods, unlike PDA and WA where spores were only produced on days 7 and 10, and 10 and 14, respectively. There was no consistent pattern with respect to harvesting spores and subsequent sporulation of *C. zeae-maydis*.

A41

PATHOGENITY AND DESTRIBUTION OF DIFFERENT PLANT PARASITIC NEMATODE SPECIES IN CORN (Zea mays) -FIELDS IN GERMANY. U. Zunke, Universität Hamburg, Institut für Angewandte Botanik, Marseillerstr. 7, D - 20355 Hamburg, Germany.

The increase of corn production in the last years was only possible by introducing corn into traditional or new crop rotation systems. This meant extension of agriculturally used areas. Corn grown on such new fields showed increased yield loss. Soil type or diseases caused by fungi or insects were major causes, but also plant parasitic nematodes seemed to be involved. To determine the importance of nematodes in corn production over 600 soil samples were taken from 43 geographically different fields during 3 years. The most common plant parasitic nematodes were Pratylenchus species (50%), Heterodera species (25%) and other species such as the Tylenchorhynchus group and Ditylenchus dipsaci. D. dipsaci was correlated with highest yield loss with visible plant damage, although they occcured in relativly low numbers and only in few fields. Also weeds were assayed and more than 20 species were found as host plants for D. dipsaci. This fact has to be recognized for farming concepts. Interactions between Pratylenchus species and D. dipsaci seemed to decrease the population of Pratylenchus species. Susceptibility and tolerance of corn to the different species and their population density were revealed.

A42

DETERMINATION OF THE EFFECTS OF POWDERY MILDEW ON THE PHOTOSYNTHETIC RATE OF WHEAT LEAVES. Celsa Garcia and Steven Leath, Department of Plant Pathology and USDA-ARS, N.C. State University, Raleigh, NC 27695

Yield loss caused by foliar organisms such as *Blumeria graminis*, affect yield by reducing photosynthetic capacity of the leaves. To model that relationship, experiments were established in the field and in a phytotron. Photosynthetic rates on healthy and diseased leaves were estimated by measuring $CO_2/m^2/s$ of a section of the flag leaf. A random

coefficient regression analysis was performed to quantify relationship between powdery mildew and photosynthetic rate. Photosynthetic rates decreased consistently over time in both field and controlled conditions. This decline reflected a reduction of photosynthesis with age of the leaf and increase in amount of disease. The R^2s were low in field trials where variations in temperature and relative humidity strongly influenced CO_2 measurements making it difficult to determine the true effect of powdery mildew. Under controlled environmental conditions, disease had the most impact on photosynthesis variation.

A43

HYBRIDIZATION OF *TILLETIA FUSCA* VAR. *BROMI-TECTORUM* WITH *TILLETIA CONTROVERSA* IN WHEAT. L.M. Carris, Department of Plant Pathology, Washington State University, Pullman, WA 99164-6430.

Tilletia fusca var. *bromi-tectorum* (TBT) causes a bunt disease of *Bromus tectorum*. Both host and pathogen are common in the Pacific Northwest. TBT was hybridized with the wheat dwarf bunt fungus, *T. controversa* (TCK), by inoculating monosporidial lines of each fungus into 'Red Bobs' wheat. Infection rate of hybrids ranged from 4-10%. Three of five F_1 hybrid lines and one F_2 hybrid line were pathogenic when inoculated back into wheat. Teliospore germination was 70, 32 and 29% in the F_1, F_2 and F_3 generations, respectively. Hybrid teliospore germination rate was similar to that of TBT, whereas dwarfing of infected plants was similar to that caused by TCK. Segregation of markers in F_1-F_3 hybrid progeny is being assessed with RAPD-PCR. This is the first demonstration of viable interspecific hybrids between TCK and a wild grass-infecting bunt species.

A44

INTERNATIONAL BARLEY GERMPLASM SCREENING FOR STRIPE RUST-RACE 24 RESISTANCE. W.M. Brown, Jr.[1], V.R. Velasco[1], J.P. Hill[1], D.M. Wesenberg[2], and H.E. Bockelman[2]. [1]Dept. of Plant Pathology and Weed Science, Colorado State University, Fort Collins, CO 80523; and [2]USDA-ARS, Aberdeen, ID 83210.

Barley stripe rust (Puccinia striiformis West) race 24 was introduced into the Western Hemisphere in 1974 and spread to the U.S. in 1991. Because of the recognized threat this fungus posed to U.S. producers, field screening of the USDA-ARS national germplasm collection, international lines, and U.S. public institution and commercial lines was initiated in 1990. Over 20,545 lines have been evaluated. Initial screening occurs in Bolivia with second year selected lines also being tested in Albania, Colorado, Ecuador, Germany, and Texas. Numerous sources of stripe rust-race 24 resistance have been identified and made available to University, commercial and international cooperators.

A45

THE COMMERCIAL POTENTIAL OF *XANTHOMONAS CAMPESTRIS* PV. *POANNUA* FOR CONTROL OF *POA ANNUA* VAR. *ANNUA*: LARGE SCALE FIELD TESTING. K.J. Jones, R.A. Haygood and M.G. Goering. Mycogen Corporation, 4980 Carroll Canyon Road, San Diego CA 92121.

There is no effective, registered postemergence herbicide for control of *Poa annua* (annual bluegrass), a serious weed of overseeded bermuda grass golf greens. *Xanthomonas campestris* pv *poannua* (XcP), when applied by mowing into *P. annua*, colonizes the plants systemically and causes a lethal wilt. The efficacy of this bacterium against the weed as well as its safety to desirable turf grasses, including *Poa trivialis*, has been demonstrated in field trials conducted over the last three years. In 1992, wide scale testing of XcP was conducted on commercial golf courses and turf research facilities at 9 sites in 7 southern states. Field tests were designed to investigate the control potential of XcP using a number of strict calendar based applications (4 fall/winter application, 4 winter/spring applications or 6 monthly applications in the fall/winter/spring). Monthly applications of XcP initiated in early fall provided the most consistent control of *P. annua*. In this use pattern XcP provided greater than 70% control in 5 trials, and 30 to 69 % control *P. annua* in 4 trials.

A46

EVALUATION OF BACTERIA FOR THE SUPPRESSION OF SUMMER PATCH AND ROOT COLONIZING ABILITY ON TURFGRASS. D. C. Thompson, D. Y. Kobayashi, and B. B. Clarke, Dept. of Plant Pathology, Rutgers University, New Brunswick, NJ 08903.

Summer patch (SP) in bluegrass and fine fescue turf is caused by the ectotrophic root-colonizing fungus, *Magnaporthe poae*. A controlled environment system was used to evaluate bacteria for their ability to suppress summer patch and colonize turfgrass roots. Several hundred strains isolated from turf were evaluated for SP suppression, root colonizing ability (RCA), and *in vitro* antifungal activity against *M. poae*. Moderate RCA of some strains was adequate to suppress SP in the controlled environment, but high RCA did not necessarily guarantee suppression. Strains that suppressed summer patch or had high RCA in the controlled environment were further evaluated in the field in 1992 and 1993. There was a high correlation between RCA in the controlled environment and RCA 2 and 5 wk after application in the field. Some strains established high populations rapidly, whereas others required many applications. Winter survival was correlated with early fall populations but high fall populations did not ensure winter survival.

A47

A NATURAL PLANT PRODUCT FOR EXTENDING VASE LIFE OF ROSES. C.A. Greer and J.J. Marois. Dept. of Plant Pathology, University of California, Davis, CA 95616.

Microorganisms that occur in vase solutions have a detrimental effect on the vase life of cut roses. Bacteria, yeasts, and filamentous fungi were isolated from vase solutions over time to determine the microbial population dynamics in vase solutions. Activity of BC-1000 (Chemie Research & Mfg. Co., Inc., Florida), a compound derived from a natural plant extract containing fatty acids as the active ingredients, was tested against isolated microorganisms using *in vitro* petri dish assays and by addition to sterilized vase solution systems. Fresh weights of cut flowers were monitored to determine vase life and microbial populations were monitored using standard serial dilution techniques. Effective concentrations of BC-1000 were tested against naturally occurring microbial populations in vase solutions. A diverse group of bacteria and yeast were isolated from vase solutions with combined total populations as high as 1.0×10^7 cfu/ml. Most microorganisms were undetectable when >75 ppm BC-1000 was added to vase solutions. Addition of BC-1000 at 50-100 ppm and sucrose at 1-2% increased the fresh weight gain of cut roses and resulted in a fresh weight maximum approximately 3 days later than control flowers. Visual flower quality also was maintained for approximately 2-3 days longer than in control treatments.

A48

EFFECT OF NATURAL PRODUCTS ON LESION DEVELOPMENT OF BLACKSPOT ON ROSE. M. R. Carter and J. C. Locke. USDA, ARS, FNCL, Beltsville, Maryland 20705-2350

An *in vitro* assay, using detached leaflets of two *Rosa* sp. cv. "Peach Brandy" (PB) and "Iceberg" (IB) dipped in treatment solutions, was used to monitor lesion development (LD) of blackspot caused *Diplocarpon rosae*. Beginning four days after inoculation (40,000 conidia/ml), assessment of the number of days to LD and sporulation (SP) were recorded for 14 days, followed by measurements of lesion width. Clarified neem seed oil (NSO) alone or with 0.05% sodium bicarbonate ($NaHCO_3$) (w/v) appeared to retard LD for 7 days on both cultivars and delay SP for 6 or 8 days, on PB and IB, respectively. All NSO treatments on PB delayed LD as well as Funginex. On IB, NSO treatments delayed LD by about 4 days more than Sunspray alone or Sunspray plus 0.05% $NaHCO_3$ treatments. Vernonia seed oil (VSO) alone and with 0.05% $NaHCO_3$ did not effectively retard LD on IB, however, on PB, LD was delayed for as long as 6 days. All VSO treatments on both cultivars retarded SP as well as NSO. All NSO treatments on both cultivars reduced lesion width.

A49

FIELD EVALUATION OF CLARIFIED NEEM SEED OIL, SUNSPRAY 6E PLUS HORTCULTURAL OIL AND FUNGINEX FOR CONTROL OF POWDERY MILDEW ON PERENNIAL GARDEN PHLOX. J. C. Locke, USDA, ARS, Florist and Nursery Crops Laboratory, Beltsville, Maryland 20705-2350.

Perennial garden phlox, Phlox paniculata cv. Mt. Fujiyama, were transplanted to an outdoor bed one season prior to treatment. This allowed for establishment of the plants, confirmation of cultivar susceptibility, and the buildup of inoculum within the planting. Treatment rows consisted of four crowns which were each thinned to eight uniform shoots. Treatment rows were separated by alternate spreader rows, and treatments were replicated in each of four blocks. Treatments consisted of 1% clarified neem seed oil, 1% Sunspray 6E Plus Horticultural Oil, or 0.5 fl oz/gal Funginex each applied at 7 or 14 day intervals, and an untreated check. Applications began on 4 June 1992, when mildew first appeared, and continued for seven weeks. Field evaluation of mildew development was made on 22 July. All three materials significantly reduced both the number of mildewed leaves and intensity of mildew development. The 14 day treatment interval was as effective as the 7 day interval, providing good control of this perennial garden disease.

A50

INCIDENCE AND SEVERITY OF DOGWOOD ANTHRACNOSE ON FLOWERING DOGWOOD IN ALABAMA. A. K. Hagan and J. M. Mullen. Auburn University, AL 36849-5624.

From 1991 through 1993, flowering dogwood trees (Cornus florida) on forested sites in north Alabama were surveyed for dogwood anthracnose, caused by Discula destructiva. In 1991, the disease was observed on dogwoods in four of the nine counties surveyed. Foliar blighting was especially severe in two counties. In 1992, the disease intensified at several 1991 survey sites. Extensive shoot dieback, formation of epicormic shoots, and tree death were not uncommon. Lighter outbreaks were noted on trees in five more counties, while dogwoods in five other counties were asymptomatic. Disease occurrence was recorded at elevations as low as 600 ft. but was most severe above 1300 ft. Disease incidence and severity data for 1992 and 1993 along with new county reports will be discussed.

A51

DISSEMINATION OF DISCULA DESTRUCTIVA CONIDIA ON FLOWERING DOGWOOD BY THE CONVERGENT LADY BEETLE, HIPPODAMIA CONVERGENS (COLEOPTERA: COCCINELLIDAE). D.M. Colby, M.T. Windham, and J.F. Grant, Univ. of TN, Dept. of Ent. and Plant Path., Knoxville, TN 37901-1071

Dogwood anthracnose, caused by the fungal pathogen, Discula destructiva, has infected both native and cultivated flowering dogwoods, Cornus florida, throughout the eastern United States for the past 15 years. Modes of conidial dissemination are not known beyond dispersal from acervuli via rain droplets. Therefore, the objective of this research was to determine if the convergent lady beetle, Hippodamia convergens, could disseminate viable D. destructiva conidia. Experiments showed that adult lady beetles carried viable conidia both externally and internally and could deposit viable conidia to an agar medium up to 16 days after exposure to the fungus. Most conidia observed on the external body surface were located on mesothoraxic legs and mouthparts. Healthy C. florida trees became infected with D. destructiva after introduction of inoculum by infested lady beetles.

A52

LEAF SPOT OF COCKSCOMB CAUSED BY ALTERNARIA ALTERNANTHERAE. G. E. Holcomb, Department of Plant Pathology and Crop Physiology, LA Agr. Exp. Stn., LA State University Agr. Center, Baton Rouge, LA 70803.

A severe leaf spot that resulted in leaf death and drop was observed on a landscape planting of an unidentified cultivar of a crested form of cockscomb (Celosia cristata L.). Spores of an Alternaria species were produced on necrotic leaf tissue that averaged 262 X 16 µm and bore a slender beak that was up to 4X the length of the spore body. Koch's postulates were fulfilled with pure cultures, and the fungus identified as A. alternantherae Holc. & Ant. The fungus killed plumed and crested cockscomb cultivars at a spore concentration of 10,000/ml and had an LD_{50} at 2000/ml. Daconil 2787, Dithane M-45, Chipco 26019 and Kocide gave significant disease control in greenhouse tests. This is the first reported occurrence of A. alternantherae on a cultivated plant.

A53

PATHOGENICITY AND INFLUENCE OF TEMPERATURE ON PYTHIUM SPECIES ISOLATED FROM TURFGRASSES WITH SYMPTOMS OF ROOT AND CROWN ROT IN NORTH CAROLINA. **Z. G. Abad, H. D. Shew, L. T. Lucas. North Carolina State University, Raleigh, NC 27695-7616.**

Pathogenicity was studied in pre- and post-emergence tests on bentgrass seedlings at 28C with 33 Pythium spp. isolated from bentgrass and other turfgrasses with symptoms of root and crown rot in NC. In pre-emergence tests 13 species were highly or moderately pathogenic. In post-emergence tests P. arrhenomanes, P. aristosporum, P. aphanidermatum, P. graminicola, P. myriotylum, P. tardicrescens, P. vanterpoolii and P. volutum were highly pathogenic. P. dissotocum, P. irregulare, P. multisporum, P. paroecandrum, P. splendens, P. sylvaticum, P. ultimum (var. sporangiiferum, var. ultimum), and P. violae were moderately pathogenic. Highly and moderately pathogenic species colonize the epidermis and cortex inter and intracellularly causing severe or mild disease. In tests at 16, 28 and 32C, the higher temperatures favored pathogenicity of most species.

A54

CROSS RESISTANCE IN SCLEROTINIA HOMEOCARPA TO DMI FUNGICIDES. Jack C. Doney Jr. and Paul C. Vincelli, University of Kentucky, Dept. of Plant Pathology, Lexington KY, 40546

Sclerotinia homeocarpa, the causal agent of Dollar Spot, could not be controlled on the greens of two central Kentucky golf courses where one demethylation inhibitor (DMI) fungicide, triadimefon, had been used intensively. Three isolates suspected of DMI resistance were collected from these two courses. Two control isolates were obtained from other sites. *In vitro* growth of each isolate was tested on PDA amended with 0.01, 0.10, 1.00 or 10.00 µg/ml of one of the following DMI fungicides: cyproconazole, fenarimol, propiconazole, tebuconazole or triadimefon. EC_{50} values were calculated by regression for each fungicide-isolate combination. All isolates suspected of being resistant to DMI fungicides were significantly ($P = 0.05$) less sensitive than control isolates to all DMI fungicides tested, excepting one isolate which was as sensitive to cyproconazole as was a control isolate. Two of three resistant isolates from triadimefon-treated sites were significantly more resistant to tebuconazole then triadimefon; one of these was also more resistant to propiconazole and fenarimol then triadimefon. The relative ranking of isolates from most to least sensitive was identical for all fungicides tested. The *in vitro* tests indicate *S. homeocarpa* can develop cross resistance to multiple DMI fungicides including fungicides to which it has not been exposed.

A55

CHARACTERIZATION OF ISOLATES OF DISCULA DESTRUCTIVA AND DISCULA SPECIES USING DNA AMPLIFICATION FINGERPRINTING. R. N. Trigiano, B. Bassam, G. Caetano-Anollés, and M. T. Windham. Agric. Exp. Stat., University of Tennessee, Knoxville, TN 37901-1071.

DNA Amplification Fingerprinting (DAF) was used to characterize and compare ten isolates of Discula destructiva Redlin and three isolates of an undescribed species of Discula, the causal organisms of dogwood anthracnose. Isolates of both species were obtained from eastern states throughout the range of the disease and DAF profiles generated using 12 arbitrary oligonucleotide primers. Data were analyzed using PAUP. Very few polymorphisms were detected between isolates of D. destructiva; whereas, a greater number of polymorphisms were observed between isolates of Discula species. The data indicate that the genome of D. destructiva is highly conserved throughout the distribution of the disease and suggests that the fungus was recently introduced to the eastern United States.

A56

PREDICTING RHIZOCTONIA BLIGHT AND REDUCING FUNGICIDE INPUTS ON CREEPING BENTGRASS USING ENVIRONMENTAL AND IMMUNOASSAY-BASED FORECASTS. B. B. Clarke[1] and G. L. Schumann[2], Dept. of Plant Pathology, [1]Rutgers Univ., New Brunswick, NJ 08903, and [2]Univ. of Massachusetts, Amherst, MA 01003.

Three disease forecasting systems were evaluated in MA and NJ for their potential to accurately predict Rhizoctonia blight and reduce fungicide applications on turf. Fungicides were applied to creeping bentgrass according to environment-based (EB), immunoassay-based (IB), and EB+IB forecasting systems during 1991-1992. Forecast-based applications were compared to preventive calendar sprays. The EB parameters most indicative of disease outbreaks included relative humidity ≥ 95% for ≥ 10 h, precipitation ≥ 2.54 mm within a 36 h period, and minimum and mean air and soil temperatures ≥ 15 C, 20 C, 18 C, and 21 C, respectively. The EB, IB, and EB+IB systems provided acceptable levels of disease control and reduced fungicide applications an average of 10, 28, and 40%, respectively.

A57

A SURVEY OF PLANT-PARASITIC NEMATODES IN SOUTHERN CALIFORNIA GOLF COURSE PUTTING-GREEN SOILS. Larry J. Stowell[1] and Michael A. McClure[2]. [1]PACE Turfgrass Research Institute, 1267 Diamond St., San Diego, CA 92109 and [2]University of Arizona, Department of Plant Pathology, Tucson, AZ 85721.

Two composite samples, one from a good performing green and a second sample from a poor performing green were collected from 22 Southern California golf courses between September 18 and 24, 1992. Six genera of plant-parasitic nematodes were identified (data in parenthesis represent the mean nematode count per 100 cc soil for good (G) and poor (P) performing greens): *Criconemoides* (G=99, P=108), *Helicotylenchus* (G=288, P=234), *Meloidogyne* (G=175, P=82), *Paratylenchus* (G=78, P=84), *Trichodorus* (G=42, P=24) and *Tylenchorynchus* (G=202, P=107). Nematode populations were not significantly higher in poor performing greens (Fisher protected LSD p=0.05, data normalized using $\log_{10}$[count/100 cc]). Moreover, significant correlations were identified

between populations of nematodes found in the good vs. poor rated greens. Combined, these data suggests that nematodes were not the primary cause of the poor green performance.

A59

Rose Rosette Disease-Over Wintering and Increase of the Vector as Related to Spread of the Disease in Iowa. A. H. Epstein, and J. H. Hill. Iowa State University, Plant Pathology, 351 Bessey, Ames, Iowa.

Rosa multiflora Thunb. (multiflora rose), a thorny shrub introduced into North America from Northeastern Asia, now negatively impacts over two million acres of non-tilled land in Iowa. Conventional methods of controlling it have been used only sparingly because of their high cost and negative environmental effects. Rose rosette disease, lethal to multiflora rose, has been proposed as a biological control of this plant. Little is known about the pattern of spread of the disease and there is a great deal of concern about possible effects of the disease on flowering rose should the mite vector spread (thought to be passively airborne) to neighboring gardens. Our data suggests that the pattern of spread is not consistent with that of a wind-borne agent. Populations of the vector are very sporadic during the early part of the growing season indicating that the vector does not over-winter very successfully in Iowa. The data suggests that most if not all of Iowa is in a relatively low hazard zone that would benefit from proper augmentation of the disease.

A60

EVALUATING THE SUITABILITY OF *XANTHOMONAS CAMPESTRIS* PV *POANNUA* AS A BIOCONTROL AGENT FOR ANNUAL BLUEGRASS (*POA ANNUA*). S.D. Savage, R.A. Haygood and K.D. Mitchell, Mycogen Corporation, 4980 Carroll Canyon Road. San Diego, CA 92121

A previously undescribed pathovar of *Xanthomonas campestris*, pv *poannua* (XcP) will, upon artificial inoculation, cause a lethal systemic wilt of annual bluegrass (*Poa annua*, ABG), a difficult-to-control turf weed. Before conducting large scale tests in commercial turf, it was necessary to characterize the infection biology, host range and ecology of this organism. Isolates of this bacterium have now been found throughout the United States and it appears that it is a common, cryptic colonizer of grasses. Though related to the European pathovar, *X.c. poae*, XcP can be distinguished on the basis of its specific host range and FAME profile. Of >300 species and cultivars tested, only a few grasses are attacked, even under optimal conditions for disease. Under field conditions, only *Poa annua ssp. annua* of ABG is affected. The rate of systemic colonization and control of ABG are closely tied to temperature. Only limited spread of XcP is seen in field settings. This organism is an excellent candidate for use as a biocontrol agent.

A61

CLONING AND EXPRESSION OF HERBICOLIN O BIOSYNTHESIS GENES IN ESCHERICHIA COLI. L. A. Davis and C. A Ishimaru, Department of Plant Pathology and Weed Science, Colorado State University, Fort Collins, CO, 80523.

Erwinia herbicola C9-1 is a biological control agent of E. amylovora, causal agent of fire blight. Previous studies determined that E. herbicola C9-1 produces multiple antibiotics, termed herbicolins, in chemically defined medium. To initiate a molecular analysis of the role of herbicolins in biocontrol, direct cloning and expression of herbicolin genes in E. coli was pursued. A genomic DNA library of E. herbicola C9-1 was constructed in the cosmid cloning vector pLAFR3 and used to transfect E. coli DH5α. Of 2,000 clones screened, one clone, AA818, produced a zone of inhibition in lawns of the indicator E. amylovora. The antibiotic expressed was identified as herbicolin O by its loss of activity in medium amended with histidine and by its antimicrobial activity spectrum. Southern analysis showed that herbicolin O biosynthesis genes were not encoded on high molecular weight plasmids, as were genes for pigmentation and thiamine prototrophy.

A62

CHEMOTATIC RESPONSE OF BACILLUS MEGATERIUM B153-2-2 TO SOYBEAN ROOT EXUDATES. Xiangyang Zheng and J.B. Sinclair, Dept. of Plant Pathology, Univ. of Illinois at Urbana-Champaign, 1102 S. Goodwin Ave., Urbana, IL 61801-4709

Bacillus megaterium strain B153-2-2, a root colonizer of soybeans, showed a significant (P = 0.01) chemotactic response (CR) to soybean root and seed exudates as measured by exudate -in-1 μl capillary. Of 19 amino acids present in the exudates, only alanine, asparagine, serine, and threonine induced a significant (P = 0.01) CR at the concentrations found in the exudates. Screening of selected organic acids and sugars with concentrations ranging from 10^{-2} to 10^{-6} M showed that only malate was a strong attractant (P = 0.01) at the concentration found in the exudates. Fumarate and succinate were attractants only at a concentration higher than in the exudate. The presence of the same compounds in appreciable concentrations in soybean root and seed exudates suggested that these compounds serve as chemoattractants for B153-2-2.

A63

POPULATION DYNAMICS OF PSEUDOMONAS FLUORESCENS STRAIN A506 IN PEAR FLOWERS FOLLOWING INOCULATION. Steven E. Lindow, Department of Plant Pathology, University of California, Berkeley, CA 94720.

Pear trees in commercial orchards were sprayed with freeze-dried cells of *P. fluorescens* strain A506 (10^8 cells/ml) once at either 5%, 20%, 50%, or 90% bloom, as well as at both 20% and 90% bloom. Newly-opening flowers were tagged every 3 days to identify flowers that had opened at different times prior to, and after, inoculation with strain A506. Bacterial populations were measured on a subset of the tagged flowers every 3 days. The average population of A506 in flowers 5 days or more after inoculation was independent of the time the flower had been open before inoculation for flowers 10 days or less old. Nearly all flowers that opened within 21 days of inoculation of strain A506 were colonized by this bacterium. The average population size of strain A506 in flowers progressively decreased with the time elapsed between spraying and flower opening in flowers that had opened more than 14 days after inoculation. The incidence of natural infection by *Erwinia amylovora* was reduced about 75% by strain A506, irrespective of treatment time or frequency. The efficient distribution of strain A506 among pear flowers indicated that single applications are probably sufficient to achieve fire blight control.

A64

THE POTENTIAL USE OF *PSEUDOMONAS CORRUGATA* FOR BIOLOGICAL CONTROL OF FUNGAL AND BACTERIAL DISEASES OF PLANTS. W. Chun, G. Yong, and K. Kettle. Plant Pathology Division, PSES, University of Idaho, Moscow, ID 83844.

Pseudomonas corrugata (*Pc*) inhibits growth of many fungal and bacterial plant pathogens. Spore germination of *Tilletia caries* (common smut of wheat) and *T. contraversa* (dwarf bunt of wheat) was reduced by 90% in the presence of cell-free culture fluids of *Pc*. Application of *Pc* to wheat seed significantly reduced the severity of take-all symptoms on developing wheat roots in non-sterile field soil. In co-inoculation studies the bacterium reduced the numbers of the potato ring rot pathogen (*Clavibacter michiganensis* subsp. *sepedonicus*) by 10- to 100-fold while maintaining its own population of at least 10^5 bacteria per gram fresh weight of plant tissue. Since pathogenicity in tomatoes and the individual antimicrobial activities appear to be independent genetic traits, *Pc* shows wide promise for controlling a number of plant diseases.

A65

EFFECTS OF SOIL PASTEURIZATION, CUCUMBER CULTIVAR AND TIMING OF INDUCTION ON INDUCED SYSTEMIC RESISTANCE MEDIATED BY PLANT GROWTH-PROMOTING RHIZOBACTERIA. G. Wei, S. Tuzun, and J.W. Kloepper, Department of Plant Pathology, Biological Control Institute, Auburn University, AL 36849-5409.

Cucumber plants ('SMR-58', unless otherwise indicated) were treated by soil drench with plant-growth promoting rhizobacteria (PGPR) strain T4 (*Bacillus pumilus*) and subsequently challenge-inoculated on the leaves with *Colletotrichum orbiculare*. Induced systemic resistance (ISR) responses following PGPR treatment were compared in pasteurized and nonpasteurized soil. Greater and more consistent ISR was achieved when plants were grown in pasteurized soil compared to nonpasteurized soil. PGPR-mediated ISR was evident on two cultivars, 'SMR-58' and 'Straight 8'; however, the effect of ISR was more pronounced on 'SMR-58' than 'Straight 8'. Intervals of 2, 7, and 14 days between PGPR root treatment and pathogen challenge-inoculation were used to determine the timing of expression of PGPR-mediated ISR. Results showed that PGPR-mediated ISR was not fully expressed until 14 days after PGPR treatment. Mechanisms of PGPR-mediated ISR are currently being investigated in our laboratories.

A66

INDUCTION OF SYSTEMIC RESISTANCE AGAINST CUCUMBER BACTERIAL ANGULAR LEAF SPOT CAUSED BY *PSEUDOMONAS SYRINGAE* PV. LACHRYMANS WITH TWO PLANT GROWTH-PROMOTING RHIZOBACTERIAL STRAINS. L. Liu, J.W. Kloepper, and S. Tuzun, Department of Plant Pathology, Biological Control Institute, Auburn University, AL 36849-5409.

Two strains of plant growth-promoting rhizobacteria (PGPR) which previously demonstrated induced systemic resistance (ISR) against cucumber anthracnose caused by *Colletotrichum orbiculare* were tested for their ability to induce systemic resistance against cucumber angular leaf spot, caused by *Pseudomonas syringae* pv. *lachrymans*. Seed treatment and cotyledon injection were used as inoculating methods for PGPR. Second true-leaves of cucumber were challenge inoculated with a suspension of *P. s.* pv. *lachrymans* (Log 8 cfu/ml in 0.85% NaCl) three weeks after planting. Challenged plants were kept in 100% RH in the dark at room temperature for 24 h and then moved to a greenhouse. Total lesion number (TLN) and total lesion area (TLA) (mm^2) per leaf were recorded 6 d after challenge. Treatments of cotyledons with both PGPR strains resulted in significant reductions (P=0.05) compared to disease controls in TLA; treatment with one strain significantly reduced TLN. With seed treatment, both strains significantly reduced TLA and TLN. These and past results suggest that some PGPR strains can induce systemic resistance against various pathogens including fungi, bacteria, and virus.

A67

SUPPRESSION OF PHYTOTOXIN PRODUCTION BY GLIOCLADIUM VIRENS WITH STEROL INHIBITING FUNGICIDES. C. R. Howell and R. D. Stipanovic, USDA, ARS, Southern Crops Research Laboratory, Route 5, Box 805, College Station, Texas 77845.

The steroid phytotoxin viridiol, produced by Gliocladium virens, is a major limiting factor in the amount of fungus/carrier preparation that can be used to control damping-off of seedlings by Rhizoctonia solani and Pythium ultimum. Treatment of developing cultures of G. virens with sublethal concentrations of the fungicides propiconazole (PC), flusilazol (FS), triadimenol (TD), or myclobutanil (MC) suppresses production of viridiol without significant adverse effects on growth or on production of nonsteroid antibiotics by the fungus. The efficacy of viridiol suppression by sterol inhibitors is dependent on culture conditions and on the strain of the biocontrol agent. PC and FS are inhibitory to G. virens at concentrations above 2 ppm, while TD and MC may be used at up to 10 ppm without significant adverse effect, and with greater suppression of viridiol production than that obtained with PC or FS.

A68

AN EXPERT SYSTEM INTEGRATING FUNGICIDE APPLICATIONS CONTROLLING FOLIAR AND SOILBORNE DISEASES OF PEANUT. J. C. Jacobi and P. A. Backman. Department of Plant Pathology, Auburn University, Auburn, AL 36849-5409.

Field trials were conducted during 1991 and 1992 to determine the efficacy of scheduling fungicide applications using the AU-Pnuts advisory for control of early leaf spot (*Cercospora arachidicola*), late leaf spot (*Cercosporidium personatum*), Rhizoctonia limb rot (*Rhizoctonia solani* AG-4), and southern stem rot (*Sclerotium rolfsii*). Fungicide treatments evaluated under both the 14-day calendar and AU-Pnuts advisory schedules were: 1.) chlorothalonil (1.26 kg a.i./ha) for all applications, and 2.) chlorothalonil (1.26 kg a.i./ha) for all applications except that cyproconazole (0.23 kg a.i./ha) was substituted for two chlorothalonil applications. In 1992, leaf spot incidence was lower for AU-Pnuts advisory treatments than 14-day schedule treatments. In both trials, stem rot incidence was lower and yields were higher in plots receiving cyproconazole and chlorothalonil in comparison to chlorothalonil alone using either fungicide schedule. No differences were observed among treatments in limb rot incidence. The AU-Pnuts advisory treatments provided equivalent or better disease control and yields in comparison to the 14-day schedule with fewer total fungicide applications in one of two trials.

A69

INTEGRATION OF HOST RESISTANCE WITH A WEATHER-BASED FUNGICIDE SCHEDULING PROGRAM FOR CONTROL OF TOMATO ANTHRACNOSE. B. A. Fulling, E.C.Tigchelaar, and R.X.Latin. Purdue University, West Lafayette

Five fungicide application intervals were used on five tomato cultivars with different levels of resistance to anthracnose. Tomcast, a weather-based fungicide scheduling program, was used to generate daily disease severity values (DSV), to indicate the favorability of environmental conditions for disease development. Fungicide application intervals were determined by the accumulation of DSVs to a critical level or action threshold value. Five action threshold values, ranging from 12 - 32 DSVs, were used in the experiment. The linear relationship between the percentage fruits with anthracnose lesions at harvest and the action threshold values was determined for each cultivar. The slope of the linear regression for each cultivar was designated as a Tomcast anthracnose coefficient (TAC). An anthracnose index was determined for each cultivar in a disease nursery. In order to predict the TAC for cultivars not included in the Tomcast study, a linear model was developed that showed the relationship between anthracnose index values and TACs. Preliminary results using a second group of cultivars indicated that the TAC could be estimated from the anthracnose index for each cultivar.

A72

SEVERITY OF SOILBORNE DISEASES OF PEANUT AS INFLUENCED BY CROPPING FREQUENCY. [1]K.L. Bowen, [1]A.K. Hagan, [2]J.R. Weeks, and [3]D. Hartzog, Depts. of [1]Plant Pathology, [2]Entomology, and [3]Agronomy and Soils, Auburn University, AL 36849.

Trials were started in 1991 in farm fields to assess the severity of southern stem rot (caused by *Sclerotium rolfsii*) and other soilborne pests as influenced by the frequency of peanut production. In two years of the study, peanut yields were significantly influenced by incidence of southern stem rot, nematode pest populations, and cropping frequency. Peanuts cropped behind bahiagrass (perennial pasture) suffered almost no southern stem rot damage and averaged 4327 and 4495 kg/ha in 1991 and 1992, respectively. In continuously cropped peanuts, incidence of southern stem rot was greatest and yields were significantly lower (3612 and 3990 kg/ha) in both years. When southern stem rot was minimized with flutolanil, yields among cropping frequencies did not differ significantly. Cropping frequency influenced nematode populations in 1991, but did not influence occurrence of Rhizoctonia limb rot or incidence of *Aspergillus flavus* in seed.

A73

IMPROVED SOIL DISINFESTATION USING BIOTOXIC VOLATILES GENERATED FROM ORGANIC AMENDMENTS AND SOLARIZATION. J. J. Stapleton, A. Gamliel, R. A. Duncan, C. Thomassian, and B. T. Bonilla. University of California, Kearney Agricultural Center, Parlier, CA 93648

The regulatory loss of soil fumigants has necessitated development of alternative methods of agricultural soil disinfestation. Sealing and heating soil amended with organic fertilizers and crop residues can provide levels of disinfestation greater than with either method alone. Concentrations of biotoxic volatiles, including alcohols, aldehydes, sulfides isothiocyanates, and others were found in low levels in field soil amended with organic fertilizers and residues when analyzed by gas chromatography. Concentrations increased when soil was sealed with polyethylene film or spray mulch, and increased further when amended soil was heated. Reductions of pathogens including *Pythium ultimum, Sclerotium rolfsii,* and *Meloidogyne incognita* were greatest when treated with amended, heated soil.

A74

IMMUNOLOGICAL AND MOLECULAR GROUPING OF MYCOPLASMALIKE ORGANISMS (MLOs) ASSOCIATED WITH GRAPEVINE YELLOWS AND CLOVER PHYLLODY DISEASES. K. H. Chen and T. A. Chen, Dept. of Plant Pathology, Rutgers University, New Brunswick, NJ 08903

Immunofluorescent staining, dot blot hybridization, polymerase chain reaction, random amplified polymorphic DNA markers, and restriction fragment length polymorphism techniques were employed to study the genetic relatedness among MLOs associated with samples of grapevine yellows (GY) disease from Italy (CA1, CH1, SA1, SA2 from Bologna; GYU from Udine; GYR from Roma) and Germany (GYG). The relationship between these and MLOs associated with clover phyllody in Italy (CPhB and CPhC) and Canada (CPhCa) was also examined. The MLOs were distinguished from one another and classified into five subgroups: Subgroup I, CA1 and CH1; II, SA1 and SA2; III, GYU, CPhB, and CPhC; IV, GYG; and V, GYR and CPhCa. Four of the GY-MLOs belonged to the MLO virescence group while one belonged to the decline group. We also found a close phylogenetic relationship between GYU and the Italian clover phyllody MLOs (CPhB and CPhC). Since clover plants showing

typical phyllody symptoms are frequently found in Northern Italy where most of the Italian vineyards are also located, clover plants may play an important role in GY disease epidemiology.

A75

DEVELOPMENT OF MONOCLONAL ANTIBODIES TO THE CLOVER PHYLLODY MYCOPLASMALIKE ORGANISM. Y.H.GUO and T.A. Chen, Dept. of Plant Pathology,Rutgers University,New Brunswick,N.J.08903

Sixteen hybridomas stably secreting monoclonal antibodies (McAbs) against the mycoplasma-like organism (MLO) associated with clover phyllody (CPH) were obtained by fusing spleen cells from BALB/c mouse immunized with partially purified CPH-MLO with NS-1 myeloma cells. Antigen used for immunizing mouse was prepared by digesting leaf veins of CPH-MLO infected periwinkles with pectinase and followed by one cycle of low and high speed centrifugations. Isotypes of these McAbs were determined to belong to IgM, IgG, IgG_{2a}, IgG_{2b}, and IgG_3. In ELISA and immunofluorescence tests, our McAbs reacted only with the Eastern CPH-MLO strain, but not with 2 CPH-MLO strains from Italy and 14 other MLOs, including aster yellows (NJ-AY, MD-AY OK-AY and Western AY) ash yellows, apple proliferation, elm yellows, Eastern X, Western X,grapevine yellows, loofa witches' broom, Panlowonia witches' broom, peanut witches' broom and sweet potato witches' broom.

A76

AT LEAST THREE GENETICALLY DISTINCT MLOs CAUSE PEAR DECLINE AND PEACH YELLOW LEAFROLL DISEASES IN CALIFORNIA. B. C. Kirkpatrick, A. H. Purcell*, J. L. Gao, G. F. Fisher and J. K. Uyemoto#. Dept. of Plant Pathology, University of California, and #USDA/ARS, Davis, 95616 and *Dept. of Entomological Sciences, University of California, Berkeley, 94720.

DNA was extracted from numerous pear and peach trees exhibiting foliar symptoms of pear decline (PD) and peach yellow leafroll (PYLR), respectively. Nucleotide sequence analyses of PCR-amplified 16S ribosomal RNA and 16/23S spacer regions showed that 3 distinct MLOs were associated with PYLR and 4 distinct MLOs were associated with PD. The 16S rRNA and spacer sequences of the most prevalent PYLR strain were identical to one of the PD-MLOs and this MLO was transmitted to peach seedlings in the insectary using field-collected pear psylla. These results confirm that pear is a reservoir and pear psylla are vectors of at least one of the PYLR-MLO strains present in California.

A77

HEAT ELIMINATION OF PLASMIDS FROM WESTERN ASTER YELLOWS MLO. C.D. Smart, A.H. Purcell*, S.R. Saunders* and B.C. Kirkpatrick. Dept. of Plant Pathology, University of California, Davis 95616 and *Dept. of Entomological Sciences, University of California, Berkeley 94720.

Aster leafhoppers (Macrosteles fascifrons) infected with the severe strain of western aster yellows (SAY) MLO were kept on ryegrass at 43 C for 24 or 48 h. Heat-treated vectors were fed individualy on healthy Plantago major. DNA was extracted from healthy, asymptomatic and symptomatic P. major, followed by Southern blot analysis using SAY genomic and plasmid probes. DNA from all symptomatic plants hybridized with the genomic probe, but the majority of the symptomatic plants inoculated by heat-treated insects did not hybridize with the SAY plasmid probe. Healthy and asymptomatic leafhopper-inoculated plants did not hybridize with either of the SAY probes. Symptoms expressed by heat-treated and nonheat-treated controls were indistinguishable. These results indicate that heat treatment can eliminate plasmids from SAY-MLO.

A78

ANALYSIS OF A WORLDWIDE COLLECTION OF *XANTHOMONAS CAMPESTRIS* PV. *VESICATORIA* STRAINS. H. Bouzar, S. R. Payne, J. B. Jones, N. C. Hodge, G. V. Minsavage, R. E. Stall, A. M. Alvarez, and A. A. Benedict. Gulf Coast Research & Education Center, University of Florida, 5007 60th Street East, Bradenton, Florida 34203

Physiological, chemical, and serological tests performed on 161 *X. c. vesicatoria* strains identified two phenotypic groups (i.e., A and B) within this pathovar. Group B strains hydrolyzed starch and degraded pectate, whereas group A strains did not. Silver-stained protein profiles, and cluster analyses of fatty acid content (MIDI) and carbon source utilization patterns (Biolog GN MicroPlate™) also differentiated the two groups. In addition, ELISA reactions using a panel of six monoclonal antibodies (MAbs) identified three serovars within each group. Three group B strains did not react with any MAbs.

A79

EPIPHYTIC BACTERIA FROM SEVEN BEAN (PHASEOLUS VULGARIS L.) GENOTYPES AND THEIR POTENTIAL FOR BIOCONTROL OF XANTHOMONAS CAMPESTRIS PV. PHASEOLI. R.B. Mabagala, Department of Crop Science, Sokoine University of Agriculture, P.O. Box 3005, Morogoro, TANZANIA.

Twenty two naturally occurring epiphytic non-pathogenic bacteria were isolated from reproductive tissues of seven bean genotypes grown in the field and screened both in vitro and in vivo for antagonism to Xanthomonas campestris pv. phaseoli. Three isolates exhibited antagonism to X.c. phaseoli. Two of the three isolates were identified as Bacillus sp and the third as Pseudomonas fluorescens. When screened in the green house in vitro, all the three bacterial antagonists delayed common blight symptom appearance for 2-3 days when spray-inoculated 2 days prior to X.c. phaseoli. The rate of common blight disease development was significantly reduced. These results suggest, phylloplane microflora from beans influence the development of common bacterial blight on the bean crop. These antagonists are promising potential biocontrol agents for bean common bacterial blight disease.

A80

EFFECT OF MINERAL NUTRIENTS ON PHENAZINE GENE EXPRESSION IN TWO *PSEUDOMONAS* SPP. BIOCONTROL STRAINS. D. K. Fujimoto and L. S. Thomashow, USDA-ARS Root Disease and Biological Control Unit, 362 Johnson Hall, Washington State University, Pullman, WA 99164-6430.

The disease severity of take-all on wheat in the field can be significantly reduced by the introduction of *Pseudomonas* spp. which produce phenazine-1-carboxylic acid (PCA) or its derivatives. To increase biocontrol efficacy, there must be a greater understanding of how soil characteristics may affect PCA production. We studied the effects of zinc and iron on phenazine gene expression in derivatives of *P. fluorescens* 2-79 and *P. aureofaciens* 30-84 which have *lacZ* fusions to the PCA biosynthetic locus. Strains were cultured in a defined medium where growth and gene expression, measured as β-galactosidase activity, were monitored over a 48 h incubation period. Both cell density and nutrient composition of the growth medium were manipulated. Significant differences were seen in the accumulation of the gene product between the two strains at different time points, although the onset of gene expression were similar.

A82

ISOLATION AND EXPRESSION OF A GENE INVOLVED IN INDOLE-3-ACETIC ACID BIOSYNTHESIS IN AN EPIPHYTIC *ERWINIA HERBICOLA* STRAIN. Maria Brandl, Ellen Clark, and S.E. Lindow. University of California, Department of Plant Pathology, Berkeley, CA 94720.

An epiphytic strain of *Erwinia herbicola* (299R) isolated from pear in California was shown to produce indole-3-acetic acid (IAA) via indole-3-pyruvic acid (IPyA) and

indole-3-acetaldehyde (IAAld) in culture amended with L-tryptophan. A gene that conferred the ability to synthesize IAAld and tryptophol (TOL) in *E. coli* DH5α, was isolated from a cosmid library of strain 299R. Marker exchange mutagenesis of a Tn3-Spice insertion into the putative indolepyruvate decarboxylase gene impaired production of IAAld and TOL in strain 299R and decreased IAA biosynthesis in culture by 10-fold. In radish root bioassays, the marker exchange mutant inhibited root elongation significantly less than strain 299R, indicating that it was also reduced in its ability to produce IAA on root surfaces. Fusion of this region to a promoterless ice nucleation gene revealed that the indolepyruvate decarboxylase gene was induced during late log-phase of growth in culture and at a low level on radish roots.

A83

A Viable Cell Enrichment, Two-step, Direct PCR Technique for Detection of Pseudomonas syringae pv. phaseolicola in Bean Seeds. N.W.Schaad[1], S.S.Cheong[1,2], S.Tamaki[2], E.Hatziloukas[2], & N.J. Panopoulos[2]. [1]USDA/ARS, Ft. Detrick, MD., and [2]Department of Plant Pathology, University of California, Berkeley, CA., 94720, USA.

A technique combining viable cell enrichment of the target pathogen on a general plating agar medium with a two-step, direct PCR was developed for the detection of P. s. pv. phaseolicola (PSP), causing bean halo blight. Bean seeds are washed in 0.001% Tween 20 for 15 hr and 100 μl aliquots are plated in triplicate onto King B agar medium. After incubation for 45 hrs at 25 °C, the plates are washed three times with 1 ml water and the pooled samples are directly used for PCR or stored at -20 °C for later processing. Ten-μl aliquots are amplified directly (w/o DNA extraction) for 30 cycles using "external" phaseolotoxin gene primers (Prosen et al. 1993, Phytopathology 83: in press). Two-μl of the amplified aliquot is then re-amplified for 25 cycles using "nested" primers. Ethidium bromide staining of the final PCR products was 10 times more sensitive than Southern blot analysis of one-step PCR products and enabled us to detect single cells of PSP in water. The method gave consistently positive results with seed washes containing 1-10 colony forming units/PCR aliquot, even though colonies of PSP could not be seen on the plates. Viable cell enrichment coupled with a two-step, direct PCR using nested primers greatly improves and simplifies detection and should prove very useful for routine detection of seedborne bacteria.

A84

ANTIBIOTIC PRODUCTION BY *ERWINIA HERBICOLA* STRAIN EH318 AND BIOLOGICAL CONTROL OF FIRE BLIGHT. S. V. Beer, S. Wright-Dobrzeniecka, R. S. Wodzinski, and C. H. Zumoff. Department of Plant Pathology, Cornell University, Ithaca, NY 14853 USA.

Strain Eh318 was isolated from a symptomless apple stem. In laboratory, controlled environment chamber and research orchard tests, it reduced the incidence and severity of fire blight caused by *E. amylovora*. When grown in minimal medium, Eh318 produced low molecular weight substances that inhibited the growth of *E. amylovora*. Several cosmid clones from a library of genomic DNA were identified that bestowed on *Escherichia coli* the ability to produce antibiotics that inhibited the growth of *E. amylovora*. One clone was mapped, subcloned, mutagenized and marker-exchanged into Eh318. The resulting mutant inhibited *E. amylovora in vitro*, indicating production of additional antibiotics. Other clones were identified that bestowed antibiotic production. The several antibiotics were distinguished by their bacterial spectra of activity and the effect of specific amino acids on inhibitory activity.

A85

COEXISTENCE AMONG EPIPHYTIC BACTERIAL POPULATIONS RESULTING FROM NUTRITIONAL RESOURCE PARTITIONING. M. Wilson and S. E. Lindow. University of California, Berkeley, 94720.

Replacement series experiments were used to assess the level of coexistence between *Pseudomonas syringae* and other members of the epiphytic bacterial community. Nutrient addition experiments determined that epiphytic bacterial populations were limited primarily by the availability of a pool of substitutable carbon sources in the phyllosphere. The *in vitro* carbon source utilization spectrum was determined for each strain. This spectrum was used to delimit the ecological niche of each strain. The extent of coexistence in each strain pair was related to the extent of non-overlap of the nutritional niches of the strains. Coexistence of strains apparently resulted from nutritional resource partitioning, or the ability of one strain to utilize additional (abundant) carbon sources. Near-isogenic strain pairs were used in replacement series experiments to demonstrate that increased coexistence could be achieved by the utilization by one of the strains of an additional carbon source provided either exogenously or endogenously.

A85a

AUTOMATION OF AMINOPEPTIDASE PROFILES IN 96-WELL PLATES. D.M. Huber[1], T.S. McCay-Buis[1], K.J. Miller[1], F.E. Lytle[1], J.P. Robinson[1] and B.C. Hemming[2]. Purdue University, W. Lafayette, IN 47907 and Microbe Inotech Labs, Inc., St. Louis, MO 63146.

Peptidase specificity of bacteria, fungi, and parasitic worms has been used to differentiate species and sub-species types; to characterize ecological niches and host-parasite interactions; to develop media for culture of fastidious organisms; and to identify single gene inserts in genetically engineered bacteria. Most of these studies have employed colorimetric or fluorescent substrates with incubation in small culture tubes and manual operation of detection equipment. This procedure can be automated by "fixing" fluorescent peptidase substrates in a 96-well plate, incubating a Tris-HCl (pH 8.0) suspension of a test organism (fungal spore, mycelial homogenate, or bacterium) in each cell, and reading in a 96-well plate reader with fluorescent detection capability (CytoFluor™ 2350, Millipore Corp.). "Fixed" substrates are stable for at least four months in the plates at 4 C. The required time for set up and analysis has been reduced from 30 to 2 min, and sensitivity is increased 2- to 4-fold.

A86

INFLUENCE OF CULTIVAR AND HOST AGE ON SUSCEPTIBILITY OF SOYBEAN TO *CALONECTRIA CROTALARIAE*. K. D. Kim, J. S. Russin, and J. P. Snow. Department of Plant Pathology and Crop Physiology, Louisiana Agricultural Experiment Station, Louisiana State University Agricultural Center, Baton Rouge, LA 70803.

Susceptibility of soybean cultivars to *Calonectria crotalariae* (Loos) Bell & Sobers was evaluated in the greenhouse using an isolate (SG915) determined to be virulent in laboratory inoculation tests. Ten-day-old plants of eleven soybean cultivars were inoculated with mycelial PDA discs (10 mm diameter) placed on the stem at the soil line. Areas under disease progress curves (AUDPCs) based on two disease rating measures, i.e., disease severity and lesion length, showed differential susceptibility to disease among tested cultivars. AUDPCs based on disease severity were lowest on Bedford, Deltapine 726, Hartz 6200, and Hartz 7126. Disease severity and lesion length were significantly correlated (Pearson correlation coefficient = 0.6195, P = 0.0001). Initial disease severity or lesion length on cultivars 5 days after inoculation determined further disease development over time. The effects of plant age at time of inoculation (10, 20, 30, 40 days after planting) on disease development following greenhouse inoculation were evaluated in three cultivars, Braxton, Deltapine 726, and Riverside 699. A significant quadratic relationship was described between plant age at inoculation and both lesion length (r^2 = 0.42, P = 0.0001) and perithecial production (r^2 = 0.031, P =0.046). Maximum lesion length and perithecial production occurred on inoculated 30- and 20-day-old plants, respectively. Host age significantly affected disease development regardless of cultivar.

A87

VARIATIONS IN VIRULENCE OF CULTURAL TYPES OF *RHIZOCTONIA SOLANI* AG-2-1 ON LETTUCE AND OTHER HOSTS. L.J.Herr. Dept. of Plant Pathology, OARDC/Ohio State Univ, Wooster, OH 44691.

Isolates of two cultural types of *Rhizoctonia solani* (designated types **a** and **b**), from field lettuce grown on organic soil, were classified as AG-2-1 by anastomosis (Phytopathology 82: 1046-1050). Determinations of temperature growth (<6-32C) and thiamine requirements (autotrophic), were both characteristic for AG-2-1. In greenhouse assays on lettuce, 10 of 11 AG-2-1 type **a** isolates were nonpathogenic (DR=1.00) and one was minimally virulent (DR=1.25) on a DR scale of 1=healthy, 2=diseased, 3=dead. Whereas, nine of nine type **b** isolates were virulent (DRs=1.50-3.00). In other assays, three isolates of each type **a** and **b** and two culturally distinct AG-2-1 tester isolates (type **c**) were tested on nine crops. The DR scale was 1=healthy, 2=mild lesions, 3=severe lesions, 4=dead plant. Type **b** isolates were consistently more virulent on lettuce (DR=2.78) than the type **a** isolates (DR=1.47) or type **c** isolates (DR=1.00). All isolates were nonpathogenic on corn, whereas, virulence on the remaining hosts was variable among isolates.

A88

INFLUENCE OF PRATYLENCHUS PENETRANS ON THE INFECTION AND COLONIZATION OF RUSSET BURBANK POTATO BY VERTICILLIUM DAHLIAE. Malek, C. J., A. E. MacGuidwin, and D. I. Rouse, Department of Plant Pathology, University of Wisconsin, Madison WI 53706.

Potato ('Russet Burbank') was grown in fumigated field plots which were infested with Pratylenchus penetrans (Pp) and Verticillium dahliae (Vd). Treatments were soil infestation with Vd alone, Vd plus Pp, and no pathogens (control). Roots and stems were assayed weekly for both organisms. Root segments (1 cm) were placed onto agar plates and scored for the presence or absence of the nematode and fungus. Sap was taken from sections of the stem and incubated on Sorensen's media. Colonies of Vd were counted. Nematode infection had no effect on the timing or incidence of root infection by the fungus. Nematode infection had no effect on the timing of stem infection by the fungus but had a positive effect on the quantity, as confirmed by greenhouse studies. In plots infested with Vd,

the biomass of the fungus increased in stems throughout the season, with the most pronounced increase at 15 weeks after planting.

A89

HISTOLOGICAL STUDIES OF CLADOSPORIUM CARYIGENUM INFECTION OF PECAN SHUCK TISSUES. H. Lee Campbell and A. J. Latham, Dept. of Plant Pathology, Auburn University, Auburn, AL 36849.

Scab causes loss of pecans unless *C. caryigenum* is controlled with fungicides. To study the histology of *C. caryigenum* infection of pecan shucks, Schley nuts were inoculated with conidia of the fungus. Nuts were covered with plastic for 24 hr incubation then uncovered. Shuck samples were collected at 12, 18, 24, and 36 days for chemical treatment and tissue study. Twelve days after inoc., *C. caryigenum* conidia were formed from conidiophores borne on superficial stroma on the shucks. Hyphae were found invading epidermal cells at the 18-day interval. At 24 days, mycelium of *C. caryigenum* occurred between sub-epidermal cells. At 36 days, epidermal cells had collapsed and mycelia had penetrated 6 to 8 cells deep and toward the vascular system. Studies of host-parasite relations beyond 36 days after inoculation are needed to evaluate how scab affects the shuck tissue systems to reduce or stop nut maturation.

A90

CHARACTERIZATION OF PHYTOTOXIN(S) INVOLVED IN SUDDEN DEATH SYNDROME OF SOYBEAN. H. Jin[1], J. M. Widholm[2], and G. L. Hartman[3]. [1]Dept. of Plant Pathology, [2]Dept. of Agronomy, [3]USDA/ARS, University of Illinois at Urbana-Champaign, 1102 S. Goodwin Ave., Urbana, IL 61801.

The phytotoxin(s) of a blue isolate of *Fusarium solani*, the causal agent of sudden death syndrome of soybean, were characterized using a bioassay employing calli derived from soybean hypocotyls. Calli of susceptible cvs. A3427 and Spencer were cultured on a medium containing 5% culture filtrate of *F. solani*. Two days after transferring the calli to the medium, tissue browning was recorded visually and by the absorbency at 330 nm of acetone extracts of calli. Absorbency was significantly ($P < 0.05$) positively correlated ($r = 0.92$) to the volume of culture filtrate incorporated into the medium. The reaction of calli derived from cvs. A3427, Chamberlain, Jack, Ripley and Spencer to fungal culture filtrate were consistent with the reaction of these cvs. as seedlings inoculated with the fungus in the greenhouse with A3427 and Spencer being most susceptible and Ripley being least susceptible. Culture filtrates were extracted by ethyl acetate; treated by autoclaving, dialysis, ion exchange, gel filtration, 10% charcoal, and proteinase K; and precipitated by ammonium sulfate. Following these treatments, calli were used to detect the toxin(s). The main phytotoxin(s) was water soluble, heat unstable, and negatively charged with a molecular weight around 12-30 kDa. The phytotoxin(s) was absorbed by 10% charcoal, precipitated by ammonium sulfate (60-100%), and destroyed by proteinase K.

A91

RAPD ANALYSIS DIFFERENTIATES RACE NON-CLASSIFIABLE ISOLATES FROM KNOWN RACES OF *PHYTOPHTHORA SOJAE*. M. J. Jones, D. H. Scott, P. W. Reeser, and L. D. Dunkle*. Department of Botany and Plant Pathology, and *USDA-ARS, Purdue University, West Lafayette, IN 47907-1155.

A group of fast-growing isolates with taxonomic features consistent with *Phytophthora megasperma* f.sp. *glycinea* (*P. sojae*) were previously designated race-non-classifiable (RN), because they displayed unusual virulence patterns on a set of soybean differentials (Reeser *et al.* Phytopathology 81:1201. 1991). These isolates were analyzed for polymorphic DNA by PCR amplification with arbitrary primers (RAPD). The amplification products were compared with those from isolates of defined races of *P. sojae*. Amplification products from isolates of the identifiable races were nearly identical with a given primer but distinctly different from those of the RN isolates. Amplification products of the RN isolates were very similar, indicating a definite relationship among them. The results suggest that these pathogenic isolates are not *P. sojae* but may be another species of *Phytophthora* and a new pathogen of soybeans.

A92

THE ROOT ROT DISEASE OF COCOYAM IN CAMEROON. R. P. Pacumbaba, J. T. Tambong, and L. M. Nyochembeng. Department of Plant and Soil Science, Alabama A&M University, Normal, AL 35762.

Pythium myriotylum was isolated from the rhizosphere of root rot-infected cocoyam and from infested soil by the cocoyam leaf disc baits. *Fusarium solani* and *Rhizoctonia solani* were also isolated from the same soils by the water dilution method as well as from the roots of diseased cocoyam, and were always accompanied by *P. myriotylum*. Pathogenicity tests of the isolated pathogens on 3 and 7 months-old-cocoyam plantlets indicated that only *P. myriotylum* caused cocoyam root rot disease (CRRD). *P. myriotylum* isolates grew faster in a 24 h period at 31 C on lima bean sucrose agar, V-8 juice sucrose agar, and potato sucrose agar than on potato dextrose agar or water agar. *P. myriotylum* propagules entered the roots of cocoyam directly by means of an infection peg within 6 to 7 h and the invasion was inter- and intracellular. The concentrations of *P. myriotylum* propagules causing infection and symptoms of CRRD was 200 zoospores/ml or 180 mycelial strands/ml in 3 to 6 days, indicating that the pathogen is very destructive in cocoyam. The production of zoospores for maintaining pure isolates of *P. myriotylum* is an additional alternative to mycelial tip transfer method if cultures have been contaminated with other microorganisms. Screening period of cocoyam suckers against *P. myriotylum* was 21 days in the greenhouse and 3 months in the field with natural CRRD incidence. There are at present 42 resistant and 35 tolerant identified cocoyam accessions of the Root and Tuber Research Project in Cameroon.

A93

STATUS OF WEEDS AS HOSTS FOR *RHIZOCTONIA SOLANI* AG-1, CAUSAL AGENT FOR AERIAL BLIGHT OF SOYBEAN (*GLYCINE MAX*) IN LA. B. David Black, J. S. Russin, J. L. Griffin and J. P. Snow. Louisiana State University Ag. Center, Dept. of Plant Path. & Crop Phys. Baton Rouge, 70803.

Greenhouse experiments were conducted in 1993 to determine host status of weed species for *Rhizoctonia solani*, causal agent of aerial blight on soybean. Two isolates of the fungus (intraspecific group IA and IB) were grown on potato dextrose agar, macerated using a Waring blender, and applied to plants using an air-pressurized sprayer until runoff occurred 49 and 52 days after planting. Inoculum suspension ranged from 421,250 - 618,750 mycelial fragments / ml. After inoculation, plants were grown under polyethylene covered greenhouse benches containing a misting system to provide 100% relative humidity. Weed hosts were identified by infected tissue and the presence or absence of sclerotia / microsclerotia 81 days after second inoculation. Colonized tissue and/or sclerotia were identified on *Sorghum halepense* (IA & IB), *Cassia obtusifolia* (IA), *Sesbania exaltata* (IA & IB), *Echinochloa crus-galli* (IA), *Brachiaria platyphylla* (IA & IB), *Digitaria sanguinalis* (IA), *Rottboellia cochinchinensis* (IA & IB), *Amaranthus hybridus* (IB), *Sida spinosa* (IA & IB), *Xanthium strumarium* (IA & IB), *Aeschynomene virginica* (IA & IB), *Ipomoea hederacea* var. *integriuscula* (IA & IB), *Melochia corchorifolia* (IA & IB), and *Cyperus rotundus* (IA & IB). In 6 of 7 weed species observed, *R. solani* moved from the weed to a noninfected soybean plant in close proximity.

A94

SEEDLING EMERGENCE, PLANT HEIGHT, AND SHOOT FRESH AND DRY WEIGHTS OF FESCUE FREE OR INFECTED WITH ACREMONIUM COENOPHIALUM AND/OR COCHLIOBOLUS SATIVUS. L. E. Trevathan, Dept. of Plant Pathology and Weed Science, Mississippi State University, Mississippi State, MS 39762.

The object of this study was to determine the response of tall fescue (Festuca arundinacea) varieties to the endophytic fungus, Acremonium coenophialum, and Cochliobolus sativus. Triumph and Kentucky 31 free of the endophyte, and Kentucky 31 infected with the endophyte, were grown in the greenhouse in fumigated soil or fumigated soil infested with milled corncob grits coated with C. sativus inoculum. Emergence, seedling establishment, and growth of leaves and shoots were determined weekly for 6 wk. Seedling survival, plant growth, and shoot fresh and dry weights were reduced as a result of infection by either or both fungi. The interactive effects of A. coenophialum and C. sativus were less than additive for all parameters measured.

A108

COMPETITIVE INFECTION ABILITY AND GENETIC DIVERSITY WITHIN AND AMONG VEGETATIVE COMPATIBILITY GROUPS OF NONPATHOGENIC ISOLATES OF FUSARIUM OXYSPORUM FROM TOMATO. M. M. Lear, R. W. Schneider, and K. S. Elias. Dept. Plant Path. & Crop Physiol., La. Agric. Exp. Sta., LSU Agric. Cen., Baton Rouge, LA 70803.

Nonpathogenic isolates of Fusarium oxysporum were isolated from symptomless tomato roots and assessed for vegetative compatibility and competitive infection ability against the tomato wilt pathogen, F. oxysporum f. sp. lycopersici. There were significant differences in competitive infection ability among VCGs. Nonpathogenic isolates also were subjected to isozyme analysis (starch gel electrophoresis) in order to estimate genetic diversity within and among vegetative compatibility groups (VCGs). A total of 39 isolates, including three pathogenic strains, and 15 enzymes were assessed. Principal components analysis revealed that each nonpathogenic VCG is a distinct clonal population which suggests that competitive infection ability is a fitness trait that is prone to selection pressures.

A113

RESPONSE OF HIGHLY RESISTANT SOYBEAN PLANT INTRODUCTIONS TO *MELOIDOGYNE ARENARIA* RACES 1 AND 2. E. M. R. Pedrosa, R. S. Hussey, and H. R. Boerma, Dept. Plant Pathology, University of Georgia, Athens, GA 30602-7274.

Two soybean plant introductions, PI230977 and PI200538, highly resistant to *Meloidogyne arenaria*, were evaluated for seed yield, and their effects on second-

stage juvenile soil population densities and root penetration in field microplots. The plant introductions were compared to moderately resistant Jackson, and susceptible CNS, at increasing initial soil population densities (Pi) (0, 31, 125, 500 eggs/100 cm^3 soil) of *M. arenaria* races 1 and 2. Yield suppressions of CNS, Jackson, PI230977, and PI200538 were 55, 28, 31, 29%, and 99, 86, 66, 58% respectively, for races 1 and 2, at the highest Pi when compared with uninfested control plots. Juveniles present in roots 14 days after planting did not differ among the three resistant genotypes at the same Pi level, although at the highest Pi fewer juveniles were present in roots of both plant introductions than in roots of CNS and Jackson. Soil second-stage juvenile population densities differed between races 1 and 2 rather than among soybean genotypes within race. For the resistant genotypes, juvenile soil densities 135 days after planting were 96% greater in microplots infested with race 2 than with race 1.

A116

EPIDEMIOLOGY OF STEMPHYLIUM LEAF SPOT AND PURPLE SPOT IN NO-TILL ASPARAGUS. M.K. Hausbeck. Dept. of Botany and Plant Pathology, Michigan State University, East Lansing, MI 48824.

Atmospheric concentrations of conidia and ascospores of the asparagus purple spot and leaf spot pathogen *Stemphylium vesicarium* (teleomorph *Pleospora allii*) were monitored with Burkard volumetric spore traps in two no-till asparagus fields near Hart, MI from 21 April to 31 August, 1992. Weather recording instruments in each field provided hourly records of temperature, rainfall, relative humidity, and leaf wetness. Peak concentrations of ascospores were usually associated with rainfall events. Purple spot disease developed on spears in the field when harvesting occurred $\geq$48 hours following ascospore discharge prompted by rainfall. During the harvesting period of 10 May to 19 June, atmospheric concentrations of conidia were low ($<5/m^3$/hr) but increased later in the season. From 14 July to 31 August, leaf spots on asparagus fern increased following peak concentrations of ascospores and conidia. Ascospores from pseudothecia on overwintering asparagus debris on the soil surface were the primary source of inoculum for infection of spears. Conidia developing later in the season served as an additional inoculum source for infection of the fern.

A117

ETIOLOGY OF FUSARIUM WILT OF BASIL IN SOUTH CAROLINA. A. P. Keinath, Clemson University, Dept. of Plant Pathology & Physiology, Coastal REC, Charleston, SC 29414.

Fusarium wilt of basil (*Ocimum basilicum*) was first detected in South Carolina in a commercial greenhouse in spring 1992. Four isolates of *Fusarium oxysporum* were obtained from stem or root vascular tissue of bush 'Minimum' and sweet basil. The pathogen was seedborne in bush basil; the source of inoculum in sweet basil has not been determined. All isolates were highly virulent to both types of basil when inoculated with 10 mL of 10^7 microconidia/mL. Height and leaf area of sweet basil were significantly ($P \leq 0.01$) reduced by 30% and 40%, respectively, 15 days after inoculation. *F. oxysporum* from sweet basil also was weakly virulent to lemon basil (*O. basilicum* 'Citriodora'), but was nonpathogenic to other herbs in the Lamiaceae family, including catmint (*Nepeta cataria*), lemon balm (*Melissa officinalis*), oregano (*Origanum vulgare*), rosemary (*Rosmarinus officinalis*), sage (*Salvia officinalis*), and thyme (*Thymus vulgaris*). *F. oxysporum* was recovered from the stems of 0 to 60% of inoculated nonsusceptible herbs. Fusarium wilt of basil is a potentially destructive disease of *O. basilicum*, but not of other herbs in the Lamiaceae.

A118

YIELD SUPPRESSION IN PROCESSING TOMATO CAUSED BY THE INTERACTION OF VERTICILLIUM DAHLIAE AND PRATYLENCHUS PENETRANS. D. R. Hanmer and R. M. Riedel, Department of Plant Pathology, The Ohio State University, Columbus, OH 43210.

A field study was done using 30 cm diam. clay tile microplots in fumigated (MBC-33, 76 kg/ha) Kokomo silty clay loam (pH 7.1). Treatments (15 reps each) were: control, High Verticillium dahliae, race 1 (Vd) [100 microsclerotia (ms)/cm^3], Low Vd (10 ms/cm^3), Pratylenchus penetrans (Pp) (20 vermiforms/100 cm^3), High Vd and Pp, Low Vd and Pp. Tomato transplants (cv. H 7155) were Vd race 1 resistant. Total fruit yield and fresh weight of vines were significantly reduced by the combination of high Vd and Pp. Neither Vd nor Pp alone had a significant effect on either variable. Percent fruit set was significantly reduced only in those treatments that had both Vd (high or low) and Pp present. There were no significant treatment effects for: number of blossoms per inflorescence, date of first pink fruit, foliar symptoms, or vessel discoloration. Vd and Pp interact synergistically and result in significant reductions in growth and yield on a Verticillium-resistant processing tomato.

A119

QUANTIFICATION OF VASCULAR COLONIZATION OF POTATO STEM BY *VERTICILLIUM ALBO-ATRUM*. Tarkus Suganda and Neil A. Anderson, Department of Plant Pathology, University of Minnesota, St. Paul, MN 55108.

Verticillium albo-atrum Rheinke & Berth. (VA) and *V. dahliae* Kleb. (VD) are the causal agents of Verticillium wilt (VW) disease of potato. Levels of vascular colonization by VD have been used as an indication of resistance in potato. Quantification of vascular colonization of potato stems by VD is quantified by plating basal stem juice in a water agar-ethanol-streptomycin-penicillin G medium. However, such a medium does not detect VA. The Christen medium with chloramphenicol, chlortetracycline and streptomycin sulfate, has been successfully used to detect and quantify vascular colonization of potato stems by VA. Colony counts from basal stem of Kennebec, a susceptible cv., infected with VD were ca. 50,000 CFU/ml stem juice and ca. 2,000 CFU/ml when infected with VA. In Reddale, a resistant cv., vascular colonization was ca. 100 CFU/ml when inoculated with VD and ca. 30 CFU/ml with VA.

A120

A NEW RACE OF *FUSARIUM OXYSPORUM* F. SP. *MELONIS* CAUSING WILT OF MUSKMELON IN NEW YORK. T. L. Zuniga and T. A. Zitter, Dept. of Plant Pathology, Cornell University, Ithaca, NY 14853.

Fusarium wilt (*Fusarium oxysporum* f. sp. *melonis*) was very severe on muskmelon in New York in 1992 with the cultivar Saticoy, which is resistant to race 2, collapsing in Washington Co. Three isolates were collected and identified as race 1 on the differentials Topmark, Topmark FR, CM 17-187, and Perlita FR (Risser et al. 1976 Phytopath. 66:1105; Martyn et al. 1987 Plant Dis. 71:469). Three isolates from the same farm in Washington Co. collected in 1985, two from Monroe Co. (1985), and three from Erie Co. (1986) were all identified as race 2. The only previous occurrence of race 1 in the United States was in Maryland in 1985. A survey is underway to determine the current distribution of races within the state.

A121

EFFECTS OF MECHANICAL PLANT DESTRUCTION ON POTATO TUBER BLACK SCURF CAUSED BY RHIZOCTONIA SOLANI. D. A. Inglis and L. Johnson, Washington State University-Mount Vernon REU, 1468 Memorial Hwy, Mount Vernon, WA 98273.

Haulm pulling, root undercutting, propane burning, stem cutting (at soil line) and vine chopping were compared to chemical vine killing and natural senescence in 1991 and 1992 experimental field studies that investigated mechanical methods for scurf control. On cv Red LaSoda only chopping was comparable to natural senescence in significantly reducing the incidence of Rhizoctonia sclerotia on tubers. Scurf indices for haulm pulling and chemical desiccation treatments were similar but significantly lower compared to those for propane burning and stem cutting. Sclerotial deposition increased the most between 7 and 14 days following the time of vine kill and appeared to be correlated with periderm maturation. At harvest, 21 days later, plants exposed to vine destruction treatments in which skin set was delayed had tubers with the least Rhizoctonia sclerotia.

A122

LEAF SPOT OF ERUCA VESICARIA CAUSED BY CHOANEPHORA SP. R. T. McMillan, Jr. and W. R. Graves, University of Florida, IFAS Tropical Research and Education Center, Homestead, FL 33031

Leaf spot caused by Choanephora sp. has been observed this summer on Eruca vesicaria, commonly called rugula, grown in a plastic covered quonset screen house. The rugula are hydropnicaly grown as a salad green, for the fresh herb market. The plants have been grown throughout several years but this is the first season that Choanephora was a problem. The invaded leaf tissue of the plant was water-soaked with a soft wet-rot but without any white mycelial weft being present. Under dry conditions the leaf spot appeared as a bleached whitish lesion. Conidiaphores formed on the lesions which were unbranched and had a spherical head which produced capituli that were rounded and bore sterigmata. Lemon-shaped, continuous, striate conidia formed on the sterigmata.

A123

THE OCCURRENCE OF RACE 3 OF FUSARIUM OXYSPORUM F.SP. LYCOPERSICI IN ARKANSAS. **M. Marlatt and J. C. Correll. Department of Plant Pathology, University of Arkansas, Fayetteville, AR 72701.**

In 1992, isolates of Fusarium oxysporum were recovered from symptomatic tomato plants from two commercial fields in Arkansas. The cultivar Bradley (resistant to FOL race 1) was being grown in one field and the cultivar Mt. Delight (resistant to FOL race 1 and 2) was being grown in the second field. Monoconidial isolates from both fields were tested for pathogenicity on a set of differential tomato cultivars (Bonny Best, VC82-L, MH-1, and I3R-1) and for vegetative compatibility using nitrate nonutilizing mutants. Known reference isolates of races 1, 2, and 3 were included as positive controls. All of the isolates recovered from the cv. Bradley were identified as race 2 and belonged to a previously identified VCG (VCG 0032). The isolates recovered from cv. Mt. Delight were identified as race 3 and 50 isolates, collected from symptomatic plants from throughout the field, belonged to one VCG (VCG 0033). The race 3 isolates from Arkansas were not vegetatively compatible with any race 3 isolates from FL, CA, or Australia that were tested. A single race 3 isolate obtained from North Carolina also was in VCG 0033.

A124

SCREENING CUCUMBERS FOR ANTHRACNOSE DISEASE RESISTANCE. **L. A. Wasilwa, J. C. Correll, and T. E. Morelock. Depts. of Plant Pathology and Horticulture and Forestry, University of Arkansas, Fayetteville, AR 72701.**

The Colletotrichum orbiculare population is made up of two genetically distinct races, race 1 and 2, which can be differentiated by both virulence and vegetative compatibility. Race 1 isolates originated from cucumber or cantaloupe and race 2 isolates originated from watermelon or cucuzzi gourd. Eighty cucumber cultivars and breeding lines were evaluated for resistance to race 1 and 2 in a cotyledon inoculation test. Cotyledons were inoculated with a spore suspension (8×10^4 spores/ml) of a composite of six geographically diverse race 1 isolates or race 2 isolates. Symptoms were assessed on cotyledons on a scale of 0 -7 with 0 = healthy and 7 = 100 % disease. Cultivars were classified as highly resistant (0.5-2.5), moderately resistant (2.6-5.0), or highly susceptible (5.1-7.0). Most cucumber cultivars were highly susceptible to race 1 and only four were moderately resistant. However, many of the cultivars were either moderately or highly resistant to race 2. The highly resistant cucumbers have either been observed or reported to be resistant to race 1 under field conditions. Thus, the watermelon pathogen (race 2) was more effective for screening for resistance to race 1 in cucumbers than the race 1 pathogen; apparently the race 1 pathogen is too aggressive under these conditions and may overwhelm any inherent resistance. Work is in progress to see if resistance at the cotyledon and true leaf stage is correlated and if the resistance observed in the greenhouse is correlated with field resistance.

A125

CONTROL OF SEEDLING DISEASES OF SNAP BEANS IN WEST TENNESSEE. C. H. Canaday, Dept. of Entomology & Plant Pathology, and J. E. Wyatt, Dept. of Plant and Soil Science, Univ. of Tennessee, West Tennessee Experiment Station, Jackson, TN 38301.

The effects of seed treatment fungicides, herbicides, and nitrogen fertilizers on snap bean seedling diseases caused by *Pythium* spp. and *Rhizoctonia solani* were evaluated in a series of field experiments conducted in 1989 through 1992 in fields naturally infested with the pathogens. No interactions between seed treatments and the herbicides (metoachlor, trifluralin, or pendimethalin) were detected. Use of metolachlor instead of trifluralin depressed yields but not stands in summer plantings while not affecting yields in spring tests. In several of the tests, stands and yields were increased by >45% when calcium nitrate instead of ammonium nitrate was used with a metalaxyl-quintozene dust. Best disease control overall (in both spring and summer plantings) was obtained with a captan + metalaxyl seed treatment. For summer plantings in warm soils, highest stands and yields were with a chloroneb + metalaxyl seed treatment. The latter was comparable to use of in-furrow fungicides.

A126

VERTICILLIUM WILT OF CAULIFLOWER IN CALIFORNIA. S. T. Koike[1], K. V. Subbarao[2], R. M. Davis[2], and T. R. Gordon[3]. [1]University of California Cooperative Extension, Salinas 93901, [2]Dept. of Plant Pathology, Univ. of Calif., Davis 95616, and [3]Dept. of Plant Pathology, Univ. of Calif., Berkeley 94720.

During the past two years, commercial cauliflower in coastal California has been severely affected by a vascular wilt disease. Symptoms consist of chlorosis, defoliation, stunting, wilting, and vascular discoloration. *Verticillium dahliae* was consistently isolated from xylem tissue in stems and roots of affected plants. Disease was widespread and caused significant damage in summer and fall crops. Pathogenicity was established by dipping roots of 30-day-old seedlings (cv. 'White Rock') into conidial suspensions (10^7 conidia/ml) for 5 min. Control plants were dipped into sterile distilled water. All plants were potted into autoclaved soil and incubated in a greenhouse (23/10 C day/night regime). After 3 wk, inoculated plants became stunted and chlorotic, and *V. dahliae* was reisolated from them. Control plants were symptomless. Soil from commercial fields was assayed for microsclerotia (ms) using the modified Anderson Sampler and plating onto NP-10 selective medium. Assays showed that *V. dahliae* was widely distributed in the Salinas Valley, and propagule densities were as high as 93 ms/g soil. Evaluation of cauliflower cultivars in infested fields indicated that all were susceptible. This new disease has become a major threat to cauliflower production in coastal California.

A127

PERSISTENCE OF *COLLETOTRICHUM COCCODES* ON TOMATO ROOTS AND IN SOIL. H. R. Dillard and A. C. Cobb, Cornell University, New York State Agricultural Experiment Station, Dept. of Plant Pathology, Geneva, NY 14456.

Overwintering of *C. coccodes* (CC) on tomato roots was determined in 6 commercial fields of tomatoes grown for fresh market and processing in 1990 and 1991. Three different locations were selected each autumn. The tomato fields were divided into 10 sections, and 10 plants were collected randomly from each section, for a total of 100 plants per field. Thirty root pieces, 1 to 1.5 cm long, were subsampled from each root system, surface disinfected and incubated on V-8 juice agar plates. After 2 to 3 weeks, the number of root pieces from which CC was recovered was determined. The same fields were sampled in the same manner in the spring of 1991 and 1992. Populations of CC were determined using the same technique. In all but one field, there was no signficant decrease in viability of the fungus after the winter months. In a separate study, sclerotia of CC and colonized tomato fruit skins were placed 0, 10 and 20 cm deep in soil on November 15, 1988. Viability of CC was determined at regular intervals. In December of 1992, survival remained near 50% at all depths, with greater variability in the tomato skin.

A128

THE OCCURRENCE OF ALTERNARIA RADICINA IN SOIL AND THE EFFECT OF DEEP TILLAGE ON THE INCIDENCE OF BLACK ROT DISEASE OF CARROTS. B. Pryor, R. M. Davis, and R. L. Gilbertson, Department of Plant Pathology, University of California, Davis, CA 95616.

Alternaria radicina, causal agent of black rot of carrots, was recovered from soil by plating soil dilutions directly onto a semi-selective medium, Alternaria Radicina Selective Agar (ARSA). Characteristic A. radicina growth developed on ARSA and was readily distinguished from that of other soilborne fungi. Soil populations of A. radicina in selected fields in the Cuyama Valley, CA, ranged from 0-317 cfu/g soil and occurred in a clumped distribution. A. radicina soil populations were positively correlated with severity of black rot ($Y=0.94+(0.013)X$, $r=0.95$). Deep tillage was evaluated for black rot management. Pre-plant tillage to a depth of 12" using a moldboard plow significantly reduced black rot incidence in a field with a high soil population of A. radicina but had little effect on disease development in a field with a low soil population.

A129

INFLUENCE OF SOIL TEMPERATURE AND MICROFLORA ON THE INCIDENCE OF PYTHIUM-INDUCED ROOT DIEBACK OF CARROT. R. M. Davis and J. J. Nunez. Department of Plant Pathology, University of California, Davis 95616.

Pythium-induced root dieback of carrots in the Central Valley of California often occurs in fields planted in late summer or early fall following a fallow period of 2-4 mo. In a 3 yr study, replicated plots were covered with straw or irrigated by sprinklers every 4-6 days throughout the summer to moderate temperatures in the top of the soil profile. The 2 mo average temperature 7.6 cm deep was 28°C in the shaded plots and 45°C in the nonshaded plots. In one year, carrot stands and yields were increased by shading the soil prior to planting and the incidence of forked carrots was reduced from 5.7% to 3.6%. Total bacteria and microbial activity (determined by fluorescein diacetate hydrolysis assay) were reduced in fully exposed soil. It is suggested that the number of Pythium antagonists was reduced in the naturally solarized soil.

A131

EVIDENCE FOR PENTAKETIDE WALL-BOUND PIGMENTS IN PENICILLIUM SPP. AND ASPERGILLUS SPP. M. H. Wheeler and M. A. Klich, USDA, ARS, Cotton Pathology Research Unit, Rt. 5, Box 805, College Station, TX 77845 and USDA, ARS, SRRC, P. O. Box 19687, New Orleans, LA 70179.

The fungicide tricyclazole, at 8 or 30 ug/ml, when added to malt extract agar (MEA), potato dextrose agar, Czapek yeast autolysate agar, and a specialized alkaline agar medium (AAM), inhibited the production of green pigments in conidial cell walls of all fifteen Penicillium spp. tested and three out of six Aspergillus spp.. Tricyclazole also caused the accumulation of the pentaketide melanin- related metabolite flaviolin in AAM cultures of these fungi. This suggests that some Penicillium and Aspergillus species have a biosynthetic pathway similar to the tricyclazole-sensitive pathway previously demonstrated in several brown to black imperfect and Ascomycetous fungi. Homogenates from most of the Penicillium and Aspergillus species grown on MEA and AAM converted scytalone to 1,3,8-trihydroxynaphthalene. The presence of this known dehydratase conversion that occurs in the pentaketide melanin pathway, suggests that pigment biosynthesis in the green colored fungi may be similar to that in the brown to black fungi.

A132

MATING POPULATIONS OF *GIBBERELLA FUJIKUROI* IN ASPARAGUS FIELDS. W. H. Elmer, The Connecticut Agricultural Experiment Station, Box 1106, New Haven, CT 06504.

Isolates of two morphologically distinct anamorphs of *Gibberella fujikuroi, Fusarium moniliforme* and *F. proliferatum* in mating populations A and D, respectively, were highly pathogenic on asparagus in greenhouse trials (*Mycologia* 84:253-257). The occurrence of these mating populations in asparagus fields was not known. During 1985-1992 *Fusarium* spp. were isolated from asparagus stalks and feeding insects from several fields in CT, MI, and MA, The majority of these isolates bore conidia in long chains and lacked chlamydospores on KCl agar; these were single spored and placed in storage. Conidia of 238 wild type isolates were paired on carrot agar against known female tester strains of *F. moniliforme* or *F. proliferatum*. The appearance of perithecia with exuded ascospores was evidence for assigning an isolate into a mating population. Ninety-five % of the isolates were assigned to mating population D; the remaining 5% could not be classified into either mating population. Of those assigned to mating population D, between 62-95% (average = 82%) per field were typed as the + strain. *F. moniliforme* (mating population A) may have no role in asparagus crown and root rot.

A133

ASSESSMENT OF VEGETATIVE COMPATIBILITY GROUPS OF VERTICILLIUM DAHLIAE ISOLATED FROM ORNAMENTAL WOODY PLANTS. W. Chen. Illinois Natural History Survey, 607 East Peabody Drive, Champaign, IL 61820.

Forty-five strains of Verticillium dahliae isolated from diverse ornamental woody plants obtained from Illinois, Indiana, New Jersey, Ohio and Massachusetts were studied by assessing their VCGs. More than 120 nitrogen non-utilizing mutants were generated and their phenotypes were determined. Frequently only one type of mutants was obtained from a given wild type strain. The nit 1 mutants were tested for compatibility with the nitM mutants in all possible combinations. In addition, every nit 1 mutant was tested with previously determined nitM tester strains. Most of the V. dahliae strains from woody ornamental plants belonged to VCG1. Implications of the results on the inoculum source of V. dahliae in ornamental woody plants will be discussed.

A135

REASSESSMENT OF HETEROKARYON FORMATION IN *THANATEPHORUS PRACTICOLA* (ANAMORPH: *RHIZOCTONIA SOLANI* AG-4). M. A. Cubeta, R. Briones-Ortega and R. Vilgalys, Dept. of Botany, Duke University, Durham, NC 27708.

Heterokaryon formation was evaluated using homokaryotic strains of *Thanatephorus practicola* (anamorph: *Rhizoctonia solani* AG-4). Previous mating studies with *T. practicola* have utilized hyphal tuft formation between paired homokaryons as the primary criterion for detection of heterokaryons. In this study, somatic incompatability reactions and genetic markers were used to confirm heterokaryon formation. Sixteen, single basidiospore strains from 10 different heterokaryotic field isolates of *T. practicola* were paired in all possible combinations and assessed for tuft formation. Of 136 pairings, 25% (7 of 28) and 54% (58 of 108) of sib and nonsib pairings, respectively, produced visible tufts that varied in size and color. Morphologically and somatically distinct heterokaryons were detected from either aerial tuft hyphae or the hyphal interaction zone in 20% of the nonsib pairings. Heterokaryons were also detected on one or both sides of the heterokaryotic tuft, indicating that heterokaryotization was not restricted to the zone corresponding to the tuft. Randomly amplified polymorphic DNA (RAPD) markers were used to confirm 22 putative heterokaryons (16 from tufts and 6 not from tufts). These results suggest that heterokaryons can also develop in the absence of tuft formation.

A136

CHARACTERIZATION OF NUCLEAR SMALL-SUBUNIT RIBOSOMAL DNA FROM *PLASMODIOPHORA BRASSICAE*. L.A. Castlebury and L.L. Domier*, Dept. of Plant Pathology, Univ. of Illinois, Urbana, IL 61801 and *USDA-ARS, Urbana, IL 61801.

Plasmodiophora brassicae Woron., the causal agent of clubroot in cabbage, is a member of the Plasmodiophoromycetes, a group of obligate parasites of various higher plants, algae and Oomycetes. Plasmodiophoromycetes have been classified in various ways as both protists and fungi since Woronin established the genus *Plasmodiophora* in 1877. In this study, *P. brassicae* was propagated in Chinese cabbage plants, resting spores were purified from host material and other contaminants, and DNA was extracted. The nuclear small-subunit ribosomal RNA genes were amplified using PCR, producing an approximately 3 Kb DNA fragment which has been cloned into a plasmid vector. Sequences at the highly conserved ends of both strands of this unusually long fragment have been determined and show close similarities to other ribosomal DNA sequences in GenBank. The remainder of the sequence will be also be characterized.

A137

K. O'Donnell[1], G. Samuels[2], H. Nirenberg[3]. Molecular Genetic Relationships within Fusarium solani. NCAUR, USDA, ARS[1], Peoria, IL 61604; BARC, USDA, ARS[2], Beltsville, MD 20705; and Federal Biological Research Centre[3], D-1000 Berlin, Germany.

Current classification schemes for Fusarium are based exclusively on cultural characteristics together with microanatomy of the anamorph(s). In the treatment of fusaria within sections Martiella and Ventricosum, the taxonomic systems of Gerlach and Nirenberg [1982] and Nelson et al. [1983] are at opposite ends of the taxonomic spectrum, while the scheme proposed by Booth [1971] occupies an intermediate position. We have sequenced three regions of rDNA from over 60 members of these sections to determine the relationship between the morphologically-based taxonomic schemes and the rDNA gene trees. The sequence data show that the system of Gerlach and Nirenberg [1982] is by far the most accurate reflection of the phylogeny of these fusaria while the taxonomic system of Nelson et al. [1983] is the most artificial.

A138

SYNTHESIS OF ERICOID MYCORRHIZAE IN CRANBERRY BY *OIDIODENDRON GRISEUM* AND A DARK NON-SPORULATING FUNGUS. S.E. Keates and L.M. Carris, Dept. of Plant Pathology, Washington State University, Pullman, WA 99164-6430.

Fungi isolated from roots of cranberry, *Vaccinium macrocarpon*, were tested for mycorrhizal synthesis with 10-12 d old cranberry seedlings. *Oidiodendron griseum* and a dark non-sporulating fungus (DNF) formed hyphal coils typical of ericoid mycorrhizae in root cortical cells of inoculated seedlings. Mycorrhizal colonization percentages were significantly higher in seedlings inoculated with the DNF (39%) as compared to those inoculated with *O. griseum* (24.5%). This is the first report of mycorrhizal synthesis in cranberry by cranberry root-colonizing fungi. Neither fungus tested was present in aerial tissues of the host. Based on these findings, the practice of planting stem cuttings into fumigated soils to establish new cranberry beds may need to be reassessed.

A139

AN ASSESSMENT OF THE RELATEDNESS OF SUBPOPULATIONS WITHIN *FUSARIUM OXYSPORUM* f. sp. *MELONIS* BASED ON DNA FINGERPRINTING. D.T. Schroeder and T. R. Gordon. Dept. of Plant Pathology, Univ. of California, Berkeley, CA 94720.

DNA fingerprinting was used to assess the relationships among previously defined subpopulations within *Fusarium oxysporum* f. sp. *melonis*. Disparities in genomic structure were visualized as banding pattern variations. A quantitative interpretation of the degree of similarity between isolates was based on character state profiles representing the presence\absence of bands as compared against a composite

reference. DNA fingerprints of isolates from each of 3 VCGs were analyzed by this method. In almost all cases, isolates within a VCG tended to approximate a common profile. The greater genetic distance observed between than within VCGs is consistent with the presumed function of vegetative incompatibility as a contributing mechanism in the isolation of populations. A similar analysis of the 4 races within this special form revealed an absence of race-specific profiles. In several cases multiple races shared similar or identical DNA fingerprints. Conversely, races composed of 2 or more distinct haplotypes were observed. The data from this study suggest that the generation of race phenotypes is a recurrent process.

A140

PCR AMPLIFICATION, SEQUENCE, AND RFLP ANALYSIS OF THE RIBOSOMAL TRANSCRIBED SPACER REGIONS OF *MONOSPORASCUS* SPECIES. B. R. Lovic, R. D. Martyn, and M. E. Miller. Department of Plant Pathology and Microbiology, Texas A&M University, College Station 77843, and Weslaco 78596.

Monosporascus cannonballus and *M. eutypoides* are recently described soilborne ascomycetes that cause root rot/vine decline of cucurbits. The internal transcribed spacer regions (ITS1 and ITS2) of the rDNA were amplified *via* PCR using universal primers from conserved regions of the 28S, 5.8S, and 18S ribosomal genes. The size of the entire spacer regions, including the 5.8S gene, was estimated to be 610 bp. The entire region was cloned into a pUC18 vector and sequenced. The sequence was visually aligned with published sequences from other fungi to identify gene-spacer junctions. There was 90% homology with the 5.8S gene while no homology within the ITS regions was found with other fungi. The ITS regions from 15 isolates of *M. cannonballus* and *M. eutypoides* representing the major geographic areas where they are known to occur except Israel, were amplified and digested singularly with 10 restriction enzymes, all of which had restriction sites within the ITS. RFLP analysis indicated no sequence divergence among the 15 isolates. The conserved ITS sequences in *Monosporascus* offers potential for the development of genus-specific probes.

A141

EVIDENCE THAT *MONOSPORASCUS CANNONBALLUS* AND *M. EUTYPOIDES* MAY BE SYNONYMOUS. R. D. Martyn, B. R. Lovic, and M. E. Miller. Department of Plant Pathology and Microbiology, Texas A&M University, College Station, 77843 and Weslaco 78596.

The genus *Monosporascus* gen. nov. was erected in 1974 by Pollack and Uecker and now contains two species, *M. cannonballus* and *M. eutypoides*, both of which are pathogenic on muskmelon and watermelon. *M. cannonballus* has been reported from the USA and Japan and *M. eutypoides* has been identified from Israel and Spain. As originally described, the two species were similar except that *M. cannonballus* produced only one ascospore/ascus which germinated only rarely while *M. eutypoides* typically formed two ascospores/ascus that germinated readily. We have examined a number of isolates identified as either *M. cannonballus* or *M. eutypoides* from the USA, Japan, and Spain for ascospore number and germination, pathogenicity to muskmelon, and sequence homology and RFLPs of the rDNA ITS regions. No differences were detected among any of the isolates with any of the criteria used, and, based on the descriptions, they all should be considered *M. cannonballus*. Possible explanations for this are: 1) the two species are distinct, but *M. eutypoides* no longer is a major component of the populations, and 2) the two species are synonymous. At the present time, we favor the synonym hypothesis.

A142

BIOCONTROL OF OF RICE SHEATH BLIGHT USING INDIGENOUS RICE-FIELD MICROORGANISMS. R.D. Cartwright, D.L. Crippen, and G.E. Templeton, Dept. of Plant Pathology, University of Arkansas, Fayetteville, AR 72701.

Numerous fungi and bacteria isolated from rice-field collected sclerotia of *Rhizoctonia solani* AG1-IA, crop residue, and water-line stem segments of growing rice plants were assayed in the laboratory and greenhouse for biocontrol activity. Dual cultures revealed several types and levels of antagonism, however, *R. solani* dominated most interactions. A limited number of fungi and bacteria were then co-inoculated with *R. solani* on late tillering rice in the greenhouse. Plants were misted at to permit vertical development of sheath blight until 100% heading. Of the candidate organisms tested, *Sclerotium hydrophilum* was the most effective, significantly reducing sheath blight severity and sclerotial production, resulting in an increased number of headed tillers and dry panicle weights. *Trichoderma* spp. (3) and several fungi considered minor rice pathogens also reduced sheath blight severity in these tests.

A143

***GLIOCLADIUM VIRENS* AS A BIOCONTROL OF DAMPING-OFF OF VINCA (*CATHARANTHUS ROSEUS*) CAUSED BY *PHYTOPHTHORA PARASITICA*.** D. M. Benson, N. C. State University, Raleigh 27695.

A prill formulation of *Gliocladium virens*, Gl-21 (Glioguard, WR Grace) was compared to two isolates of *G. virens* in wheat bran for biocontrol of pre-emergence damping-off of vinca caused by *Phytophthora parasitica*. Damping-off was 32 and 59% with isolates G10B and G7, respectively, compared to 55-69% over three rates of Gl-21, 35% in the fosetyl-Al control and 75% in the infested control. Population of *P. parasitica* in the peat:vermiculite mix was suppressed with G7 and G10B compared to that with Gl-21 or the infested control. Populations of *Gliocladium* were highest in the order: Gl-21, G7, G10B, and fairly stable up to 30 days after seeding. First true leaf length was greatest for vinca in mix with isolate G10B compared to Gl-21 or the control without wheat bran at 18 but not 21 days after seeding. Vinca seedlings were taller (P=0.05) in mix with isolates G7, G10B, or Gl-21 at 58 days after seeding than in the control.

A144

HYPOVIRULENCE AND DOUBLE-STRANDED RNA IN *SCLEROTINIA HOMOEOCARPA*. Ting Zhou and Greg J. Boland. Dept. of Environmental Biology, Univ. of Guelph, Guelph, Ontario, Canada, N1G 2W1.

Of 132 isolates of *Sclerotinia homoeocarpa* evaluated for virulence on swards of bentgrass (*Agrostis palustris*), 13 isolates (9.8%) did not produce disease symptoms after 4 weeks and were considered hypovirulent. Double-stranded RNA was detected (dsRNA$^+$) in 15 isolates (11.4%) and six of these isolates were hypovirulent. On detached bentgrass leaves, 13 of the dsRNA$^+$ isolates developed lesions that were 19.2-99.6% smaller than lesions initiated by a standard virulent isolate, Sh48B. On PDA, hypovirulent dsRNA$^+$ isolates Sh09B and Sh12B grew slower and displayed atypical colony morphologies compared to isolate Sh48B. Via hyphal anastomosis, hypovirulence and dsRNA were transmitted from Sh12B to Sh48B but were not transmitted from Sh09B to Sh48B. Coinoculation of detached bentgrass leaves or apple fruits with colonized agar plugs of Sh48B and Sh12B resulted in lesions of intermediate size compared to the virulent and hypovirulent treatments alone. No reductions of lesion size were obtained when isolates Sh09B and Sh48B were coinoculated.

A145

EVALUATION OF TRICHODERMA ATROVIRIDE IN CONTROLLING BLACK SCURF OF POTATOES UNDER COMMERCIAL FIELD CONDITIONS. J. H. McBeath, C. Chen and J. Bittner. Department of Plant, Animal & Soil Sciences, University of Alaska Fairbanks, Fairbanks, AK 99775-0080.

Four isolates of a wide spectrum, cold tolerant, mycoparasitic Trichoderma atroviride were evaluated under commercial field conditions for their efficacy in controlling potato black scurf, caused by Rhizoctonia solani. Six treatments were tested in a randomized complete block design with three replications. Results from the 1991 field trial indicated that under high tuberborne R. solani inoculum levels, T. atroviride isolates--CHS 861, CHS 901, Biotype 453 and Biotype 603--could protect an highly susceptible potato locally known as "Peanut" against R. solani equal to or better than fungicide (PCNB). In 1992, tuberborne and soilborne inoculum levels were low. Cultivar "BakeKing" was moderately resistant to R. solani. In the plants treated with T. atroviride, yield was not significantly different from that of the control. A significant reduction in yield was noticed in plants treated with PCNB.

A146

EFFECTS OF TRICHODERMA ATROVIRIDE ON PYTHIUM DAMPING OFF OF PEA. C. Chen and J. H. McBeath. Department of Plant, Animal and Soil Sciences, SALRM, University of Alaska Fairbanks, Fairbanks, AK 99775-0080.

Trichoderma atroviride isolates--CHS 861, CHS 901, biotype 603 and biotype 453--were evaluated for effectiveness in controlling pre-emergence damping off of pea plants caused by Pythium dissotocum, P. oligandrum, P. violae, and P. ultimum at 10°C and 23°C. T. atroviride isolates reduced the damping off disease incidence by 22 to 52% at 10°C and by 23 to 62% at 23°C. Although all four isolates can control the disease effectively, certain T. atroviride isolates are better at controlling particular Pythium sp. at certain temperatures. The disease incidence of pea treated with T. atroviride isolates was not significantly different from fungicide Captan treatment. In the medium uninfested by Pythium spp., seed treatment with T. atroviride isolates had no significant effect on seed emergence percentage, but Captan significantly reduced the seedling emergence percentages, both at 10°C and 23°C.

A147

GLIOCLADIUM VIRENS, A MYCOPATHOGENIC PHYTOPATHOGEN OF CORN (ZEA MAYS). N.G. Vakili, National Soil Tilth Laboratory, ARS, USDA, 2150 Pammel Drive, Ames, IA 50011.

Gliocladium roseum, G. virens, and Trichoderma harzianum were tested for their efficacy as biocontrol agents of seed - and soilborne fungi colonizing corn kernels; and for their interaction with different corn genotypes. Corn kernels were coated with conidial suspension of mycopathogens and germinated in germinators or soil. Gliocladium roseum and T. harzianum increased germination and seedling dry weight, and reduced colonization of kernels and seedlings by pathogens. Whereas, G. virens was moderately phytopathogenic to some genotypes, causing root lesions and reducing root length, number, and dry weight, while reducing colonization of roots by other fungi. Gliocladium virens could be used as an endophytic mycopathogen with slightly susceptible corn, thereby producing stalk rot suppressive corn.

A148

Biocontrol of *Phytophthora citrophthora* Root Rot of Citrus. J. K. Turney and J. A. Menge. Department of Plant Pathology, Univ. of Calif., Riverside 92521.

Spontaneous rifampicin mutants of ten bacterial isolates that significantly reduced soil populations of either *Phytophthora parasitica* or *P. citrophthora* (Pc) in previous greenhouse tests were used in a 12 week time course experiment with Pc and Troyer citrange. The experiment was conducted in lathhouse beds under field environmental conditions with all irrigation supplied by seasonal rainfall. Isolates N8.8, C8.2, and *Pseudomonas putida* (Pp) reduced Pc propagules per gram (ppg) by 66%, 42%, and 41% respectively, over the entire course of the experiment. Ppg of Pc were reduced by isolates N8.8, C8.2, and Pp on each of 4 takedown dates. N8.8 reduced root infection by 22% compared to the infected control over the course of the experiment. Visual ratings of root rot were not improved by any of the bacterial isolates over the course of the experiment. Initial bacterial populations for N8.8, C8.2, and Pp were 53, 26, and 64.8 x 10^6 cfu's per gram of soil respectively and declined to 2.43, 0.11, and 0.41 x 10^6 cfu's per gram of soil after 12 weeks.

A149

COLONIZATION BY *BACILLUS SUBTILIS* INOCULANTS TO AUGMENT SEEDLING DISEASE CONTROL AND SEASON-LONG ROOT HEALTH OF MULTIPLE COTTON VARIETIES. P. M. Brannen and P. A. Backman, Department of Plant Pathology, Auburn University, AL 36849-5409.

In a 2-year study, GUS2000 and GUS376 strains of *Bacillus subtilis* (Gustafson, Inc., Plano, TX) were applied at log 7.0 $CFUg^{-1}$ to cotton seed pretreated with metalaxyl-PCNB-carboxin. Both bacteria colonized roots throughout the growing season (>log 3.0 $CFUg^{-1}$ root). In 1992, significant colonization differences were observed for both strains among five cultivars tested ($p<0.05$). However, differing colonization could not be attributed to cultivar alone, since seed pH of the cultivar was also correlated with colonization. Cultivars with high colonization levels had less *Rhizoctonia* damage, improved stands, and yields. In 1993, 10 cultivars were evaluated, and all seed were neutralized with a saturated solution of sodium carbonate prior to planting. As a result, initial seed pH was not a factor in 1993 trials. Only one cultivar showed a significantly different level of colonization, indicating that cultivar is probably not a major limiting factor in colonization. Stands were improved with either strain of *B. subtilis* in 1993 ($p<0.05$). Acid residues from cotton delinting may reduce colonization and subsequent impact of biologicals applied to cotton seed.

A150

ALGINATE PELLET FORMULATION OF THE NEMATOPHAGOUS FUNGUS HIRSUTELLA RHOSSILIENSIS. LACKEY, B. A., A. E. Muldoon, and B. A. JAFFEE. Department of Nematology, University of California, Davis, CA 95616.

Macerated hyphae of Hirsutella rhossiliensis were pelletized in 1% alginate. Pellets dried for 1 day at 22 C were 1.7 mm in diameter, weighed 0.58 mg, and contained 0.14 mg dry weight of hyphae. Dried pellets were added to 17 cm^3 loamy sand and packed into vials. After 14 days at 20 C, Heterodera schachtii juveniles were added to each vial, recovered after 66 h, and examined to determine the percentage with at least one fungal spore attached (transmission). Transmission with four pellets per vial was 73 ± 6%. Transmission decreased when pellets were stored at 22 C for > 13 days, but remained high when pellets were stored at 5 C for 13-252 days before addition to soil. In other tests, addition of pellets to 100 cm^3 loamy sand suppressed root penetration by H. schachtii and Meloidogyne javanica, but suppression was less when nematodes were naturally present than when juveniles were added.

A151

FIELD EFFICACY AND SURVIVAL OF *PHOMA PROBOSCIS* PATHOGENIC ON FIELD BINDWEED. D. K. Heiny. Department of Plant Pathology, University of Arkansas, Fayetteville, AR 72701, U.S.A.

Phoma proboscis was evaluated under field conditions for effectiveness in controlling field bindweed (*Convolvulus arvensis*) in small plots. At five intervals during the growing season of 1992, seven treatments of spore formulations were applied by spray inoculation of young and mature field bindweed plants. The most successful inoculations, with high spore concentrations (10^8 spores/ml) and young plants, resulted in an average death rate of 83% of individuals and 95% of bindweed tissue necrotic. Necrosis among treatments of mature bindweed ranged from 30 to 72%. Survival of the pathogen was determined using stems and nylon membranes infested with *P. proboscis*. These were buried at three depths in soil in a field during November of 1991 and 1992, and assayed at one month intervals for six months. Propagules of *P. proboscis* survived longer and in greater numbers on membranes than on stems, and survival was not affected by depth of burial. Overall, survival was higher in the winter of 1992 than in 1991 ($P=0.0001$), but declined rapidly between the fifth and sixth month in both years. In the sixth month, no isolates were recovered from stems in the first season, but 11% of stems contained viable *P. proboscis* in the second season.

A153

MICROBIAL COLONIZATION OF PRUNING WOUNDS OF GRAPEVINES IN CALIFORNIA. P. M. Coleman and J. J. Marois, Department of Plant Pathology, University of California, Davis, CA, 95616.

The fungus *Eutypa lata*, cause of Eutypa dieback, infects xylem vessels of grapevines pruned in winter. Because colonization of wounds by non-pathogenic microorganisms may prevent infection by *Eutypa*, the microbial community of pruning wounds was monitored to provide insight into biological control of Eutypa dieback. Wounds of vines pruned in the dormant season were initially free of microorganisms. Numbers of total bacteria, *Bacillus* spp., and yeasts recovered from wounds increased rapidly for 7-14 days after pruning, peaked at 10^8, 10^6, and 10^6 CFU/wound, respectively, then decreased. The majority of filamentous fungi recovered were species of *Cladosporium* and *Aureobasidium*, which reached maxima of 10^4 and 10^5 CFU/wound. Populations of *Cladosporium* spp. peaked at 50 days when vines were pruned early in the dormant season, and at 7-14 days when vines were pruned 5-9 weeks later.

A154 Withdrawn

A155

DEVELOPMENT OF A BIOLOGICAL CONTROL ASSAY AND SCREENING OF SELECTED GEOCARPOSPHERE BACTERIA AGAINST CONTAMINATION OF PEANUT BY AFLATOXIGENIC FUNGI. C.J. Mickler, K.L. Bowen, and J.W. Kloepper, Dept. of Plant Pathology, Auburn University, AL 36849-5409.

The geocarposphere, or area immediately surrounding peanut pods, is a unique ecological habitat. Of 159 bacteria previously isolated, 20 were selected based on their biocontrol potential against aflatoxigenic fungal colonization using *in vivo* assays. These strains are now being screened in the greenhouse and field using a recently developed assay based on: (1) inoculum rate of *A. flavus*-type fungi and proper drought regime for optimum colonization of pegs and pods, and (2) optimum time of application of bacterial candidates for reducing *A. flavus*-type fungal infestation of seed and aflatoxin production. A suspension of 10^4 spores/ml with drought from 70 DAP to harvest produced highest peg and pod colonization by *A. flavus*. Drought length was the only factor contributing significantly to these results, indicating that the best candidate will be required to survive extended periods without irrigation. Therefore, rifampicin-resistant and unmarked candidate bacteria used in greenhouse and field screens, respectively, are being applied at planting, on the seed, and at mid-peg by soil drenching. Results of these screens will be presented.

A156

BIOCONTROL OF RHIZOCTONIA STEM ROT OF POINSETTIA IN POLYFOAM ROOTING CUBES WITH *PAECILOMYCES LILACINUS*. D. Kelly Cartwright and D. M. Benson. Dept. of Plant Pathology, N. C. State University, Raleigh.

Paecilomyces lilacinus (strain 6.2F) isolated from natural soil was efficacious in control of Rhizoctonia stem rot of poinsettia in polyfoam rooting cubes. In repeated trials, infection and mortality of poinsettia cuttings in cubes treated with *P. lilacinus* ranged from 7-20% compared to 73-100% in infested controls. Excellent control was achieved with *P.*

lilacinus over a 2 or 3-wk period. No infection or mortality was observed on cuttings in benomyl treated cubes. *Gliocladium virens* (strain GL-21), a known biocontrol agent of *Rhizoctonia solani*, gave no control (100% mortality) of stem rot. Root development was suppressed on cuttings treated with *P. lilacinus*. Strain 6.2F inhibited *R. solani* more on full strength potato dextrose agar (PDA) than on dilute (0.13%) PDA. Strain 6.2F of *P. lilacinus* has potential as a biocontrol agent of stem rot of poinsettia.

A157

IDENTIFICATION BY SITE-DIRECTED MUTAGENESIS OF AMINO ACIDS CONTROLLING DISASSEMBLY OF TOBACCO MOSAIC VIRUS.
James N. Culver[1] and Gerald Stubbs[2]. (1) Center for Agricultural Biotechnology, University of Maryland, College Park, Maryland 20742. (2) Department of Molecular Biology, Vanderbilt University, Nashville, Tennessee 37235.

The structure of intact tobacco mosaic virus has been determined in molecular detail by x-ray diffraction methods (Namba, Pattanayek and Stubbs, J. Mol. Biol. 208, 307-325, 1989). In the intact virus, Glu50 from one subunit is located only 4 Å from Asp77 in an adjacent subunit. The mutual electrostatic repulsion of the two charged residues could provide a large component of the driving force in disassembly of the virus. In order to test this hypothesis, we separately replaced Asp77 by Asn and Glu50 by Gln. The Gln50 mutant virus formed virion-like particles without RNA. The Asn77 mutant virus was approximately four times as stable as wild-type virus to alkaline degradation. These results confirm the involvement of both residues in viral disassembly.

A158

TEMPERATURE-DEPENDENT COMPLEMENTATION OF MOVEMENT-DEFICIENT MUTANTS OF TOBACCO MOSAIC VIRUS BY GENE I OF PEANUT CHLOROTIC STREAK CAULIMOVIRUS. R. D. Richins, J. Donson, D. J. Lewandowski, D. A. Ducasse, W. O. Dawson and R. J. Shepherd. Dept. of Plant Pathology, University of Kentucky, Lexington, KY 40506, and Citrus Research & Education Center, Lake Alfred, FL. 33850

Tobacco (*Nicotiana tabacum* vars. Xanthi and Xanthi-nc) plants systemically infected with peanut chlorotic streak caulimovirus (PClSV) supported the systemic infection of a movement-defective mutant of tobacco mosaic virus (TMV). Complementation occurred only at temperatures which were conducive to the systemic spread of PClSV (> 30°C). A recombinant virus, consisting of a movement-defective mutant of TMV and the putative PClSV movement protein gene, was capable of cell-to-cell movement. The TMV/PClSV recombinant virus demonstrated a temperature dependency similar to that of PClSV. The results indicate that ORF I of PClSV encodes a movement protein which is able to complement a defect in the TMV movement protein. Furthermore, the PClSV movement protein appears to be at least partially responsible for the unique temperature dependency of the virus.

A159

TRANSCAPSIDATION IN TRANSGENIC PLANTS EXPRESSING BEAN YELLOW MOSAIC VIRUS OR CHIMERIC COAT PROTEINS IS NOT CORRELATED WITH RESISTANCE TO VARIOUS POTYVIRUSES. John Hammond. USDA-ARS, FNCL, Beltsville, MD 20705.

Transgenic *Nicotiana benthamiana* expressing the coat protein (CP) of bean yellow mosaic virus (BYMV), or chimeric CPs having N-terminal domains of BYMV and C-terminal domains of potato virus Y (PVY) or zucchini yellow mosaic virus (ZYMV) were challenged with BYMV, PVY, pepper mottle (PeMV), tobacco etch (TEV) or turnip mosaic (TuMV) viruses. Partial resistance to BYMV infection and/or recovery from infection was observed with each CP construct. One BYMV/PVY chimeric transformant recovered from PVY infection in upper leaves. Some transformants of each construct were partially resistant to TEV and TuMV. Virions of BYMV, PVY, PeMV, TEV and TuMV purified from various transgenic plants were found to have incorporated the transgene CP (the different transgene CPs are serologically identifiable with monoclonal antibody PTY 43, which is specific for the N-terminus of BYMV isolate GDD from which the transgenes were derived. Transcapsidation was not correlated with degree of resistance or recovery from infection. The potential contribution of the transgene CP to aphid transmission of normally non-aphid transmitted potyvirus isolates is being investigated.

A160

CONSTRUCTION, EXPRESSION AND CHARACTERIZATION OF A FUNCTIONAL RECOMBINANT MONOCLONAL Fab ANTIBODY THAT BINDS BEAN YELLOW MOSAIC POTYVIRUS COAT PROTEIN. Leslie Palmer and Ramon Jordan, USDA-ARS, PSI, Florist and Nursery Crops Lab, BARC-W, Beltsville, MD

The hybridoma genes encoding a murine monoclonal antibody which recognizes a coat protein epitope present on bean yellow mosaic potyviruses have been cloned and expressed as an assembled and functional Fab. mRNA from the hybridoma cell line was converted to cDNAs and amplified by polymerase chain reaction using primers specific for the Fab region of the IgG_1 heavy chain (Fd) and κ light chain. The resulting 700-bp products were cloned into modified lambda bacteriophage immunoexpression vectors and expressed in *Escherichia coli*. Production of Fd and light-chain proteins was determined by immunoscreening of plaque lifts. When the Fd and light-chain gene constructs were combined in a single vector and coexpressed in *E. coli*, a 55 kDa protein which co-migrates with an Fab molecule in Western blot analysis and is immunoreactive with antibodies specific for expressed light chain and heavy chain was obtained. The expressed recombinant Fab was shown to bind coat protein subunit of bean yellow mosaic potyvirus in an indirect antigen-coated plate ELISA. The binding specificity corresponded to that of the parent monoclonal antibody. Current experiments dealing with the transient expression of the recombinant Fab in tobacco suspension cells will also be presented.

A161

COMPLEMENTATION OF MOVEMENT DEFECTIVE MUTANTS IN TRANSGENIC TOBACCO EXPERSSING THE CUCUMBER MOSAIC VIRUS MOVEMENT GENE. I. B. Kaplan, L. Zhang, M. H. Shintaku, L. E. Marsh, and P. Palukaitis. Dept. of Plant Pathology, Cornell University, Ithaca, NY.

The 3a gene of cucumber mosaic virus (CMV), is believed to be involved in potentiating the cell-to-cell movement of CMV. Transgenic tobacco plants expressing this gene were inoculated with RNA transcripts of a series of Fny-CMV RNAs 1, 2 and 3, in which deletion and frame-shift mutants were made in the 3a gene. Most of the deletion and frame-shift mutants did not infect non-transformed plants, but they all infected the 3a-transgenic plants, indicating complementation of the defective gene function. Two of the defective 3a gene mutants systemically infected the 3a-transgenic plants but produced a unique symptom response limited to vein-clearning. Thus, the expression of higher levels of the movement protein appears to be involved in symptom expression. Data on the ability of the 3a-transgenic plants to complement the movement of other viruses will be presented.

A162

STUDIES ON THE INTERACTION OF CUCUMBER MOSAIC VIRUS (CMV) REPLICASE-MEDIATED RESISTANT TOBACCO PLANTS WITH A RESISTANCE BREAKING CMV STRAIN. Karl-Heinz Hellwald and Peter Palukaitis, Department of Plant Pathology, Cornell University, Ithaca, NY 14853.

The transformation of tobacco plants with a truncated form of CMV-RNA 2, one of the CMV-replicase genes, derived from the CMV subgroup I strain Fny, renders these plants highly resistant against most of the subgroup I strains. During the screening of various subgroup I strains only CMV-K was shown to break the replicase-mediated resistance. The ability to break the resistance maps to CMV-K RNA 2. An infectious in vitro transcript to CMV-K RNA 2 was constructed using PCR. Sequence data from the CMV-K RNA 2 obtained so far showed about 10% difference at the nucleotide level. The construction of recombinants between CMV-Fny RNA 2 and CMV-K RNA 2 showed that the resistance-breaking domain of CMV-K RNA 2 is located in the 5' proximal half of RNA 2. Further results of genomic mapping will be presented.

A163

SEQUENCES IN LS-CMV CONTROLLING INFECTION OF MAIZE MAP TO THE 5' HALF OF RNA 3. Lee Zhang and Peter Palukaitis, Dept. of Plant Pathology, Cornell University, Ithaca, NY 14853.

The genome of cucumber mosaic virus (CMV) consists of 3 RNAs, namely RNA 1, RNA 2 and RNA 3 as well as a CMV subgenomic RNA 4. CMV can be divided into two subgroups, I and II. Full-length cDNA clones of those RNAs of a subgroup II strain, LS-CMV, were constructed and infectious RNAs were made from these clones. Infectious RNAs were also made from Fny-CMV (a subgroup I strain) cDNA clones (which were previously constructed in this laboratory). The Fny-CMV can infect maize and cause systemic symptom while LS-CMV cannot infect maize. By psuedorecombination among the infectious transcripts made from cDNA clones of LS-CMV and Fny-CMV, we found that the maize plants were infected when the inoculum consisted of any combination that included Fny-CMV RNA 3. Recombination between cDNA clones of Fny-CMV RNA 3 and LS-CMV RNA 3 showed that 5' half of LS-CMV RNA 3 determines its inability to infect maize.

A164

GENOMIC MAPPING OF B-CMV RNA 2 TO DETERMINE THE DOMAIN RESPONSIBLE FOR THE INFECTION OF COWPEA. Chung Ho Kim and Peter Palukaitis, Dept. of Plant Pathology, Cornell University, Ithaca, NY 14853.

B-CMV, a member of cucumber mosaic virus (CMV) subgroup I, is able to infect cowpea systemically, whereas Fny-CMV is not able to infect this plant systemically. This host range determinant maps to RNA 2 of B-CMV. To determine the genomic sequence in B-CMV RNA 2 responsible for the ability to infect cowpea systemically, a full-length cDNA clone of B-CMV RNA 2 was constructed and used to make recombinants between B-CMV RNA 2 and Fny-CMV RNA 2. According to these results, the domain of B-CMV RNA 2 responsible for systemic infection of cowpea is located between nucleotide 1626 and 2132. By analyzing the nucleotide sequence of this part of the genome, four amino acid changes between Fny-CMV and B-CMV were predicted. Site directed mutagenesis will be used to determine the amino acid changes(s) responsible for the ability of B-CMV to infect cowpea systemically.

A165

IN VITRO AND *IN VIVO* SYNTHESIS OF CUCUMBER MOSAIC VIRUS SATELLITE RNA. A. Gal-On, I. Kaplan and P. Palukaitis, Dept. Plant Pathology, Cornell University, Ithaca, NY 14853.

Most strains of cucumber mosaic virus (CMV) such as Fny-CMV support the accumulation of satellite RNA. The Sny-strain does not support satellite RNA accumulation in zucchini squash, but does do so in tobacco. Protoplast isolated from tobacco support the replication of satellite RNA by both strains of CMV, where as in zucchini squash protoplasts only Fny-CMV supports satellite RNA replication. RNA-dependent RNA polymerases isolated from both Fny- and Sny-CMV infected zucchini squash were able to synthesize satellite RNA *in vitro* from added (+) satellite RNA, suggesting that the block in replication, is not in the synthesis of (-) RNA. The effects of satellite RNA replication on the synthesis of CMV RNAs and the four encoded proteins in protoplasts from both plant species were examined. These data will be presented.

A166

CHARACTERIZATION OF THE INDEPENDENT AND DEPENDENT INTERACTIONS OCCURRING BETWEEN THE GENOMIC RNAS OF PEA ENATION MOSAIC VIRUS (PEMV). S.A. Demler, O.N. Borkhsenious, D.G. Rucker and G.A. de Zoeten. Department of Botany and Plant Pathology, Michigan State University, East Lansing, MI 48824

The genome of PEMV can be characterized as a symbiotic association between a luteovirus-like RNA (RNA 1) and an RNA related to the carmo- and tombusvirus groups (RNA 2). Current studies have addressed the relative contribution of each RNA to this interaction. In protoplasts, both phases are capable of autonomous replication, although RNA 1 is individually responsible for the characteristic PEMV replication complex. RNA 1 is also solely responsible for the expression of all structural functions. In transmission studies, RNA 1 is incapable of mechanical passage, although inoculations with RNA 2 leads to the development of a symptomless systemic infection. Combined inoculation of RNA 1 and 2 results in the systemic invasion of both RNAs, suggesting that RNA 2 complements the deficiencies of RNA 1 in systemic invasion.

A167

AGROINFECTION OF BEAN, TOBACCO, AND TOMATO WITH TWO BIPARTITE GEMINIVIRUSES ISOLATED FROM TOMATO. R. L. Gilbertson[1], E. J. Paplomatas[1], P. D. Grieco[1], V. P. Patel[1], and D. P. Maxwell[2]. [1]Department of Plant Pathology, University of California-Davis and [2]Department of Plant Pathology, University of Wisconsin-Madison.

Infectious clones of two bipartite geminiviruses, tomato mottle (ToMoV) and TGV-MX1, were isolated from infected tomatoes from Florida and Mexico, respectively. Dimers or 1.5-mers of the DNA-A and DNA-B components were each transferred into an Agrobacterium binary vector and recombinant plasmids transformed into *Agrobacterium tumefaciens*. Bean, tobacco, and tomato plants were agroinoculated with all possible combinations of the DNA components. Agroinoculated ToMoV produced severe symptoms in tomato and tobacco, identical to those induced after mechanical inoculation of the cloned DNA components. Agroinoculated ToMoV was also infectious in bean, producing mild symptoms. Agroinoculated TGV-MX1 produced severe symptoms in tobacco and bean, identical to those induced by mechanical inoculation of virions or the cloned DNA components. TGV-MX1 was not highly infectious in tomato. Pseudorecombinants made by exchanging the components of the two geminiviruses were infectious, but produced delayed and attenuated symptoms.

A168

MAPPING OF SEED TRANSMISSIBILITY DETERMINANTS OF BARLEY STRIPE MOSAIC VIRUS. Michael C. Edwards. USDA-ARS Cereal Crops Research Unit, Northern Crop Science Lab, Fargo, ND 58105-5677.

With no known vectors, BSMV depends exclusively on seed transmission for its survival in nature. The primary determinant of seed transmissibility was mapped to RNAγ using infectious RNAs transcribed from full-length cDNA clones of BSMV strains ND18 and CV17. More precise mapping by analysis of chimeric γ RNAs indicated that seed transmissibility may be affected by three different regions of RNAγ, including the 5'-noncoding region, the repeated region of the γa gene, and the γb gene. The presence of a ~370 nt tandem repeat, beginning just upstream of the γa gene and extending into it, was found to reduce seed transmissibility. Evidence obtained with RNAγ point mutants supports the view that the observed 5'-leader effects on seed transmission are due to sequence differences between the parental strains, rather than to the presence of small ORFs located within the CV17 RNAγ leader. It appears that variation in seed transmissibility of BSMV strains may be due to the differential ability of strains to replicate and/or move into reproductive tissues.

A169

A SHIFTY HEPTANUCLEOTIDE SEQUENCE AND A SECONDARY STRUCTURE ARE REQUIRED FOR EFFICIENT RIBOSOME FRAMESHIFT EXPRESSION OF AN RNA PLANT VIRUS POLYMERASE. K. H. Kim and S. A. Lommel, Department of Plant Pathology, Box 7616, North Carolina State University, Raleigh, NC 27695-7616.

The RNA-1 of the bisegmented genome red clover necrotic mosaic dianthovirus (RCNMV) encodes the 88 kDa viral polymerase which is translated by a -1 ribosomal frameshifting event. A β-glucuronidase reporter system assay identified a 118 nucleotide element containing both the shifty heptanucleotide and the predicted secondary structure that was required for efficient -1 ribosomal frameshift expression in *Nicotiana benthamiana* protoplasts. Secondary structure sensitive chemical probing illustrated that a 118 nucleotide region required for ribosome frameshifting formed a complex and stable secondary structure. Deletion analysis and site-specific structure destabilizing and compensatory mutation analysis of the base-paired regions of the secondary structure showed that expression of the RCNMV polymerase required the specific secondary structure for ribosomal frameshift.

A171

EFFECT OF FLUAZINAM ON GROWTH AND SPORULATION OF *PHYTOPHTHORA CAPSICI* AND DEVELOPMENT OF CROWN AND ROOT ROT ON CHILE PEPPER. M.E. Matheron, J.C. Matejka and M.S. Porchas. Yuma Agric. Center, Univ. of Ariz., Yuma, 85364

In vitro tests were initiated to examine the effects of fluazinam on growth and sporulation of *Phytophthora capsici*. Growth of the pathogen was completely inhibited on corn meal agar containing 3,000 ppm fluazinam. Sporangium production by *P. capsici* in 1.5% soil extract was totally suppressed in the presence of 1,000 ppm of fluazinam. Zoospores of *P. capsici* ceased motility within 1 minute after treatment with fluazinam at 10 ppm; however, these encysted zoospores remained viable and germinated when placed in 5% V-8 juice. In greenhouse trials, 6-wk-old chile pepper (*Capsicum annuum* L.) plants survived for 83 days when grown in soil naturally infested with *P. capsici* and drenched with 1,000 ppm fluazinam, while plants grown in infested soil without fluazinam survived for only 5 days.

A172

CONTROL OF LATE BLIGHT DISEASE IN PROCESSING TOMATOES CONSIDERING SOME PHYSICAL PARAMETERS FOR FUNGICIDE APPLICATIONS. R. Felix-Gastelum. Campbell Research and Development, Apartado Postal No. 185, Guasave, Sinaloa, Mexico, C.P. 81000.

The forecast system included in the potato crop management (PCM) package (Stevenson, W.R. 1993. Plant Dis. 77: 309-311) was used for the first prophylactic application of chorothalonil or metalaxyl+chlorothalonil to control late blight disease, caused by Phytophthora infestans, in processing tomatoes. Subsequent spray applications were based on Disease Severity Values (DSVs) modified from the TOMCAST system (Madden, L. et al. 1978. Phytopathology 68: 1354-1358). Number of fungicide applications were 9, 6, and 5 for PCM+10, PCM+20, PCM+30 DSVs, respectively. The most cost effective treatment for the control of the disease was PCM+20 DSVs using chlorothalonil alone. Nine applications of chlorothalonil or 9 applications of metalaxyl+chlorothalonil applied at 10-day intervals starting 2 wk after transplanting, gave a control (1-1.2% blighted foliage and 0.7-0.9% blighted fruit) similar to that obtained with PCM+20 DSVs using chlorothalonil alone. Plants sprayed with metalaxyl-2E and unsprayed plants showed 99% defoliation and 77% fruit blighted, suggesting that P. infestans strains affecting tomatoes in Sinaloa, Mexico, are resistant to metalaxyl.

A174

CONTROL OF *PYTHIUM APHANIDERMATUM* BY EXTRACTS OF *ACACIA* SPP. A.J. Khan, A. Zouba and A.O.M.Al-Matrushi. Department of Plant Sciences, College of Agriculture, Sultan Qaboos University, Al-Khod-123, Muscat, Sultanate of Oman.

Three species of *Acacia* has been screened for their antifungal activity. Aqueous extract of *A. nilotica* supplemented with potato-dextrose agar medium in the concentration of 80, 160, 240, and 320 mg fresh weight per ml of medium showed significant mycelial growth inhibition of *Pythium aphanidermatum*.The growth of the fungus was completely ceased at 160 mg fresh weight per ml of medium. Whereas very little or no growth inhibition was observed with aqueous extracts of *A. senegal* and *A. tortilus*. Air dried leaves of *A. nilotica* were extracted in acetone-water mixture (60:40 v/v). Acetone was removed *in vacuo* and aqueous extract was fractioned with dichloromethane, ethyl acetate and n-butanol. The growth of *P. aphanidermatum* was completely ceased with ethyl acetate (25 mg) and n-butanol (55 mg) extracts on potato-dextrose agar by disc diffusion assay. Whereas, dichloromethane extract showed no inhibition of the mycelial growth. Three compounds were partially purified from ethyl acetate extract by thin layer and column chromatography on silica gel 60 and Sephadex LH-20.Significant control of cucumber damping-off disease caused by *P. aphanidermatum* was achieved when aqueous leaf extract of *A. nilotica* was drenched at the rate 240 mg fresh weight per gram of soil infested with damping-off pathogen. No phytotoxicity of cucumber seedlings was observed with leaf extract.

A178

MONOTERPENES OF NATURAL ORIGIN FOR CONTROL OF PHYTOPARASITIC NEMATODES A. Soler, R. Rodríguez-Kábana, C.F. Weaver, P.S. King, and J.A. McInroy. Department of Plant Pathology, Alabama Agricultural Experiment Station, Auburn University, Alabama 36849.

As part of an ongoing effort to determine the nematicidal activity of naturally occurring allelopathic compounds, a greenhouse experiment was performed combining increasing dosages of geraniol (0, 50, 100, 150, and 200 µl/kg soil) and thymol (0, 50, and 100 mg/kg soil). Ten days after application, the terpenes showed synergistic effects in suppressing populations of *Meloidogyne incognita* and *Hoplolaimus galeatus*, and increasing bacterial counts. Geraniol and thymol caused significant reductions in populations of fungi and actinomycetes. Populations of *Pseudomonas* spp. shifted from 15% of total bacterial counts in control pots to 70-86% in treated soils. We could confirm that plant allelochemicals are effective tools for the manipulation of the soil microbiota.

A179

ELECTRON BEAM ANALYSIS OF COPPER HYDROXIDE SPRAY DRIFT APPLIED TO DORMANT MALUS spp. C.R. Krause, R.D. Brazee, R.D. Fox, D.L. Reichard and C. Tappan. US Department of Agriculture, ARS, Application Technology Research Unit, Dept. of Plant Pathology OARDC/The Ohio State University, Wooster. OH. 44691.

Dormant, semi-dwarf apple trees (Malus spp.) were sprayed with $Cu(OH)_2$ 50 WP in H_2O using an axial flow orchard sprayer while microclimatic data were recorded. Apple buds and inert sample surfaces (carbon-coated stubs), located downwind from the line of application, were collected after each spray and evaluated with electron beam analysis (EBA), a combination of scanning electron microscopy and energy dispersive X-ray analysis. Spray droplet residue was detected on the basis of shape, size and the presence of Cu. Droplet size and frequency and Cu quantity significantly decreased with distance from the spray drift line on buds and inert stubs. Electron beam analysis provides a useful tool for the evaluation of fungicide drift as a function of environmental and application variables. Such knowledge could improve crop protection methods while easing environmental concerns.

A181

BIOLOGICAL CONTROL OF BLUE STAIN FUNGI IN WOOD. C.J. Behrendt[1], R.A. Blanchette[1] and R.L. Farrell[2]. [1]Dept. of Plant Pathology, University of Minnesota, St. Paul 55108, [2]Sandoz Chemicals Biotech, Lexington, MA 02173.

Ophiostoma piliferum, a common blue stain fungus, is an aggressive pioneer colonist of wood. Biological control of the sap staining fungus was attained by spraying the ends of *Pinus resinosa* logs in the field with a "colorless", non-staining strain of *O. piliferum*, which lacks dark hyphal pigments. Spore suspensions (2.5 g/1420 ml water) of the colorless strain were sprayed on logs 1 day after cutting, and exposed to inocula of natural blue stain fungi. Observations of stain development and chip isolations were used to determine the establishment of blue stain fungi in the log. After 4 and 8 wk, 21% and 6% of the chips isolated from inoculated logs had blue stain fungi, while untreated-control logs had 64% and 73%. The mean distance of blue stain into wood of inoculated and control logs was 0.2 and 1.5 cm, respectively, at 4 wk; and 0.02 and 3.1 cm at 8 wk after inoculation. These results show that application of the colorless strain effectively reduced incidence of blue stain.

A182

TOMATO MOSAIC VIRUS OF RED SPRUCE ON WHITEFACE MT. IN NEW YORK. J.D. Castello, G.D. Bachand, State Univ. of New York, College of Env. Sci. & Forestry, Syracuse NY 13210, P.M. Wargo, USDA For. Serv., Hamden CT 06514, and V. Jacobi, Rothamstead Exp. Sta., Harpenden, UK AL5 2JQ.

Tomato mosaic virus (ToMV) has been detected previously (Jacobi et.al., 1992. Plant Dis. 76:518-522) in needle and root tissues of red spruce growing on Whiteface Mt. NY. Because crown dieback of red spruce increases with elevation on this mountain, the objectives of this study were to determine if: (1) the frequency of infection and/or the concentration of the virus in root tissues is greater in spruce located above compared to those located below cloud base, and (2) virus presence or concentration in root tissue is associated with crown dieback in red spruce. In August 1991, 322 root samples were collected from 86 trees, approximately four root samples/tree, located on four research plots, two above and two below cloud base, on Whiteface Mt. All samples were assayed for ToMV by enzyme-linked immunosorbent assay. ToMV was detected in approximately 21 of 46 trees above and 5 of 40 trees below cloud base. The virus was unevenly distributed in both fine and coarse roots, appeared to occur in higher concentration in the roots of trees above cloud base, and was detected in trees with and without crown dieback.

A183

SNOW DEPTH IDENTIFIES LATE WINTER AS THE "WINDOW" FOR FREEZING INJURY OF RED SPRUCE. P.D. Manion and J.D. Castello, State Univ. of New York, College of Env. Sci. & Forestry, Syracuse NY 13210.

Why is it called red spruce (*Picea rubens* Sarg.)? The obvious red color of the 1992 foliage of red spruce in the spring of 1993 suggests a natural reason for the common name. Efforts to relate red coloration in the spring with a reduction in cold tolerance by acid mist (DeHayes et.al., Can. J. For. Res. 21:1292-1295) may have incorrectly characterized winter freezing in the spruce decline system. We established 25 plots throughout the Adirondacks and Tug Hill regions in April 1993 and demonstrated that the damage is not limited to cloud-based acid mist high elevation sites. Extreme damage was distributed throughout the region at all elevations and aspects, on large and small trees, and on exposed canopy as well as protected understory trees. Because the lower, snow-covered branches (60-120

cm) were unaffected, snow depth records identify mid-February to mid-March as the window for the major freeze event. Similar and widespread damage to red spruce also occurred during the winter of 1948 (Curry and Church, J. For. 50: 114-116). The unique feature of both these events was the occurrence of two consecutive days of rapid temperature drop from above to well below freezing (but well above the reported critical freezing temperature).

A184

PURIFICATION AND CHARACTERIZATION OF AN ENDOPOLYGALACTURONASE FROM CRYPHONECTRIA PARASITICA. S. Gao and L. Shain. Dept. Plant Pathology, Univ. of Kentucky, Lexington, KY 40546-0091.

An extracellular endopolygalacturonase (E.C.3.2.1.15) was purified to apparent homogeneity from culture filtrates of Cryphonectria parasitica strain Ep 155 grown with 1% sodium polypectate as an inducer in complete medium (Puhalla and Anagnostakis 1971, Phytopathology 61:169-173). Purification involved ultrafiltration, cation exchange chromatography on CM-cellulose, and gel filtration on Sephdex G-75. The molecular weight was approximately 41 kDa as estimated by SDS-PAGE and gel filtration. The pI of the enzyme was about pH 7.9 and it had a pH optimum of 5.0. The temperature optimum was 40 C. The enzyme acted in an endo fashion; a 50% reduction in viscosity of polygalacturonic acid resulted in fewer than 5% hydrolysis of the glycosidic bonds.

A185

***TRICHODERMA HAMATUM* AND *T. HARZIANUM* VS. *PYTHIUM* SPP. IN STORED LONGLEAF SEEDLING ROOT SYSTEMS.** Xiaoan Sun[1], J.P. Jones[1], and J.P. Barnett[2]. [1]L.S.U. Agric. Center, La. Ag. Expt. Ctr., Baton Rouge, LA 70808 and [2]U.S.D.A. Forest Service, So. For. Expt. Stn., Pineville, LA 71360

T. hamatum, T. harzianum, T. pseudokoningii and *T. koningii* were the common species of *Trichoderma* associated with longleaf seedling roots cold-stored 4 wks. Of 1000 *Trichoderma* isolates tested, 292 were able to grow well at 4°C. Through a dual culture technique, 64 of these were determined to be antagonistic to 14 *Pythium* isolates which were pathogenic to newly germinated slash pine seeds in petri dishes. The 14 *Pythium* isolates and 4 *Trichoderma* species were inoculated on pine seedlings which were then cold-stored for 4 weeks. After storage the seedlings were outplanted; those inoculated with *Pythium* suffered relatively high levels of mortality while those inoculated with *Trichoderma* had very high survival rates. The results confirmed that *Pythium* spp. are one cause of pine seedling loss during cold storage and that some *Trichoderma* isolates may be useful for biocontrol of this disease. Field studies are being implemented to evaluate the potential of *Trichoderma* for the biological control of seedling mortality of southern pine during cold storage.

A186

EVALUATION OF CYPROCONAZOLE FOR CONTROL OF FUSIFORM RUST ON LOBLOLLY PINE SEEDLINGS. W.A. Carey and W.D. Kelley. School of Forestry, Auburn University, AL 36849-5418.

Cyproconazole seed treatments and foliar sprays each protected loblolly (Pinus taeda L.) seedlings from fusiform rust (causal agent Cronartium quercuum (Berk.) Miyabe ex Shirai f. sp. fusiforme). In laboratory tests (controlled inoculations), cyproconazole seed treatments at rates of 1.25 and 2.5 g ai/kg seed were effective for at least 20 and 30 days, respectively, after sowing; foliar sprays at rates of 84 or 112 g ai/ha were effective for 14 but not 21 days after application. In nursery trials (natural inoculation), no seedlings receiving only foliar applications of cyproconazole (four applications at test rates of 56, 84, and 112 g ai/ha) were galled at lifting, and only 0.1% of seedlings receiving only the seed-treatment (1.25 g ai/kg seed) were galled. At lifting, 54% of seedlings in non-treated control plots in the field study were galled. Neither seedling heights, diameters, dry weights, nor mycorrhizal development differed among treatments.

A188

INCIDENCE AND SEVERITY OF BUTTERNUT CANKER IN WISCONSIN IN 1976 AND 1992. Jane Cummings Carlson, Mark Guthmiller, Department of Natural Resources, 3911 Fish Hatchery Rd., Madison, WI 53711.

Butternut, Juglans cinerea is being examined for listing as a threatened or endangered species. Its demise is caused primarily by a fungus, Sirococcus clavigignenti-juglandacearum. Butternut canker was first reported in Wisconsin in 1967. In 1976, 2,882 trees on 83 plots in 36 counties were examined; 31% and 9% of the trees surveyed were found to be cankered and dead, respectively. In 1992, 32 counties were resurveyed to determine disease incidence, severity, spread, and impact on reproduction. Of 1,394 trees surveyed, 92% were cankered and 27% dead. Reproduction was frequently affected; 65% of the sprout clumps and 75% of the seedlings were cankered. Apparently disease resistant individuals were rare. Butternut canker was observed in 18 of 36 counties in 1976 and in all 32 counties surveyed in 1992. Rare, noncankered butternut > 10" in diameter is currently being used for preservation of genetic material. Silvicultural techniques are being applied to encourage reproduction of butternut.

A189

PATHOGENICITY OF 3 DECLINE FUNGI ON WATER-STRESSED CORK OAK. K. A. Jacobs[1], C. Colinas[2], I. F. Alvarez[3]. [1,3]Dep. Patol. Veg., IRTA, 08348 Cabrils, Spain, [2]ETS Eng. Agr., Univ. Lerida, 26006 Lerida, Spain.

Two hundred cork oak seedlings were divided into two treatments: well-watered, and watered every 10-20 days. When mean leaf water potentials of the low water treatment were maintained at -2.0 MPa for 8 wks, stems were inoculated with mycelial agar plugs of Botryosphaeria stevensii, Hypoxylon mediterraneum, Coryneum sp., or agar alone. Rate of symptom development was monitored, and shoot growth and canker size were measured after 6 wks. Shoot death occurred in nearly all seedlings inoculated with B. stevensii, but the rate of symptom development and death were not affected by the watering treatment. Water-stressed seedlings inoculated with H. mediterraneum developed significantly (p=0.01) larger cankers (3.4 cm) than non-stressed plants (1.3 cm). Coryneum sp. was not pathogenic to seedlings from either watering treatment.

A190

MORPHOTYPE AND WATER STRESS EFFECTS ON DISEASE DEVELOPMENT BY *SPHAEROPSIS SAPINEA* ON RED PINE. J.T. Blodgett and G.R. Stanosz, Dept. of Plant Path., Univ. of Wisconsin, Madison, WI 53706.

Sphaeropsis sapinea (syn. *Diplodia pinea*) causes a shoot blight and canker disease of pines and other conifers. Severe losses have been reported on trees predisposed by stresses, including drought. Two *S. sapinea* morphotypes ("A" and "B") are recognized and have been suggested to differ in virulence. A greenhouse study was conducted to compare the aggressiveness of "A" and "B" isolates on water-stressed and nonstressed red pine (*Pinus resinosa*). Three-year-old potted seedlings were either watered daily or when the mean predawn water potential fell below -1.64 MPa. Growing shoots were inoculated by placing a colonized agar plug on a wound made by removing a needle fascicle. Two "A" and two "B" isolates were used. After four weeks, "A" isolates caused more severe symptoms and could be recovered further from the inoculation site than "B" isolates (which produced less severe or no symptoms). "A" isolates also caused greater symptom development on water-stressed trees. Symptom development was positively correlated (R^2 = 0.90) with distance of recovery from the inoculation point.

A190a

A needle-cast disease complex in Southern pines. F.F. Jewell, Sr., School of Forestry, Louisiana Tech University, Ruston, LA 71272.

Since 1985, periodic observations and collections have been made of fungi associated with needle-cast symptoms on the four major Southern pines in Alabama, Florida, Louisiana, and Texas. The prominent fungi observed (anamoprh and/or teleomorph) were Lophodermella cerina, Lophodermium australe, Ploioderma lethale, and P. hedgcockii. Similar patterns of seasonal symptom appearance were observed regardless of geographic locality. L. cerina was usually the earliest needle cast observed, with the affected foliage a bright red, which continued until casting (coined "red needle cast"). Characteristic symptoms of L. australe, P. lethale, and P. hedgcockii, which normally appeared >3-5 weeks later than L. cerina, initially appeared reddish then tan to tan/grey prior to casting (grey needle cast). Seldom was L. cerina observed simultaneously with the other pathogens on an individual host. L. australe, P. lethale, and P. hedgcockii were observed both individually or in various combinations on affected foliage of host pines.

A192

GENETIC STABILITY IN POPULATIONS OF *SEPTORIA TRITICI*. R.S. Chen and B. A. McDonald, Department of Plant Pathology and Microbiology, Texas A&M University, College Station, Texas 77843-2132.

Local populations of plant pathogenic fungi potentially undergo repeated extinction and recolonization events each year as a result of the annual cycle of planting and harvesting in agricultural ecosystems. This may lead to large shifts in gene frequencies from year to year in any particular field. Allele frequencies for ten RFLP loci were monitored over a 3-year period in *Septoria tritici* populations sampled from a single wheat field. There were no significant changes in allele frequencies for any of the RFLP loci across years. DNA fingerprints showed that no genotypes were conserved across years, suggesting that asexual spores were not a significant source of primary inoculum in this field. It is likely that the primary inoculum each year was wind-borne ascospores of the teleomorph, *Mycosphaerella graminicola*. The source of ascospores may be *M. graminicola* populations resident on indigenous alternate host species such as *Poa annua* (annual bluegrass). This experiment showed that field populations of plant pathogenic fungi can be genetically stable over several years despite repeated recolonization events.

A193

RECENT MIGRATION, WIDESPREAD DISTRIBUTION, AND PROBABLE SOURCE OF THE A2 MATING TYPE OF *PHYTOPHTHORA INFESTANS* IN THE UNITED STATES AND CANADA. S. B. Goodwin, L. S. Sujkowski, A. T. Dyer and W. E. Fry. Dept. of Plant Pathology, Cornell University, Ithaca, NY 14853.

For the first time in many years, late blight epidemics, caused by *Phytophthora infestans*, were widespread on potatoes and tomatoes in the U.S. and Canada in 1992/1993. Mating type, allozyme, and DNA "fingerprint" analyses of more than 240 isolates collected from nine states and three provinces revealed only eight genotypes. There was a striking change in the genetic composition of some *P. infestans* populations in that six of the eight genotypes were new to the US and Canada. Two A2 genotypes appeared to be recent migrants from northwestern Mexico. One of these was widely distributed (BC, FL, NC, NY, TN) and common (36% of the total sample). The second was common in upstate NY. DNA fingerprint analysis suggests that three additional A2 genotypes in British Columbia may have arisen by *in situ* sexual reproduction. The sixth (A1) genotype was probably introduced from Europe. Most fields contained only a single genotype. The end result of these most recent migrations was a geographic mosaic of genotypes.

A194

RFLPS IN nuDNA DIFFERENTIATE TEXAS ISOLATES OF CERATOCYSTIS FAGACEARUM FROM DIFFERENT OAK WILT CENTERS. P.A.I. Guthrie, B.A. McDonald, and D.N. Appel. Dept. Plant Pathology and Microbiology, Texas A&M University, College Station, TX 77843.

DNA from 20 Texas isolates of Ceratocystis fagacearum originating from 18 disease centers was digested with three restriction enzymes. Southern blots were hybridized with eight probes from a nuclear genomic library that detected RFLPs in previous screening of a geographically diverse group of isolates. Two probes hybridized to moderately repetitive elements which were noteworthy for their high degree of homogeneity. The other six hybridized to single loci or low copy number sequences (two or three copies per genome). The number of alleles at polymorphic loci ranged from two to four, with allele frequencies of 10% to 50% per locus. Combining data from all probes to create multi locus haplotypes, 14 different genotypes were found among the 20 isolates. Two disease centers where the fungus was known to reproduce sexually, separated by half a kilometer, could be differentiated.

A196

SELFING AND VIRULENCE EVALUATION OF SINGLE-OOSPORE CULTURES OF VARIOUS PHYSIOLOGIC RACES OF *PHYTOPHTHORA SOJAE*. R. G. Bhat, and A. F. Schmitthenner, Dept. of Plant Pathology, OARDC/Ohio State Univ., Wooster, OH 44691.

Twenty-five field isolates of *Phytophthora sojae*, comprising of physiologic races 1, 3, 4, 7, 10, 12, 14, 16, 25 and newly designated races 28, 29, 30 and 31, were selfed. Oospores were harvested after one month, and single-oospore colonies were obtained on 1.5% Bacto-agar medium containing 10 µg/ml of cholesterol and rifampicin. A minimum of 30 single-oospore cultures from each isolate was evaluated for virulence on soybean seedlings using a hypocotyl inoculation method. Soybean differentials included Williams near-isolines for *rps*, *Rps*1-a, *Rps*1-b, *Rps*1-c, *Rps*1-k or *Rps*3 genes, Harosoy near-isolines for *Rps*7 or *Rps*6+7 and P.I. 103.091 for *Rps*1-d. Field isolates were heterogenic. Sexual progenies from many field isolates were composed of different virulence phenotypes, including a small percentage of undescribed races. Races 1, 3, 4, 7, 14 and 25 were selfed further, and cultures from second generation bred true for parental phenotypes.

A197

CHROMOSOMAL LOCATION OF GENES CONTRIBUTING TO NET AND SPOT BLOTCH RESISTANCE BASED ON A MOLECULAR MAP OF BARLEY. B. J. Steffenson and P. M. Hayes*. Dept. of Plant Pathology, North Dakota State University, Fargo, ND 58105 and *Dept. of Crop & Soil Sciences, Oregon State University, Corvallis, OR 97331.

Genes conferring resistance to *Pyrenophora teres* f. *teres* (net blotch pathogen) and *Cochliobolus sativus* (spot blotch pathogen) have been described in barley, but little is known about their chromosomal location. Cultivars Steptoe and Morex differ markedly in their reaction to these two pathogens. A doubled haploid population derived from these cultivars was evaluated for reaction to *P. t.* f. *teres* and *C. sativus* in the greenhouse and field. Infection response and disease severity data were merged with molecular mapping data (Kleinhofs et al., 1993 Theor. Appl. Gen.) to identify regions of the barley genome contributing to net and spot blotch resistance. The regions flanked by ABG3-WG1026B on chromosome 4 and ksuA3D-Nar7 on chromosome 6 contributed to net blotch resistance, and the region of ABG500A-ABC164 on chromosome 5 contributed to spot blotch resistance. The use of these flanking molecular markers will increase the efficiency of selection for net and spot blotch resistance in barley.

A198

MENDELIAN INHERITANCE OF TELIOSPORE PRODUCTION IN *UROMYCES APPENDICULATUS*. B.D. McCallum, J.V. Groth, and A.P. Roelfs. Department of Plant Pathology and USDA/ARS Cereal Rust Laboratory, University of Minnesota, St. Paul, MN 55108.

The conversion from urediniospore to teliospore production is a crucial developmental stage the life cycle of most rust fungi, because it represents the transition from asexual to sexual reproduction. *Uromyces appendiculatus* typically produces five spore stages, but isolates exist that have apparently lost the ability to produce teliospores and instead continually cycle as uredinia. A cross was made using urediniospores from an asexual isolate to fertilize pycnia of a sexual isolate. The ability to

produce teliospores on a common host was assessed quantitatively for the parents of the cross, the F_1, and the F_2 progeny. Telia production of the F_1 was intermediate between the two parents, and the F_2 progeny divided into three discontinuous groups. The F_2 progeny fit a 1:2:1 ratio of low : intermediate : high telia production, indicating that a single incompletely dominant gene governs telia production in this cross.

A199

KARYOTYPIC VARIATION WITHIN TILLETIA INDICA ISOLATES FROM INDIA, PAKISTAN, AND MEXICO. P. W. Tooley, R. Beck, G. Peterson, and M. R. Bonde, USDA-ARS, Frederick, MD 21702.

Pulsed-field gel electrophoresis was used to resolve chromosomes of T. indica, causal agent of Karnal bunt of wheat. Mycelium of single-teliospore cultures grown in Difco YM broth for ca. 5 days at room temperature was protoplasted using Sigma lysing enzymes and β-glucoronidase. Contour-clamped homogeneous electric field (CHEF) gel electrophoresis was performed using a Bio-Rad CHEF DR-II system with buffer maintained at 14 C. Chromosome sizes were in the range of 1.0 to 4.6 Mb. A minimum of eight bands were resolved per isolate, with substantial variation in banding intensity observed. Eight isolates tested (five Mexican, two Indian, and one from Pakistan) were found to vary substantially in their karyotypes, indicating the potential for a high degree of genetic variability within T. indica.

A200

PATHOGENICITY AND PHYTOTOXICITY OF ALTERNARIA ALTERNATA AND ITS AAL-TOXIN, FUSARIUM MONILIFORME AND ITS FUMONISIN B_1 ON TOMATO CULTIVARS. H. K. Abbas, T. Tanaka, S. O. Duke, and R. N. Paul. SWSL, USDA-ARS, Stoneville, MS.

Phytotoxicity of AAL-toxin and fumonisin B_1 to six cultivars of tomato was compared with the pathogenicity of their fungal sources, A. alternata and F. moniliforme, respectively. These included two susceptible (asc/asc), three resistant (Asc/Asc) and one heterozygous cultivar (Asc/asc). A. alternata spores were pathogenic to the susceptible but not to the resistant or heterozygous cultivars. F. moniliforme was not pathogenic to any of the tomatoes. Filtrates of both rice grown fungi, containing their respective toxins, caused necrosis within 48 h and mortality on susceptible cultivars but not on resistant. The heterozygous cultivar Asc/asc was minimally susceptible to both toxins with no mortality after 14 days exposure. Dose-response studies on the susceptible cultivar with both toxins showed increasing cellular leakage and decreasing chlorophyll content with increasing concentrations from 0.01 to 1000 μM. Minimal changes occurred on the resistant and heterozygous varieties even at high dosages.

A201

YEASTS PROVIDE LIMITED BIOLOGICAL CONTROL OF *FUSARIUM SAMBUCINUM*, A CAUSAL AGENT OF FUSARIUM DRY ROT OF POTATOES. D.A. Schisler, C.P. Kurtzman, and P.J. Slininger. USDA-ARS, National Center for Agricultural Utilization Research, Peoria, IL 61604.

Thiabendazole-resistant strains of *F. sambucinum* jeopardize options for post harvest control of Fusarium dry rot. Biological control of dry rot is feasible using bacterial antagonists (Schisler et al., 1992. Phytopath. 82:1120). The impact of yeasts on dry rot is unknown. Strains of 20 species of yeasts were selected from the ARS Culture Collection (NCAUR) based on plant origin or expectation of survival in soil. The control potential of these and 14 additional strains from dry rot suppressive soils was evaluated using a whole tuber bioassay. At 2 x 10^6 cells/ml, only two unidentified strains and *Cryptococcus laurentii* strain NRRL Y-2536 reduced disease (P=0.05, P=0.10, respectively) while *Pseudomonas fluorescens* 2-79 was more effective (P=0.01). Conversely, *Wingea robertsii* increased disease (P=0.05). No yeast strains significantly controlled disease in a subsequent trial. Increasing yeast cell concentrations 10-fold did not improve the performance of several strains of *C. laurentii* and *Pichia farinosa*.

A202

Characterization of Bacteriophages of Erwinia ananas. C. G. Eayre, USDA-ARS, 2301 S. Intl. Blvd., Weslaco, TX 78596, and N. L. Robertson, Department of Plant Pathology, Texas A&M, College Station, TX 77843-2132. Phages of Erwinia ananas were characterized according to particle morphology, plaque morphology, host range, and other traits. The phages, originally isolated on 13 isolates of E. ananas, fell into 4 distinct, non-overlapping host groups. None formed plaques on E. herbicola, E. carotovora pv. carotovora, or Escherichia coli. Within one group were 3 phages of identical host range, but different particle morphology, as well as different plaque morphology. Particle morphologies included filamentous particles of different length classes (Bradley's group F) and tailed particles (Bradley's group B). A combination of different phages with the same host ranges used for biological control of bacterial pathogens should limit development of resistant pathogen populations.

A203

SANOSIL-25 (HYDROGEN PEROXIDE): A SUBSTANCE THAT CONTROLS POSTHARVEST DECAY IN FRUITS AND VEGETABLES. E. FALLIK, Y. AHARONI, S. GRINBERG, A. COPEL and M. GIL. *Dept. of Postharvest Science of Fresh Produce, ARO, The Volcani Center, Bet Dagan 50250, Israel.*

Sanosil-25 is a universally applicable disinfectant that is highly effective against pathogenic bacteria, fungi, algae and viruses. The compound is a strong oxidant which contains 48% hydrogen peroxide (H_2O_2) and 0.05% silver ion (Ag+) as a stabilizing agent. Sanosil has been approved for use in drinking water and in food industries at concentrations between 0.005 and 0.03%, and as a surface disinfectant at a concentration of up to 3%.

The ED_{50} of Sanosil-25 for the inhibition of conidial germination *in-vitro* was 0.09% and 0.18% for *Botrytis cinerea* and *Alternaria alternata*, while germination was completely inhibited at concentrations of 0.4% to 0.7%. Dipping commercially harvested eggplants and sweet red pepper in 0.5% Sanosil-25 reduced decay development by 30 to 50% compared to untreated control.

A204

DISTRIBUTION AND OCCURRENCE OF *LEUCONOSTOC MESENTEROIDES* IN CALIFORNIA FRESH MARKET TOMATO FIELDS. K.E.Conn AND J.M. Ogawa, Dept. of Plant Pathology, Univ. of California, Davis, CA 95616.

The coccoid, Gram-positive, lactic acid-producing bacterium, *Leuconostoc mesenteroides* ssp. *mesenteroides* (*LMM*), is a postharvest wound pathogen of mature-green and ripe fresh market tomato fruit in California and Mexico. In 1992, distribution and occurrence of *LMM* on fresh market tomato in California was determined in 5 furrow-irrigated fields in the San Joaquin Valley and 3 in the Salinas Valley. A minimum of 20 samples each of fruit, leaflets, decayed fruit, and leaves of weeds and 10 soil samples from each tomato field were randomly collected from all locations. A stationary, non-aerated enrichment procedure was used for isolation of *LMM* strains from plant tissues or soil. Non-surface sterilized, excised fruit tissue and leaf disks, or subsamples of soil, were incubated in MRS broth at 25C for 4 days. *LMM* was recovered from all fields, however, recovery of the organism from tissues and soils varied between fields. *LMM* was recovered more often from fungal-decayed fruit (85%) and soil (33%) than from healthy fruit (14%), or tomato leaflets (9%) and weeds (17%). Mature-green fruit, wound-inoculated with strains of LMM from various sources and fields, produced characteristic decay symptoms.

A205

EFFICACY OF IPRODIONE COMPARED TO DICHLORAN FOR POSTHARVEST CONTROL OF RHIZOPUS ROT OF SWEET POTATOES. J.E. Adaskaveg, K.E. Conn, and J.M. Ogawa, Plant Pathology Dept, Univ. of California, Davis, CA 95616.

Iprodione (Rovral 1.2 g ai/L) and iprodione plus 20% Decco Wax 255 were compared with dichloran (Botran 0.9 g ai/L) for control of postharvest Rhizopus rot of sweet potatoes. Sweet potatoes (cv. Garnet), stored at 13C, 85% RH for 6 months, were rinsed in a water dump tank, spray rinsed with potable water, and air-dried (43-51C) on a steel-mesh conveyor belt. Roots were injured at three sites (3 X 0.5 X 0.5 cm) by striking them on a steel bar before packing in cardboard boxes (5 reps of 9 kg of roots/rep). Half of the roots were dipped for 2 min in a suspension of *Rhizopus stolonifer* (62,000 spores/ml) originally isolated from sweet potato and grown on potato dextrose agar. Roots were treated using a commercial 'T-Jet' sprayer and conveyor belt. After 3 days at 20C and >95% RH, percentage of wounds with Rhizopus rot was 81.5, 22.8, 26.4, and 12.7 for inoculated roots and 16.0, 3.1, 0.2, and 1.7 for noninoculated roots for check, iprodione, iprodione-255, and dichloran treatments, respectively. Fungicide treatments were significantly different from the check ($P < 0.05$); whereas in inoculated treatments, dichloran provided the best control. Similar trends in decay were observed for 7 and 14 days.

A206

A COMPARISON OF POSTHARVEST RESISTANCE TO AFLATOXIN CONTAMINATION WITH PREHARVEST RESISTANCE IN FORTY-

FIVE MAIZE GENOTYPES R.L. Brown, T.E. Cleveland, K.W. Campbell,* and D.G. White,* USDA, ARS, Southern Regional Research Center, P.O. Box 19687, New Orleans, LA 70179 and *The Department of Plant Pathology, University of Illinois, Urbana, IL 61801.

Forty-five maize inbreds and hybrids, previously screened for resistance to aflatoxin contamination by *Aspergillus flavus* in 1991 and 1992 Illinois field trials, were screened for postharvest resistance to contamination by *A. flavus* using a laboratory assay. Whole kernels from each line were evaluated in three trials. The MAS:gk maize population, previously shown to be highly resistant to aflatoxin contamination in numerous field trials, was also tested. Results indicate a high level of consistency among the three laboratory trials. Genotypes exhibiting high levels of preharvest resistance were generally the most resistant in the postharvest assay. The laboratory assay may have potential use in screening for both postharvest and preharvest resistance.

A207

VEGETATIVE COMPATIBILITY GROUPS OF *FUSARIUM MONILIFORME* FROM SEED, PLANTS, AND GRAIN OF FIELD-GROWN MAIZE. R. A. Shelby and D. Zhang, Department of Plant Pathology, Auburn University, AL 36849-5409.

Nitrate non-utilizing (NIT) mutants were generated for in 14 isolates of *F. moniliforme* from one maize seed lot. By appropriate pairing of NIT mutants it was possible to identify vegetative compatibility groups (VCG's) that could serve as genetic markers to trace isolates of this fungus which is toxigenic in grain. NIT mutants were similarly generated and VCG's identified from *F. moniliforme* grain isolates of this seed lot. Most isolates (13/14) from the seed were all of one VCG. From the grain, 10 isolates represented 4 VCG's, none of which were common to the seed used to produce it. These data suggest that seed may not be the predominant inoculum source for this organism. We are currently following infection throughout the growing season, isolating *F. moniliforme* from all plant parts, and identifying VGG's in attempt to identify the inoculum source of the fungus in infected grain.

A208

EFFECT OF SIX *Aspergillus flavus* TYPE ISOLATES ON KERNEL INFECTION AND AFLATOXIN CONTAMINATION IN FIELD INOCULATED MAIZE IN MISSISSIPPI. N. Zummo and G.E. Scott, USDA-ARS, P.O. Drawer PG, Miss. State, MS 39762.

Kernels and cobs from maize ears, field-inoculated with individual *Aspergillus flavus* type isolates were assayed for kernel and placental infection and aflatoxin contamination. Kernels from inoculated ears did not differ visually from kernels from uninoculated ears. Isolates differed significantly in their ability to infect kernels, but not placentas, and in ability to produce aflatoxin in kernels and cobs. Isolates that produced high levels of aflatoxin in kernels also produced high levels in cobs. The ability of an isolate to infect kernels apparently did not influence the ability to produce aflatoxin in kernels or cobs. For instance, isolate NRRL 3251, which did not differ significantly from other isolates in ability to infect kernels, produced significantly more aflatoxin in kernels than all other isolates.

A209

THIABENDAZOLE RESISTANCE IN *FUSARIUM* SPP. CAUSING FUSARIUM DRY ROT OF POTATO IN NEW YORK STATE. L.E Hanson and R. Loria, Department of Plant Pathology, Cornell University. Ithaca, NY 14853

The incidence of thiabendazole (TBZ) resistance in *Fusarium* spp. causing dry rot of potato tubers in New York was estimated during 1992. Most tuber samples were randomly collected from seed tuber lots. Of the 81 samples (5 tubers per tuber lot), 52% yielded *Fusarium* isolates from dry rot lesions, all of which were pathogenic in potato tuber bioassays. Approximately 52% of the isolates were *Fusarium sambucinum*. Other isolates obtained included *F. avenaceum*, *F. culmorum*, *F. solani* and *F. oxysporum*. Of the 67 *Fusarium* isolates obtained, 33 were resistant to TBZ at 25 µg/ml *in vitro*; of those, 31 were *F. sambucinum*. One resistant isolate each of *F. oxysporum* and *F. solani* were also identified. This is the first report of TBZ resistance in *F. solani* from potato.

A210

TRANSFORMATION OF TOBACCO WITH FOUR DIFFERENT NUCLEOCAPSID CONSTRUCTS OF TOMATO SPOTTED WILT VIRUS. R. Perez, S.M. Geske, J. Speck, P. Reese, J.W. Moyer and M.E. Daub, Dept. of Plant Pathology, North Carolina State University, Raleigh, NC 27695-7616.

Nicotiana tabacum cv. 'Burley-21' has been transformed with four constructs of the nucleocapsid (N) gene of tomato spotted wilt virus. These include two full length constructs, the 784 bp N gene (N+) and the N gene plus viral flanking sequences of 71 and 84 bp (Nf), both which produce a 29 kd protein in *in vitro* translation assays. Also used was a mutated full length insert that produces a 27 kd protein (T), as well as an antisense construct (N-). Transgenic plants were generated using *Agrobacterium tumefaciens* strain LBA 4404 and plasmid vector pBI121. All constructs except Nf resulted in virus resistant R_o plants. Analysis of twelve segregating R_1 lines from selfed R_o T plants have yielded a highly resistant line (66% of the plants resistant), three moderately resistant lines (36%, 18%, 16% resistant) and eight 100% susceptible lines. R_1 plants of Nf and N+ transformants are currently being analyzed.

A211

TRANSFORMATION OF CHRYSANTHEMUM WITH THE NUCLEOCAPSID GENE FROM TOMATO SPOTTED WILT VIRUS. J. M. Sherman , L. A. Urban, S. M. Geske, J. Speck, J. W. Moyer, and M. E. Daub, Dept. of Plant Pathology, North Carolina State University, Raleigh, NC 27695-7616.

Chrysanthemum cultivar 'Iridon' was transformed with two tomato spotted wilt virus nucleocapsid (N) gene constructs using the disarmed *Agrobacterium tumefaciens* strain EHA105. One construct (N+) contained only the sequence of the N gene (nt 1983 to 2767) while the second (Nf) contained additional flanking sequences (nt 1912 to 2854). Transformed shoots were regenerated from leaf explants on Murashige and Skoog medium with 11.5 µM indoleacetic acid, 1.0 µM benzyladenine, and 50 mg/l kanamycin. To date, PCR analysis demonstrated that 32 of 78 Nf and 3 of 8 N+ putative transformants contained the N gene. Southern hybridization of PCR-positive plants indicated stable integration of the N gene at multiple sites. Transformants have been challenged with TSWV by mechanical and thrips inoculations.

A212 Withdrawn

A213

ANTISERUM TO NEW YORK ISOLATE OF WHEAT SPINDLE STREAK MOSAIC VIRUS DETECTS GEOGRAPHICALLY DIVERSE BAYMOVIRUSES. J. E. Carroll[1], G. C. Bergstrom[1], and S. M. Gray[2], Dept. of Plant Pathology, [1]Cornell University and [2]USDA-ARS, Ithaca, NY 14853.

A polyclonal antiserum produced in rabbit against a New York isolate of wheat spindle streak mosaic virus (WSSMV) was used to test the serological properties of WSSMV isolates from North America and their relationship to wheat yellow mosaic virus (WYMV), barley yellow mosaic virus (BaYMV), and barley mild mosaic virus (BaMMV) from Europe and Asia. Samples were tested in indirect ELISA, direct DAS-ELISA, and electroblot immunoassay. The antiserum reacted with the 33 kD WSSMV capsid protein and did not react with soilborne wheat mosaic virus, a furovirus, or wheat streak mosaic virus, a potyvirus. Using DAS-ELISA, differences were found among North American isolates of WSSMV suggesting that distinct strains of the virus occur here. In general, our antiserum reacted with all isolates of WSSMV, WYMV and BaYMV tested, but not with BaMMV. Our results corroborate previous results on the serological relationships of baymoviruses and demonstrate the existence of distinct strains of WSSMV in North America.

A214

IDENTIFICATION OF A WEED PLANT SPECIES AS A HOST OF TOMATO MOTTLE VIRUS IN FLORIDA. J. E. Polston[1], R. J. McGovern[2], G. M. Danyluk[1], E. Hiebert[3], and P. A. Stansly[2]. University of Florida, [1]Gulf Coast Research and Education Center, Bradenton, FL 34203, [2]Southwest Florida Research and Education Center, Immokolee, FL 33934, [3]Department of Plant Pathology, Gainesville, FL 32611.

Field surveys in southwest and west central Florida and greenhouse transmission experiments were conducted to identify naturally occurring weed hosts of the whitefly-transmitted geminivirus, tomato mottle virus (TMoV). Approximately 780 samples, representing 42 species in 14 families were collected from 39 field sites over three years. Detection procedures included nucleic acid spot hybridization assays with confirmation by polymerase chain reaction and virus inclusion visualization. Experimental transmission of TMoV to over 300 plants representing 18 species in 7

families was conducted using sweetpotato whiteflies (*Bemisia tabaci* Genn., biotype B). One exotic weed, yellow tropical soda apple, *Solanum viarum* Dunal (Solanaceae) was found to be naturally infected in the field at low incidence and could be infected in the greenhouse by whitefly transmission. This rapidly spreading weed also supports the reproduction of *B. tabaci*. Tomato (*Lycopersicon esculentum*) appears to be the most important host of TMoV in the environment at this time.

A215

TRANSMISSION OF MAIZE CHLOROTIC DWARF VIRUS BY PIN INOCULATION OF MAIZE KERNELS. R. Louie, USDA-ARS, Dept. of Plant Pathology, OARDC/The Ohio State University, Wooster, OH 44691.

Maize chlorotic dwarf virus (MCDV) is semipersistent in *Graminella nigrifrons*, its principal vector, and, until now, was obligately transmitted by leafhoppers. A severe isolate of MCDV (MCDV-S) was transmitted by pin-inoculation of kernels of Seneca Chief sweet corn (*Zea mays* L. var *saccharata*). For inoculation, 6 μl of extract (leaf tissue ground in 5 vol 0.01M potassium phosphate, pH 7.0) was placed on the coleoptile end of the embryo (seed presoaked 4 hr). Five (#1) insect pins were tied together, mounted on a engraving tool, and used to pierce the coleoptile end twice while passing thru the inoculum. Transmission rates of 1-5% occurred in seven tests of about 100 plants each. MCD symptoms developed on the 2nd or 3rd leaf within 5-7 days after inoculation. Infection was confirmed both by ELISA and by re-acquisition and transmission to healthy test plants by *G. nigrifrons*. No symptoms developed on plants of kernels pin-inoculated with buffer.

A216

DIFFERENTIATION OF WHEAT STREAK MOSAIC VIRUS ISOLATES BY PCR AND RFLP ANALYSIS. Roy French, Jill E. Petrisko, Nancy L. Robertson*, and P. Stephen Baenziger. USDA, ARS, Departments of Plant Pathology and Agronomy, University of Nebraska, Lincoln, NE, and *Department of Plant Pathology and Microbiology, Texas A&M University, College Station, TX

Isolates of wheat streak mosaic virus (WSMV) can be separated by host symptom severity and by apparent size of capsid protein but no serological variation has been reported. The polymerase chain reaction (PCR) was used to amplify 3'-terminal cDNA sequences of four isolates of WSMV, including the type isolate, (ATCC PV 57), 'Sidney' (collected 1981 from wheat in Nebraska), 'Corn' (collected 1978 from corn in Nebraska), and 'Wyoming' (collected 1989 from wheat in Wyoming). Digestion of the *ca.* 1.4 kilobase PCR products with several restriction nucleases gave identical banding patterns for all four isolates while digestion with *Alu* I gave rise to patterns unique for each isolate. *Ava* I, *Hha* I, and *Hpa* I each differentiated one or two of the four isolates. The sequence variability among these isolates suggests that it will be feasible to study WSMV epidemiology using PCR/RFLP analysis.

A217

IMMUNOLOGICAL ANALYSIS OF THE STRUCTURAL PROTEINS OF BARLEY YELLOW DWARF VIRUS. Jen-Yeu Wang & Stewart M. Gray, Department of Plant Pathology, Cornell University and USDA-ARS, Ithaca, NY 14853.

The virions of barley yellow dwarf virus (BYDV) are composed of a 22 Kd capsid protein (CP) and potentially a 72 kd protein expressed as a readthrough product of open reading frame 3 (ORF 3, CP) and ORF 5. Separation of virion proteins by SDS-PAGE resolved two predominant bands of 22 Kd (CP) and 48 Kd (thought to be derived from the 72 Kd readthrough protein). Various antibodies were used in immunoblotting assays to characterize these proteins. Polyclonal and monoclonal antibodies produced against PAV virions recognized both the 22 Kd CP and the 48 Kd protein. Additionally, a 48 Kd protein was also detected by these antibodies in a crude extract from PAV infected leaves. To further identify the origin of the 48 Kd protein, the ORF 5 of NY-PAV was cloned and subclones of different regions were ligated into the bacterial expression vector-pET to express native proteins in *Escherichia coli* . Two polyclonal antibodies, anti-O5N and anti-O5C, were produced against the expressed peptides representing the amino- (a.a. 35-220) and the carboxyl- (a.a. 221-449) halves of the ORF 5 protein, respectively. The anti-O5N antibody recognized a 48 Kd protein from purified virions as well as a 48 Kd protein from the soluble and insoluble fractions of NY-PAV infected leaves. The anti-O5C antibody detected a 72 Kd protein in the insoluble fraction from NY-PAV and NY-MAV infected leaves, but did not react with SDS-denatured proteins from purified virions. These results provide direct evidence that the BYDV particle contains not only the 22 Kd CP but also a 48 Kd protein, *in planta*.

A218

VARIABILITY AMONG PAV ISOLATES OF BARLEY YELLOW DWARF VIRUS. C. Chay, D. Smith, and S.M. Gray, USDA-ARS, Ithaca, NY 14853.

Three isolates of barley yellow dwarf virus (BYDV) that cause severe symptoms on 'Coast Black' oats were identified during a field survey in 1992. The relative severity of symptoms induced by each isolate was consistent in various spring and winter oat cultivars, but differed among the three isolates. Each of these isolates, PAV-83, PAV-129 and PAV-251, reacted with antisera to NY-BYDV-PAV in DAS-ELISA, and was transmitted by the aphid vector Rhopalosiphum padi L. We compared the relationships of these isolates to NY-BYDV-PAV by restriction enzyme analysis of PCR products generated with primers that either amplified a portion of the coat protein gene (ORF 3) or amplified sequences of a coding region 5' of the coat protein gene (ORF 5). The banding pattern of restriction fragments for the ORF 3 PCR products were identical to NY-BYDV-PAV for PAV-251, and polymorphic with at least one restriction enzyme for PAV-83 and PAV-129. Using primers that amplify ORF 5 of NY-BYDV-PAV, we obtained a PCR product for isolates PAV-251 and PAV-83, but failed to obtain a product for PAV-129. The banding patterns of the ORF 5 PCR products were either identical to that of NY-BYDV-PAV (PAV-251) or polymorphic with one restriction enzyme (PAV-83). We are currently testing whether restriction fragment polymorphisms are correlated with symptom expression and/or aphid transmission phenotypes.

A219

BIOLOGICAL AND MOLECULAR CHARACTERIZATION OF LETTUCE MOSAIC POTYVIRUS (LMV) ISOLATES FROM THE SALINAS VALLEY. F.M. Zerbini Jr.[1], S.T. Koike[2], and R.L. Gilbertson[1]. [1]Department of Plant Pathology, University of California-Davis and [2]UC Cooperative Extension, Salinas, CA.

Lettuce mosaic potyvirus (LMV) isolates were collected from infected lettuce plants in the Salinas Valley and characterized based on host range and reaction on a series of lettuce differential cultivars. There were no differences among isolates in host range or in infection of the lettuce differential cultivars. Only cultivars 'Salinas' and 'Ithaca', which lack LMV resistance genes, were infected. Different disease symptoms developed on these two cultivars. However, these differences were consistent for all LMV isolates and may be due to environmental effects. The nucleotide sequence encoding the coat protein of one LMV isolate (LMV-S) was determined and found to be 96% identical at the amino acid level to that of an LMV 'type' isolate (LMV-0). These results suggest that recent outbreaks of lettuce mosaic in the Salinas Valley are not due to new strains (pathotypes) of LMV.

A220

DIVERSITY AMONG ISOLATES WITHIN THE TOMATO SPOTTED WILT VIRUS SEROGROUP (SPECIES). S. M. Geske, J. M. Hall, J. Speck, P. Reece and J. W. Moyer. Dept. of Plant Pathology, North Carolina State University, Raleigh, NC 27695-7616.

Tomato Spotted Wilt Tospovirus (TSWV) has long been considered a monotypic taxa; however, several new viruses have been recently defined by differences in sequence homology. Serologically distinct nucleocapsid proteins (N) have been shown to be indicative of different species (= serogroup = virus). Little is known about the diversity within these new taxa. To study serogroup diversity, 16 field isolates obtained from tobacco, peanut, tomato and several floral crops, classified as TSWV based on polyclonal antibody reactions, were compared biologically and at the molecular level. Isolates had overlapping host ranges. Additionally, variation in symptom severity was noted with a variety of hosts, as well as with TSWV resistant transgenic tobacco lines. A panel of monoclonal antibodies to the N protein further differentiated the isolates into 2 serotypes, indicating epitope dissimilarity. Nucleic acid sequencing of epitope regions is ongoing.

A221

VIRULENCE VARIATION IN *SETOSPHAERIA TURCICA* POPULATIONS COLLECTED FROM MAIZE IN CHINA, MEXICO, UGANDA, AND ZAMBIA. H.G. Welz, R. Wagner, and H.H. Geiger, Univ. of Hohenheim (762), D-70593 Stuttgart, Germany.

Virulence/avirulence of *S. turcica* isolates to genes *Ht1*, *Ht2*, *Ht3*, and *HtN* was assayed in growth chambers using near isogenic maize inbred lines of H4460 and B37. Race 1 (i.e., virulent to *Ht1*) was frequent in northern China but absent in the subtropical south of China where race 0 dominated. In Mexico race 1 was also absent but virulence to *Ht2*, *Ht3*, and *HtN* nearly fixed (race 23N: 80%, race 23: 14%, race 2N: 5%). The *S. turcica* population sampled in Zambia (races 1: 0%, 23: 53%, 23N: 29%, 0: 16%) was similar to that in Mexico but quite different from the one in Uganda (races 0: 44%, N: 44%, 2: 4%). Several new races (e.g., races 2, N, 123N) were detected in this study. Virulence to *Ht1* was found only in northern China where it had probably been selected by growing corn hybrids with the *Ht1* gene. Virulence to *Ht2*, *Ht3*, and *HtN* occurred in all five populations in spite of the very limited use of these resistance genes.

A222

ASSOCIATION OF PHOTOCARCINOGENIC FUROCOUMARINS WITH SUSCEPTIBILITY OF PARSLEY CULTIVARS FOR SEPTORIA BLIGHT. R.F.

Cerkauskas and M. Chiba, Agriculture Canada, Research Station, Vineland Station, Ontario L0R 2E0.

Nineteen parsley (Petroselinum crispum (Mill.)Nym. ex A.W. Hill) cultivars (cvs.) varying in susceptibility to Septoria blight (Septoria petroselini (Lib.)Desmaz.) as determined using a scale of 0=healthy to 5=dead were evaluated for levels of naturally- occurring photocarcinogenic furocoumarins consisting of psoralen, 5-methoxypsoralen, 8-methoxypsoralen, angelicin, and isopimpinellin using reversed-phase HPLC in field plants inoculated (I) or non-inoculated (NI) with the fungus at Vineland in 1989. Significant ($P<0.05$) differences in levels of total furocoumarins were observed among cultivars (6.82-128.50 ug/g fresh weight) and between I and NI plants (I/NI ranged 0.65-3.23). There was a significant correlation between the total furocoumarin level and the final disease rating ($r=0.65$) or the area under the disease progress curve ($r=0.74$). Generally, furocoumarin levels in the resistant flat leaf and root-type cvs. were substantially lower than those in the susceptible curled leaf cvs. These observations were confirmed in a smaller scale of experiments conducted in 1990.

A223

INHERITANCE AND ALLELIC RELATIONSHIPS OF RESISTANCE TO PEPPER MOTTLE VIRUS IN *CAPSICUM*. J.R. Blauth, J.F. Murphy and M.M. Kyle, Department of Plant Breeding and Biometry, 252 Emerson Hall, Cornell University, Ithaca, NY 14853

Currently, resistance to the three major potyviral pathogens of pepper is attributed to a recessive allelic series at the locus *et.* Our data indicate that this model is not correct. We are determining the genetic relationships between three widely used sources of resistance to pepper mottle virus (PeMV), tobacco etch virus (TEV), and potato virus Y (PVY). These sources display two phenotypically distinct classes of resistance. *Capsicum annuum* 'Avelar' has a different form of resistance than do *C. chinense* PI152225 and PI159236. We have confirmed the recessive inheritance of the resistances and have elucidated the allelic relationships. We are using both symptom expression and detection of virus in uninoculated tissues to determine the responses of the different populations to virus inoculation. PeMV was selected for our first tests and populations are subsequently being evaluated with TEV. PeMV resistance in 'Avelar' maps to a locus that is distinct from the *C. chinense* sources, while the PI lines have alleles at the same locus. We are determining if there is any linkage between the distinct loci in 'Avelar' and PI152225, and assessing the breadth of viral isolates affected by each allele.

A224

IDENTIFICATION OF SOME POTENTIAL "UNIVERSAL SUSCEPTS" TO DURUM LEAF RUST ISOLATES. A.N. Mishra, A.P. Roelfs, and E.E. Saari*. Dept. of Plant Pathology and USDA-ARS Cereal Rust Lab, Univ. of Minn., St. Paul, MN 55108; *CIMMYT, Mexico.

It was recently shown that some leaf rust isolates from durum wheat did not attack bread wheat. Local Red, a durum land race from India, was susceptible to most durum leaf rust isolates. We tested 110 durums and 10 bread wheats for seedling responses to 18 durum leaf rust isolates. The isolates were from: Chile(1), Ethiopia(3), India(3), Israel(1), Mexico(1), Morocco(4), Turkey(4), and USA(1), representing 7 of the 10 world rust epidemiological zones. None of the bread wheats including Thatcher, Morocco, Little Club, Line E, Agra Local, and Pissi Local was susceptible to all the isolates. The durum wheat stocks Local Red, Arabian Durum, Baxi 411-40, Bijapur 370-4, Jhansi Local, Malvi Local, Nagpur Local, Neemuch Amber, Sanver Local, and Sarangpur Local were susceptible to all the test isolates. These lines hold promise as "universal suscepts" to durum leaf rust and are useful in genetic studies of resistance.

A225

COMPARISON OF DISEASE RESISTANCE INDUCED BY UV AND PRE-INOCULATION IN STORED CARROTS. J. Mercier, J. Arul and D. Rousselle. Dépt. de sciences et technologie des aliments, Université Laval, Ste-Foy, Quebec, Canada G1K7P4

The resistance induced by UV in carrots stored at 1°C was compared to systemic resistance induced by pre-inoculation with Botrytis cinerea. Carrots exposed to UV on the entire surface were resistant to B. cinerea with concomittant accumulation of 6-methoxymellein (6-MM) in the peel (386 µg/g). The healthy portion of pre-inoculated roots showed similar resistance when challenged, but 6-MM accumulation was lower (138 µg/g). In carrots partially exposed to UV, the non-irradiated portion showed only marginal resistance and no 6-MM accumulation. The chitinase and glucanase isozyme patterns were the same for all the treatments and the control, although chitinase activity was higher in resistant tissues. It is hypothesized that UV-induced resistance is a result of localized 6-MM accumulation, while systemic resistance may involve other mechanisms as well.

A226

TRANSFORMATION OF LETTUCE WITH NUCLEAR PROTEIN GENE OF TOMATO SPOTTED WILT VIRUS. M. Wang, J. Cho, J. Hu, Department of Plant Pathology, University of Hawaii, Honolulu, HI 96822, T. German Department of Plant Pathology, University of Wisconsin, Madison, WI 53706, and J. W. Kim, S. Sun, Department of Plant Molecular Physiology, University of Hawaii, Honolulu, HI 96822

Plasmid pBI121 N46 carrying nuclear protein (NP) gene of tomato spotted wilt virus (TSWV) was introduced into Lactuca sativa L. cultivars, Augustus, Manoa, and Cobham Green, through A. tumfaciens. Transformation Murashige and Skogg medium containing 50 mg/L of kanamycin and 150 mg/L ampicillin was used. Shoots developed from callus were transferred to rooting medium with same amount of antibiotics. Transformation efficiency for Augustus, Manoa and Cobham Green was 14%, 30%, and 23% respectively. Fifty-three percent of Augustus, 66.6% of Manoa and 87.8% of Cobham Green transformants carried the NP gene, although gene expression level varied among individuals. Percentage of plants carrying only NPT II gene was 36.6%, 20.8%, and 12.1% for Augustus, Manoa and Cobham Green, respectively. R1 generation plants will be inoculated with different TSWV isolates and resistance evaluated.

A227

USE OF THE β-GLUCURONIDASE (GUS) REPORTER GENE SYSTEM TO DETECT RESISTANCE IN WHEAT TO *PSEUDOCERCOSPORELLA HERPOTRICHOIDES*. R.C. de la Peña and T.D. Murray, Department of Plant Pathology, Washington State University, Pullman, WA 99164-6430.

Resistance in wheat to *P. herpotrichoides* was detected by inoculating 2-wk-old plants with a GUS-transformed strain of the pathogen. Disease severity in six wheat genotypes was evaluated at 2 wk intervals by visually scoring symptoms, GUS enzyme assay, and ELISA. Differences among resistant and susceptible genotypes were apparent 2 wks postinoculation, however, the greatest differentiation occurred at 6 and 8 wks postinoculation. GUS activity was 7-40 times greater in susceptible than resistant genotypes, and provided statistically significant differences among highly resistant and resistant genotypes. ELISA values were 3-10 times greater in susceptible than resistant genotypes, but did not differentiate highly resistant and resistant genotypes. The GUS reporter gene allowed rapid quantification of fungal growth within plants and was more sensitive than ELISA or visual disease ratings.

A227a

MARKERS FOR DISEASE RESISTANCE OF *CUCUMIS MELO* TO *FUSARIUM OXYSPORUM* . W.P. Wechter[1], C.E. Thomas[2] and R.A. Dean[1]. [1]Department of Plant Pathology and Physiology, Clemson University, Clemson, SC 29634. [2]USDA Vegetable Laboratories, Charleston, SC 29414.

Biochemical and genetic markers for resistance to *Fusarium oxysporum f. sp. melonis* race 1 in *Cucumis melo* are being investigated. For biochemical markers, preinfection levels of salicylic acid and peroxidase were measured. PCR generated Random Amplified Polymorphic DNA (RAPD) and DNA Amplification Fingerprinting (DAF) were employed to identify genetic markers. No correlation between peroxidase and Fusarium resistance in *C. melo* was observed. Salicylic acid levels fluctuated considerably indicating this is not a reliable marker. Bulked segregant analysis of F_2 populations of *C. melo* yielded 2 putative RAPDs from 220 primers analysed. DAF, employing PAGE and silver staining techniques for greater band resolution and sensitivity, yielded an additional 2 polymorphisms between resistant and susceptible F2 bulks from 50 primers analysed.

A228

EFFECT OF PLANT AGE, LEAF AGE AND LEAF POSITION ON INFECTION OF CARROT LEAVES BY *Cercospora carotae*. Arnout van Delden and Odile Carisse. Wageningen Agricultural University, Department of Plant Pathology, POB 8025, 6700 EE Wageningen, The Netherlands. Agriculture Canada Research Station, 430 Gouin Blvd., St-Jean-sur-Richelieu, Québec, Canada J3B 3E6.

A greenhouse study was conducted to determine the effects of plant age, leaf age and leaf position on infection of carrot by *Cercospora carotae*. The relative number of lesions decreased linearly with increasing plant age from 39- to 60-d-old plants, and remained low from 60- to 71-d-old plants. The incubation period increased from 9.0 to 16.6 d, with increasing plant age. Relative number of lesions decreased with increasing leaf age from 1 to 36 d, but the variation among leaves was high. The incubation period increased from 9.0 to 18.3 d with increasing leaf age, but lesions on few young leaves appeared relatively late. Generally, differences in relative number of lesions for leaves on different positions for 10- and 13-wk-old plants were not significant. All leaves except the two youngest were a good estimator of infection on all leaves. Effect of leaf position on incubation period was

different for the 10- and 13-wk-old plants and for the two trials. Plants younger than 60 d-old, in the seven to eight-leaf stages should be used for experiments on the initial development of Cercospora blight of carrots.

A229

VERTICAL VARIATION OF THE AERIAL CONCENTRATION OF VENTURIA INAEQUALIS ASCOSPORES IN AN ORCHARD. Donald E. Aylor, The Connecticut Agric. Experiment Station, New Haven, CT 06504.

The ability to determine the concentration of pathogenic spores in the air surrounding host plants is fundamental for developing models to predict the airborne spread of diseases. The aerial concentration of Venturia inaequalis ascospores, C (spores m^{-3}), was measured using Rotorod samplers in a young orchard of dwarf apple trees. The rotorods were deployed at several heights above the ground between 0.15 and 2.10 m. C decreased rapidly (50 to 95%) with sampling height between 0.15 and 0.40 m and less rapidly (5 to 20%) between 0.40 m and 2.10 m. The rapid decrease of C near the ground mirrored the shape of the vertical wind speed profile. Values of C were modeled as a function of height using an advective-diffusion equation. The equation described the general shape of the vertical variation of C, but tended to predict smaller values of C at heights > 0.40 m than were observed. This discrepancy appears to be due to an under-estimation of the turbulent diffusivity which is not presently well specified during rain.

A230

WHEAT FOLIAR INFECTIONS AFTER DISCRETE PERIODS OF FIELD EXPOSURE. L.J. Francl and J.G. Jordahl. Department of Plant Pathology, North Dakota State University, Fargo, ND 58105.

Infection periods are typically described for defined environmental conditions but outdoor conditions fluctuate and inocula vary in amount and infection potential. ND495, a wheat (*Triticum aestivum*) line susceptible to the tan spot pathogen *Pyrenophora tritici-repentis*, was exposed to the environment of a wheat field for 24 hr and then subjected to a wet period of 24 hr or returned directly to the greenhouse. Weather and airborne spores of *P. tritici-repentis* were monitored during exposure periods. Resulting foliar lesions were surface sterilized and cultured on water agar to induce sporulation. *Pyrenophora tritici-repentis* was responsible for the greatest number of lesions in 1992 and had the highest infection efficiency. *Alternaria* sp., *Septoria nodorum*, and *Cochliobolus sativus* were also commonly found. Incidence and number of lesions were intercorrelated among the four pathogens, but relationships were reduced when there was an added wet period. This implies that each pathogen had particular conditions that influenced suitability of infection. Duration of leaf wetness, night temperature and occurrence of airborne conidia were most important to tan spot development.

A231

MAPPING THE SPREAD AND FOCUS FORMATION OF SORGHUM ERGOT OVER TIME. R. Bandyopadhyay, X.B. Yang, and M.V. Satyanarayana. Cereals Pathology, ICRISAT, India, and Dept. of Plant Pathology, Iowa State Univ., Ames, Iowa 50011.

Formation and expansion of primary and secondary foci of sorghum ergot were mapped in fields of 33 x 33 m. A primary focus was introduced at the center of the plot. For each plant, flowering date, honey dew appearance, number of infected spikelets, and position of the plant in a field were recorded. Because the pathogen has a limited period of infection of about three days during spikelet flowering, infection maps on days after inoculation were produced by overlaying daily flowering maps with infected spikelet maps. These maps showed that there were distinctive dispersal gradients in the first 8 days of inoculation. Spatial pattern of infected spikelets changed from a focal to a random pattern as disease progressed. Dispersal gradients, obtained by fitting calculated dispersal data with power model, decreased over time. Lloyd index of patchiness for the distribution of infected spikelets also decreased as disease progressed. The secondary foci appeared to be randomly established in down-wind areas of the fields.

A232

EFFECT OF RELATIVE HUMIDITY AND TEMPERATURE ON INFECTION OF PEANUT CULTIVARS BY CERCOSPORA ARACHIDICOLA. L. J. Wu, J. P. Damicone, and H. A. Melouk. Dept. of Plant Pathology and USDA-ARS, Oklahoma State University, Stillwater, OK 74078-9947.

Spanish peanut cultivars are more susceptible to early leafspot in the field than runner cultivars. This study was initiated to determine whether the environmental conditions required for infection by Cercospora arachidicola differ among these peanut cultivars. The Spanish cv. Spanco and the runner cvs. Florunner and Okrun were exposed to temperatures of 18-30 C with wetting periods (RH≥95%) of 12-84 hours following inoculations. Components of infection were then quantified following further incubation at low RH (60-80%). Spanco required a minimum of 36 hours of RH≥95% for infection, but runners required 48-60 hours. Temperature alone and the interaction of temperature and hours of RH≥95%, had a significant ($P<0.05$) effect on the number of lesions/leaf on Spanco and Florunner. Hours of RH≥95% alone had a significant effect on the number of lesions on all three cultivars. The shortest incubation period (12 days) and highest infection efficiency (0.027, 0.011 lesions/conidium) occurred at 24 C for Spanco and Florunner, but no significant differences were found for these infection components for Okrun.

A233

TEMPORAL AND SPATIAL DEVELOPMENT OF SOYBEAN FOLIAR DISEASES WITH STRIP INTERCROPPING. K. M. Tubajika, C. A. Martinson and F. W. Nutter, Jr. Department of Plant Pathology, Iowa State University, Ames, IA 50011.

Soybean, oat, and maize were intercropped in repeated narrow (3.8-4.6 m wide) strips; strips were rotated with soybean preceding oat and following maize. Temporal and spatial development of soybean foliar diseases were quantified. Brown spot (Septoria glycines) and bacterial blight (Pseudomonas syringae pv. glycinea) were the main diseases that developed. Brown spot appeared early and bacterial blight developed later in the growing season. The highest disease incidence and most severe symptoms of both diseases occurred in the row contiguous with land planted to soybean the prior year, which was the inoculum source. The lowest disease levels were in the row farthest from the inoculum source. Brown spot disease gradients across soybean strips were linear. Brown spot was most severe with intensive tillage and least severe with no-till ridge culture of soybean. Bacterial blight disease gradients were detected early in the season and then disease intensity became more uniform with time as nearly all plants in the strip were diseased by growth stage R7. No single population growth model adequately described bacterial blight progress in each row across the strip.

A234

DISEASE DEVELOPMENT ON SOYBEAN FOLIAGE INOCULATED WITH CERCOSPORA KIKUCHII AT VARIOUS HOST GROWTH STAGES. C. E. Orth and W. Schuh. Department of Plant Pathology, The Pennsylvania State University, University Park, PA 16802

The disease severity (% leaf area) and number of latent infections per cm^2 was assessed at soybean growth stages (GS) V3, V5, R1-R6 on leaves at the uppermost, uppermost-2 and-5 leaves 21 days after inoculation. Plants were inoculated at 25C and 24 h of leaf wetness. Afterwards, plants were maintained in growth chambers at 25C and either 60 or 95% RH. Disease severity was highest at growth stage V3. Leaf position was a significant factor with respect to disease severity at GS R4-R6, with the uppermost leaf being the most susceptible. The number of latent infections was lowest at GS R4-R6 and was not influenced by the RH after inoculation at these growth stages. At GS V3 - R4, relative humidity had a significant effect on the number of latent infections. The 95% RH treatment had a significantly higher number of lesions per cm^2 as compared to the 65% treatment. There was no effect of leaf position on the number of latent infections at any growth stage.

A235

ARCHITECTURE OF SHOOTS OF BIRDSFOOT TREFOIL INFECTED WITH *RHIZOCTONIA* SPP. AND *STEMPHYLIUM LOTI*. J.T. English Dept. of Plant Pathology, Univ. of Missouri, Columbia 65211.

Shoot architectures of three varieties of birdsfoot trefoil (*Lotus corniculatus*), representing erect, semi-erect, and prostrate growth habits, changed through 165 days of growth. Branching patterns varied from herringbone to random forms early in the growing season, regardless of plant growth habit. With further growth and infection by *Rhizoctonia* spp. and *Stemphylium loti*, shoot architectures changed to reflect only random patterns of branching in the case of erect and semi-erect varieties. By topological analysis, alterations in architecture were detectable as changes in the slope of the relationship of branch system magnitude and total exterior pathlength. Differences in shoot architecture among the three growth habits reflected differences in age distributions of surviving leaves and numbers of surviving lateral shoots.

A236

THE DEVELOPMENT OF FOLIAR DISEASES OF ALFALFA IN RELATION TO ALFALFA GROWTH. K.M. Emery and J.T. English, Dept. of Plant Pathology, Univ. of Missouri, Columbia, 65211.

Alfalfa growth and foliar disease development were monitored during three regrowth periods of both 1991 and 1992. Disease was caused primarily by *Leptosphaerulina briosiana* and *Stemphylium botryosum*, and was evaluated as a complex. Estimations of disease incidence and severity were influenced by leaf production and defoliation. Disease incidence frequently increased in a rapid, nonlinear fashion early in regrowth periods. After such increases, disease incidence either remained constant or decreased linearly. Reductions in incidence occurred as noninfected leaves produced on emerging lateral branches entered the pool of sampled leaves. Disease severity was generally low and did not change significantly over the course of regrowth periods. Disease incidence and severity were greater in the lower portion of the canopy than in the upper portion, although, the patterns of disease development were similar in the two canopy regions.

A237

RELATIONSHIP AMONG THREE MEASURES OF DISEASE INTENSITY FOR ALTERNARIA BLOTCH OF APPLE. **N. Filajdić, and T. B. Sutton. Dept. of Plant Pathology, North Carolina State University, Raleigh, NC 27695.**

Correlations were examined among 3 different measures of disease intensity (incidence, severity, and defoliation) for Alternaria blotch of apple caused by *Alternaria mali*, recorded every 2-wk in 1991 and 1992 in 2 orchards with different disease histories. Defoliation (%) 1-wk prior to harvest was correlated significantly with the incidence and severity recorded in mid-June of the same year. Correlation coefficients for incidence and defoliation and severity and defoliation, respectively, were 0.52 and 0.50, at the Staton orchard which had a moderate disease intensity; 0.94 and 0.94, at the McKay orchard (severe disease intensity) in 1991; and 0.84 and 0.90 at McKay in 1992. Incidence and severity, assessed on the same days, were correlated significantly in all cases. Yield at McKay in 1991 could be predicted by incidence and/or severity assessed in mid-June. A model was constructed to predict the final intensity of Alternaria blotch based on the observations made early in the growing season.

A238

REDUCTION IN PATHOGEN GENETIC DIVERSITY FROM INOCULATION TO SYMPTOM DEVELOPMENT IN THE *Sclerotinia sclerotiorum* - CANOLA PATHOSYSTEM IN MISSOURI. A. D. Maltby and J. D. Mihail, Department of Plant Pathology, University of Missouri, Columbia MO, 65211.

In 1992, 203 isolates of *Sclerotinia sclerotiorum* were collected from infected petals of *Brassica napus* cv. Ceres (canola), *Lamium amplexicaule*, and *Lepidium* sp., and petals and stems of *Arabis virginica*, at three sites in central Missouri. Unique sets of fungal clones, as determined by mycelial compatibility, were recovered from each site with little overlap among sets. Genetic diversity of clones infecting petals was observed to be greater than diversity of clones infecting stems. The above observation is being confirmed in 1993 with clones collected from canola petals and stems in two fields, suggesting that only a subset of the clones that colonize petals can also colonize stems. Further, although a single canola plant is exposed to many different fungal clones, infection by more than one clone of *S. sclerotiorum* is probably rare.

A239

ANALYSIS OF THE *LEMA* GENE REQUIRED BY *PSEUDOMONAS SYRINGAE* FOR LESION FORMATION ON BEAN: OVERPRODUCTION OF THE GENE PRODUCT AND ISOLATION OF EXTRAGENIC SUPPRESSORS. T. Kitten[1] and D.K. Willis[1,2]. Department of Plant Pathology[1] and Plant Disease Resistance Research Unit, Agricultural Research Service, U.S. Department of Agriculture[2],1630 Linden Drive, University of Wisconsin, Madison WI 53706.

The *lemA* gene of *Pseudomonas syringae* pv. syringae is required for lesion formation on bean plants as well as for protease and syringomycin production. The *lemA* gene bears sequence similarity to the sensor kinase of a family of bacterial two-component regulatory systems. To determine whether the *lemA* gene product possesses autophosphorylation activity, we have overproduced LemA protein derivatives as fusions with glutathione S-transferase. The fusion proteins were also used for polycolonal antiserum production. To identify gene products that interact with LemA, a *Pseudomonas syringae* strain carrying a Tn5 insertion within *lemA* was mutagenized with EMS and screened for restoration of protease production. Of 43,000 colonies examined, 53 were restored in protease production. Syringomycin production appeared fully restored in three of these suppressor mutants.

A240

DEVELOPMENT OF AN ENDOGENOUS PLASMID INTO A CLONING VECTOR IN CLAVIBACTER XYLI SUBSP CYNODONTIS FOR EXPRESSING INSECTICIDAL GENES. **T. Y. Li, Y. P. Zhang, and T. A. Chen, Dept.of Plant Pathology,Rutgers University,New Brunswick, N.J. 08903**

A 51 Kb endogenous plasmid, pCXC100, from Clavibacter xyli subsp. cynodontis (CXC) has been mapped. Seven DNA fragments which covered the entire pCXC100 with 3-10 Kb overlapping between two adjacent fragments have been obtained with suitable restriction enzyme digestions. They were subcloned into E. coli plasmid PBR325. Plasmid containing each fragment was then transferred into CXC by electroporation and two subclones survived. In order to determine the exact site of the origin of replication,the subclones were further deleted and the stability of the deletion clones in the CXC transformants was confirmed. A stable shuttle vector has been constructed which contained Tc^r and Amp^r genes from PBR325 and several single restriction enzyme cloning sites. Currently, insecticidal toxin gene from Bacillus thuringiensis has been cloned into the shuttle vector and study on the pesticidal expression of CXC is being carried out.

A241

AVRBS3 HYBRIDIZES TO DNA FROM STRAINS OF *XANTHOMONAS CAMPESTRIS* PV. *VESICATORIA* EXHIBITING A RACE 3 PHENOTYPE. C. S. Kousik and D. F. Ritchie. Dept. Plant Pathology. N. C. State Univ., Raleigh, NC 27695.

Race determination was conducted on 132 strains of *X. c.* pv. *vesicatoria* (Xcv), obtained from diseased pepper. Most (47%) strains were race 1, 23% race 2 and 24% race 3. All race 1 strains induced hypersensitive reaction (HR) on ECW 30R and hybridized with a probe for the avirulence gene *avrBs3* that confers avirulence of Xcv strains on pepper line ECW 30R. All race 2 and race 3 strains failed to induce HR on ECW 30R. However, 4 strains of race 2, and most of the race 3 strains hybridized with the *avrBs3* probe. All race 2 strains hybridized with the *avrBs1* probe, whereas none of the race 1 or race 3 strains did. Three spontaneous mutant strains isolated from a race 0 infiltrated ECW 30R behaved as race 2, however, they also hybridized with the *avrBs3* probe. While two strains isolated from race 0 infiltrated ECW 10R behaved as race 1 and did not hybridize with the *avrBs1* probe. These data indicate that many strains of race 3 carry DNA homologous to *avrBs3*, and may be mutants of race 1. The data also suggest that race determination of naturally occurring strains of Xcv using avirulence gene probes alone may lead to misleading results.

A242

PLANT-INDUCED EXPRESSION OF *PEHR*, A POSITIVE REGULATOR OF POLYGALACTURONASE PRODUCTION IN *PSEUDOMONAS SOLANACEARUM*. Caitilyn Allen, Laureano Simon, and Elizabeth Hinkens. Department of Plant Pathology, University of Wisconsin-Madison, Madison, WI 53706.

Pseudomonas solanacearum, the agent of bacterial wilt disease, produces 3 polygalacturonases (PG) that contribute to virulence. An *endo*-PG, PehA, is a minor virulence factor. Two *exo*-PGs, PehB and PehC, were produced at 25-fold higher levels *in planta* than in rich broth. PehB mutant strains were substantially reduced in virulence on eggplant seedlings. Expression of the PG structural genes was positively controlled at the transcriptional level by *pehR*. *pehR* mutants produced one-tenth the *endo*-PG and half the *exo*-PG activity of wildtype bacteria. Expression of *pehR::gus* (β-glucuronidase) fusions increased about ten-fold when bacteria grew in tobacco leaves rather than in culture, suggesting that this locus is itself regulated, possibly by a plant signal. Reporter gene experiments also indicated that *pehR* is not autoregulated, but may be repressed by a global regulator, *phcA*.

A243

PHYLOGENETIC ANALYSIS OF *HRP* RELATED DNA SEQUENCES OF *XANTHOMONAS CAMPESTRIS* THAT CAUSE DISEASES OF CITRUS. R. P. Leite Jr., D. S. Egel*, and R. E. Stall. Department of Plant Pathology, University of Florida, Gainesville, FL 32611, and *American Sunmelon, P.O. Box 153, Hinton, OK 73047.

Fragments of the *hrp* gene cluster from strains of *X. campestris* that cause diseases of citrus were amplified enzymatically and restricted with frequent cutting endonucleases. The restriction pattern varied within the moderately and weakly aggressive groups of the citrus bacterial spot pathogen, *X. campestris* pv. citrumelo, but not for the highly aggressive group of this bacterium, or within each of the three groups of *X. campestris* pv. citri. The genetic relatedness among strains of *X. campestris* pv. citrumelo ranged from 77 to 100%, whereas that for groups A, B, and C of the citrus canker pathogen, *X. campestris* pv. citri, ranged from 84 to 100%. By contrast, the relatedness of *X. campestris* pv. citrumelo to *X. campestris* pv. citri ranged from 49 to 57 %. The phylogenetic trees reconstructed using parsimony and

distance methods support a polyphyletic relationship regarding the *hrp* evolution of the strains of these pathovars. Strains of the three groups of *X. campestris* pv. citri were clearly monophyletic, but those of *X. campestris* pv. citrumelo were highly related to strains of *X. campestris* of other pathovars that cause disease on different hosts and have different genetic backgrounds. This strongly supports a hypothesis of horizontal transfer of the *hrp* genes among certain pathovars of *X. campestris*.

A244

AGROBACTERIUM TI PLASMID VirB GENES ENCODE PILUS-LIKE STRUCTURE FOR CONJUGATIVE TRANSFER OF T-DNA TO PLANTS. K. Shirasu and C. I. Kado, Dept. of Plant Pathology, University of California, Davis, CA 95616.

The *virB* operon encodes eleven proteins, which are associated with the *Agrobacterium* membranes. Western blot analysis showed that *virB2*, *virB3* and *virB9* proteins are associated with both the inner- and outer-membranes. VirB2 is cleaved from a 12.3 kDa protein into a 7.2 kDa polypeptide at the signal peptide peptidase I cleavage site. VirB2 protein processing is strikingly similar to the processing of TraA protein encoded by the *tra* operon of plasmid F. TraA is cleaved into a 7.2 kDa subunit from a 13.2 kDa propilin protein. Furthermore, there is strong similarity between VirB2 and TraA (48% similarity; 16% identity), and between VirB3 and TraL (46% similarity; 16% identity). In addition, *virB* genes are strongly similar in genetic arrangement and sequences to the proteins encoded by the *pilW* (*trw*) operon involved in pilin biosynthesis of plasmid R388. TraA and TraL are part of the pilin structure of the *tra* operon involved in pilus synthesis and assembly in *E. coli*. *virB* genes are involved in pilus synthesis and assembly at the *Agrobacterium* membrane.

A245

CLONING OF A PATHOGENICITY FACTOR FROM *STREPTOMYCES SCABIES*. R. A. Bukhalid and R. Loria, Department of Plant Pathology, Cornell University, Ithaca, NY 14853.

A cosmid (pKC505) library of total genomic DNA from *Streptomyces scabies* was expressed in *Streptomyces lividans* TK24, a non-pathogen. Transformants were screened for the ability to colonize and produce necrosis on potato tuber slices. Three clones out of 2,000 produced a necrotic reaction similar to that of the pathogenic wild type strain, 84-34, from which the library was constructed. Thin layer chromatography (Merck silica gel 60 F_{254}) of the chloroform-soluble fractions of potato tuber tissue colonized by two of the clones produced a band that co-migrated with thaxtomin A, a phytotoxin produced by *S. scabies* but not *S. lividans* TK24. One clone was characterized in *Escherichia coli* and found to contain an insert of approximately 7.5 kb.

A246

Thermoregulation of the phaseolotoxin and resistant-OCT genes in *Pseudomonas syringae* pv. *phaseolicola*: A molecular analysis. K.B. Rowley, and S.S. Patil, Dept. of Plant Pathology and Biotechnology Program, University of Hawaii at Manoa, Honolulu, HI 96822.

Phaseolotoxin and the toxin-resistant ornithine carbamoyltransferase (ROCT) are produced by the bean pathogen *Pseudomonas syringae* pv. *phaseolicola* at 18°C, but not at 28°C. Previously we identified a 260 bp region of genomic DNA from the wild-type strain that complements a UV-induced Tox⁻ mutant by nonallelic complementation. This fragment contains motifs commonly found in DNA binding sites, and in mobility shift assays it forms DNA-specific protein complexes in the presence of partially purified extracts of wild-type cells grown at 28°C but not at 18°C. DNase I footprinting experiments identified the sequence GAAAGGGATCCAAAGTTAATCTT as the protein binding site. We also found that homologous sequences from the promoter region of the ROCT gene (*argK*) formed DNA-specific protein complexes in the presence of the same protein extract used in experiments with the 260 bp fragment. We propose that the DNA-binding protein produced at 28°C is a global regulator which binds to similar DNA-binding sites present in the toxin biosynthetic genes and represses toxin production in *P.s.* pv. *phaseolicola* at 28°C.

A247

CLONING OF BACTERIAL GENES ENCODING CHITINASES THAT INHIBIT MYCOTOXIN PRODUCING FUNGI. S.V. Karyala[1], P.A. Gay[1], J. Carey[2], T.E. Cleveland[2], F. Woods[3] and S. Tuzun[1], Depts. of [1]Plant Pathology and [3]Horticulture, Auburn University, AL 36849 and [2]Southern Regional Research Center ARS, USDA, New Orleans, LA 70124.

Chitinolytic bacteria, as sources of antifungal genes, were tested *in vitro* for their antagonistic effects against *Fusarium moniliforme*, *Aspergillus flavus*, and *A. parasiticus*. Eight highly chitinolytic bacterial strains were selected from the AU chitinolytic bacterial collection. *In vitro* antifungal assays indicated that a specificity may exist between bacterial chitinases and the fungal substrates. A chitinolytic strain of *Bacillus* (AU192) was identified that inhibited all fungi tested. Several lines of evidence suggested that antifungal inhibition was correlated with chitinase activity of the bacterium. As a result, chitinase genes were targeted for cloning. Genomic DNA was isolated from AU192, digested with various restriction endonucleases, and probed with heterologous chitinase genes (*chi*A-*chi*D) from *B. circulans* to identify homologous DNA fragments. A genomic library was prepared from a *Sau*3A partial digest of AU192 DNA in a λ EMBL3 vector. The library was screened with the *chi*A-*chi*D genes and positive clones were identified for further characterization. Concomitantly, secreted chitinase isozymes from AU192 are being isolated to test their antifungal activity by in-gel inhibition assays of *F. moniliforme*, *A. flavus*, and *A. parasiticus*.

A248

INTRAGENIC RECOMBINATION OF *PTHA* CAN ALTER HOST SPECIFIC AVIRULENCE INDEPENDENTLY OF ITS PLEIOTROPIC VIRULENCE FUNCTION. Yinong Yang and Dean W. Gabriel, Plant Pathology Department and Plant Molecular and Cellular Biology Program, University of Florida, Gainesville, FL 32611

The *pthA* gene from *Xanthomonas citri*, causal agent of citrus canker, has pleiotropic virulence (on citrus) and avirulence (on cotton and bean) functions and belongs to a *Xanthomonas* avirulence gene family. Near-identical, 102bp tandem repeats of *pthA* were found to determine the host specificity of both virulence and avirulence. By inserting a *npt-sac* cartridge into the tandemly repeated region of *pthA* as a selective marker, intragenic recombination between homologous repeats was demonstrated in both *Xanthomonas* and *E. coli* strains. Many intragenic recombinants of *pthA* exhibited new host specificities. When present in appropriate *Xanthomonas* strains, some recombinants lost ability to elicit a hypersensitive response on bean and/or cotton, but preserved their pleiotropic virulence function on citrus, whereas other recombinants lost virulence function, but generated new avirulence specificities. Intragenic recombination therefore provides a genetic mechanism for the generation of new host specificities and evolution of this *Xanthomonas* gene family.

A249

TRANSFORMATION SYSTEM FOR *BACILLUS SUBTILIS* STRAIN GB03. W.F. Mahaffee[1], W. Moar[2], and J.W. Kloepper[1], [1]Departments of Plant Pathology and [2]Entomology, Biological Control Institute, Auburn University, 36849.

A transformation system was developed for *Bacillus subtilis* strain GB03, a commercialized biocontrol bacterium. Several procedures for protoplast transformation, component cells, and electroporation were performed using several plasmid vectors. Transformation of protoplasts was not successful since protoplasts lysed rapidly and regeneration was not accomplished. Several competent cell procedures for transformation of GB03 were tested with either inconsistent or unsuccessful results. Parameters investigated to optimize electroporation procedures for GB03 included growth medium, presence of glycerol, incubation temperature, cell densities, method of storage, DNA purity and concentration, DNA methylation, voltage, resistance, and recovery parameters. Although there was some variability among experiments, transformation efficiencies of $1x10^5$ transformants/μg DNA have been obtained by growing cells in pennassy broth with 1% NaCl and 1% glucose by constantly shaking (350 rpm) at 30 C, using an electroporation buffer without glycerol, and electroporating demethylated DNA at 16 Kv with resistance of 300Ω.

A250

CHARACTERIZATION OF GENETIC VARIATION IN *RADOPHOLUS* SIBLING SPECIES. D. T. Kaplan[1], C. H. Opperman[2], M.C. Vanderspool[1], D. Zies[1], C. Garrett[2], and S. Chang[2]. [1]USDA-ARS, 2120 Camden Rd, Orlando, FL 32803 and [2]Department of Plant Pathology, North Carolina State University, Raleigh, NC 27695.

A molecular analysis of genetic variation in two burrowing nematode sibling species *Radopholus citrophilus* and *R. similis*, was conducted using randomly amplified polymorphic DNA (RAPD) and sequence comparisons of ribosomal DNA spacer regions. These nematode species are morphologically indistinguishable, but they differ in host range, and in karyotype (*R. citrophilus*, n=5; and *R. similis*, n = 4). Of 300 RAPD primers evaluated, six polymorphisms have been identified. The intergenic (IGS) and the internal transcribed (ITS) ribosomal spacer regions were cloned and sequenced. The sequence of the ITS fragment was highly conserved between the two species whereas differences have been identified in the IGS.

A258

FUNCTIONAL MOVEMENT PROTEIN OF TOBACCO MOSAIC VIRUS IS REQUIRED FOR EFFICIENT SYSTEMIC SPREAD IN BARLEY CO-INFECTED WITH TWO CEREAL VIRUSES. Nancy L. Robertson and Roy French. USDA, ARS, Department of Plant Pathology, University of Nebraska, Lincoln, Nebraska 68583

Tobacco mosaic virus (TMV) readily infects barley (*Hordeum vulgare*) grown at 30°C to 35°C when co-inoculated with barley stripe mosaic virus (BSMV) or brome mosaic virus (BMV), but does not systemically invade barley alone. One

current explanation is that the TMV movement function is defective in barley but can be complemented by movement functions of cereal viruses. We tested this hypothesis by inoculating the first leaf of barley (*cv* 'Black Hulless') plants with a TMV mutant with a defective 30K movement protein gene, both alone and in combination with BSMV or BMV. When co-inoculated with BSMV, systemic spread of the TMV-30K(-) mutant into the third leaf was significantly less than that observed with wild type TMV. BMV did not support movement of the mutant. The TMV-30K(-) mutant also moved into the third leaf in the presence of a BSMV mutant with a defective capsid protein (cp) gene, ruling out trans-encapsidation of TMV RNA with BSMV cp as a long range transport mechanism. Interestingly, accumulation of the TMV-30K(-) mutant was enhanced in the first leaf of these plants relative to those co-infected with wild type BSMV. Clearly cereal virus interactions with TMV are more complex than has been supposed.

A259

NUCLEIC ACID SEQUENCE OF CITRUS LEAF RUGOSE VIRUS RNA-2 AND ITS RELATIONSHIP WITH ALFALFA MOSAIC VIRUS. Xin Ge and S.W.Scott, Dept.of Plant Pathology and Physiology, Clemson Univ., Clemson, SC 29634-0377.

In this first report of extended sequence of RNA-2 of an ilarvirus, the complete nucleotide sequence of the coding region of citrus leaf rugose virus (CiLRV) has been determined. The sequenced region is 2,950 nucleotides (NT) and contains a single open reading frame (ORF) between nt 34 and 2532. This ORF encodes a gene product of 832 amino acids with a mol. wt. of 95 Kd. Both the nucleotide sequence of CiLRV and the protein sequence of the gene product show extensive homology with the sequences of the RNA-2 and its gene product of alfalfa mosaic virus (AMV). The homologies between CiLRV and other Bromoviridae were much less. A region of 90 amino acids in the central part of proteins coded for by RNA-2s of CiRLV, AMV, cucumber mosaic virus and brome mosaic virus is highly conserved.

A260

A PRELIMINARY APPRAISAL OF THE TAXONOMY OF THE ILARVIRUS GROUP AT THE MOLECULAR LEVEL. S. W. Scott, Xin Ge, Elizabeth J. Bachman*, and Vicki B. Vance*. Department of Plant Pathology and Physiology, Clemson University, Clemson, SC 29634-0377 and *Department of Biological Sciences, University of South Carolina, Columbia, SC 29208.

Sequence data to RNA-3 of members of subgroups two, four and seven of the ilarviruses have been generated. There is little homology at either the nucleotide sequence or the gene product level between subgroups two and four. Similarly there is little homology between members of these subgroups and the published sequence for RNA-3 of tobacco streak virus, the type member of the ilarvirus group, findings that confirm the groupings established by earlier serological work. Homology exists between citrus variegation virus, citrus leaf rugose virus, elm mottle virus (all subgroup 2) and lilac ring mottle virus (LRMV - subgroup 7). This leads us to speculate that LRMV should perhaps be included as a member of subgroup 2 rather than as the sole member of a distinct subgroup.

A261

EXPRESSION OF TOMATO SPOTTED WILT VIRUS NUCLEOCAPSID GENE CONFERS DISEASE RESISTANCE IN TOBACCO AND TOMATO. J.W. Kim[1], S.S.M. Sun[1] and T.L. German[2]. [1]Dept. of Plant Molecular Physiology, University of Hawaii, Honolulu, HI 96822; [2]Department of Plant Pathology, University of Wisconsin Madison, WI 53544.

Tomato spotted wilt virus (TSWV) virions are composed of four proteins of about 300 kDa, 78 kDa, 58 kDa and 29 kDa which are a putative polymerase, two membrane proteins and the nucleocapsid protein (N), respectively. The genome consists of three single-stranded RNA molecules of 2.9 kb (S), 4.9 kb (M) and 8.9 kb (L). The L RNA is of negative polarity while the M and S RNAs are ambisense. In virions and infected cells, the RNAs are associated with N protein to form ribonucloprotein complexes. Transgenic tobacco and tomato plants expressing sense or antisense constructs of the N gene showed significant resistance to TSWV.

A262

SEQUENCE ANALYSIS OF THE TRAILER REGION OF SONCHUS YELLOW NET VIRUS (SYNV) GENOMIC RNA. Tae-Jin Choi and Andrew O. Jackson. Department of Plant Pathology, University of California, Berkeley, CA 94720.

The 5' terminus of the SYNV genome (or trailer sequence) is composed of 160 nucleotides (nt) adjacent to a dinucleotide forming a portion of the "gene junction" sequence at the terminus of the L protein gene and is located at positions 13561 to 13720 relative to the 3' end of genomic RNA. The 5' trailer sequence is longer than the 3' 144 nt corresponding to the plus strand leader RNA and is the longest extracistronic trailer sequence yet reported among the non-segmented negative strand viruses. Characteristic of other rhabdovirus genomes, the 3' and 5' termini of the SYNV genome are capable of forming a panhandle structure, since 16 of the first 18 nt are complementary. However, there is no direct sequence homology between the 5' terminal sequences of SYNV and those of two animal rhabdoviruses, vesicular stomatitis virus and rabies virus. A minus strand leader RNA could not be detected in nucleic acid extracted from infected plants using hybridization conditions suitable for detection of the plus strand leader RNA.

A263

BIOCHEMICAL ANALYSIS OF CUCUMBER MOSAIC VIRUS REPLICATION. Michael V. Graves*, Peter Palukaitis†, and Marilyn J. Roossinck*. *The Samuel Roberts Noble Foundation, Ardmore, OK 73402 USA. †Dept. of Plant Pathology, Cornell University, Ithaca, NY 14853 USA.

Cucumber mosaic cucumovirus (CMV) is a tripartite, plus-stranded RNA virus. Both RNAs 1 & 2 are required for viral replication; each encode a single protein (1a and 2a, respectively) which, combined with host factor(s), form the viral replicase complex. Two virus strains, Fny-CMV and Sny-CMV exhibit several host dependent phenotypic differences including temperature sensitive replication in muskmelon. These differences have been previously mapped to RNA 1 of the viral genome using pseudorecombinant viruses. The biochemical basis for the temperature sensitive replication of Sny-CMV is being examined using an *in vitro* replicase system produced from infected muskmelons. The dynamics of the synthesis of viral proteins is also being studied in infected tobacco protoplasts utilizing specific antibodies.

A264

GENETIC MAPPING OF CUCUMBER MOSAIC CUCUMOVIRUS RNA1. Marilyn J. Roossinck, The S.R. Noble Foundation, Ardmore OK 73402, and Peter Palukaitis, Dept. of Plant Path., Cornell University, Ithaca, NY 14853.

Several phenotypic differences between two strains of cucumber mosaic cucumovirus (CMV), Fny-CMV and Sny-CMV, have been previously mapped to RNA 1: timing and severity of symptoms in zucchini squash; temperature sensitive replication in muskmelon; and ability to replicate satellite RNAs in cucurbits. Clones capable of producing infectious transcripts of Fny-CMV are available. A biologically active cDNA clone of RNA 1 of Sny-CMV has facilitated the continued genetic mapping of the phenotypic differences. A number of recombinants have been constructed between the RNA 1 cDNA clones of the two strains, and site-directed mutagenesis studies are also underway to confirm the precise mapping of some of the differences. Results of the genetic mapping, as well as possible roles of the functional domains of RNA 1 in producing symptoms in infected plants will be discussed.

A265

MOLECULAR CLONING AND 5' END ANALYSIS OF TWO CUCUMOVIRUSES. P.Scott White*, Peter Palukaitis†, & Marilyn J. Roossinck*, *The S.R. Noble Foundation, Ardmore OK, 73402, & †Dept. of Plant Pathology, Cornell University, Ithaca NY 14853.

Several studies in our laboratory, including analyses of PSV diversity, host range determinants, and incidence in the field, require clones of the V subgroup of peanut stunt cucumovirus (PSV). The precise 5' end sequences of all three genomic RNAs of PSV were determined to facilitate cloning. In addition, the 5' end sequences were determined for cucumber mosaic cucumovirus (CMV) strain Fny, and for the progeny of the biologically active transcripts from cDNA clones pFny 109, pFny 209, and pFny 309 (Rizzo and Palukaitis, 1990, Mol. Gen. Genet. 222: 249). Transcript sequences corresponding to RNA 1 differed from those of the wild-type Fny-CMV RNA 1, but progeny of the transcripts showed a reversion to the wild-type sequence. At least a 10 fold increase in infectivity was observed when the 5' end of the RNA 1 cDNA clone was modified to be identical to that of the wild-type. The role of the 5' end sequences and the results of the cDNA cloning of PSV will be discussed.

A266

IDENTIFICATION OF A MOSAIC DETERMINANT WITHIN THE REPLICASE OPEN READING FRAME OF TMV. R.S. Nelson, S.A.

Arnold and M.H. Shintaku, The Noble Foundation, P.O. Box 2180, Ardmore, OK 73402.

The Masked (M) Strain of TMV was isolated and its mild symptoms described by Holmes [Phytopathology (1934) 24: 845]. We have previously shown that the attenuated symptoms, which include a lack of a mosaic and little or no leaf rugosity compared with the more severe U1 strain, are due to mutations residing in the 126-kDa open reading frame. Site-directed mutants of the infectious clone of the M strain have been made and a mutation at nt 1872 resulting in an E → K amino acid change within the nonconserved region of the 126-kDa protein between the methyltransferase and helicase domains is involved in producing a mosaic phenotype but not the leaf deformation. Descriptions of phenotypes produced by other site-directed mutants will be presented.

A267

DETECTION *IN VIVO* OF A PUTATIVE NON-STRUCTURAL PROTEIN OF CITRUS TRISTEZA CLOSTEROVIRUS. V.J. Febres, H.R. Pappu, E.J. Anderson[1], S.S. Pappu, R.F. Lee[2], and C.L. Niblett. Plant Pathology Department, University of Florida, Gainesville, FL 32611. [1]Department of Plant Pathology, University of Arkansas, Fayetteville, AR 72701, [2]Citrus Research and Education Center, Lake Alfred, FL 33850.

The citrus tristeza closterovirus (CTV) genome contains a 720 base pair-open reading frame that can encode a protein of 27,360 Daltons (p27). This protein shares 47% similarity with the coat protein (CP), although only the CP appears to be a component of the viral capsid. The gene for p27 was amplified as cDNA from CTV-infected citrus tissue extracts and cloned into the *E. coli* pETH3b expression vector. The fusion protein produced in *E. coli* was purified using SDS-polyacrylamide gel electrophoresis and was used for the production of polyclonal antibodies. The polyclonal antiserum was used in Western blot analysis to probe for the expression of the p27 protein in CTV-infected tissue. A protein band of the expected size was detected only in extracts of CTV-infected citrus but not in uninfected citrus extracts.

A269

PRACTICAL IMPLEMENTATION OF 'BLOTCHCAST', A WEATHER DRIVEN PREDICTIVE MODEL OF WEB BLOTCH IN NEW MEXICO VALENCIA PEANUTS. J.A. Woodard, C.M. Liddell. NMSU Box 30003 Dept. 3BE, Las Cruces, NM 88003.

Accurate prediction of plant disease outbreaks provides agricultural producers with information which can potentially increase both the yield and quality of the crop, while simultaneously decreasing the quantity of chemical control agents required. We have developed a model for web blotch (*Phoma arachidicola)*, BLOTCHCAST, which predicts the incidence of the disease in peanuts (*Arachis hypogaea L.*) three days in advance, and uses easily measured weather parameters which are independent of individual management strategies. A network of seven radio telemetry linked weather stations is being installed in the Portales, NM area, each of which will function as a hub for disease management areas throughout the region. Daily forecasts of the potential disease incidence will be made available to producers via a daily radio broadcast, or prerecorded telephone message. Producers can then more efficiently target fungicide application times, and increase the effectiveness of each application.

A270

MAXIMUM LIKELIHOOD METHODS FOR THE ANALYSIS OF AGGREGATED DISEASE INCIDENCE DATA. G. Hughes and L.V. Madden. School of Agriculture, University of Edinburgh, Edinburgh EH9 3JG, Scotland, U.K., and Department of Plant Pathology, OARDC, Ohio State University, Wooster, Ohio, USA.

Maximum likelihood methods provide a basis for the analysis of experimental data when disease has been recorded on a presence/absence basis (disease incidence). In order to define a likelihood, we must specify the form of distribution of the observations. Use of the binomial distribution for disease incidence data results in misleadingly small standard errors for estimated parameters if diseased plants (or plant parts) are aggregated. The beta-binomial distribution (BBD) may be an appropriate alternative : its variance is larger than that of the binomial distribution with the same mean, and, in addition, it is consistent with the relationship between variance (v) and mean (p) incidence, $v=a(p(1-p))^b$. In comparison to a linear logistic model with binomial errors, the simplest BBD anova model includes a single random effect in addition to treatment effects, and inflates the standard errors of the treatment effects. More complicated random effects structures are also possible. Some of these are illustrated with data from virus-infected tobacco crops.

A271

SAMPLE SIZE DETERMINATION WHEN OBSERVED DISEASE INCIDENCE IS ZERO. L.V. Madden* and G. Hughes**. *Dept. of Plant Pathology, OARDC/The Ohio State University, Wooster, OH 44691; and **Inst. of Ecology and Resource Management, University of Edinburgh, West Mains Rd, Edinburgh EH9 3JG, Scotland.

It is possible to determine an upper limit of a one-sided confidence interval for disease incidence (p_u) based on the sample size (n) and underlying statistical distribution, when all plants in a sample are disease free (p=0). Conversely, a sample size can be chosen (n^*) to limit p_u to a preassigned level. Such procedures are needed in inspection and quarantine practices when zero or very low incidence of a disease is demanded. Calculating n^* or p_u is straightforward when a random distribution (binomial) can be assumed. However, incidence typically is aggregated and described by the beta-binomial distribution. As aggregation increases, p_u increases at a fixed n, indicating that n^* must be greatly increased compared to the random situation. Sample-size curves will be presented for a range of aggregation levels typically found in the analysis of plant disease epidemics.

A272

SPATIO-TEMPORAL DISTANCE CLASS ANALYSIS OF EPIDEMICS. S. C. Nelson, S. A. Ferreira, K. Y. Pitz, and V. Sanaka. University of Hawaii, Department of Plant Pathology, 3190 Maile Way, Honolulu HI, 96822.

Spatio-temporal distance class analysis (STCLASS) of binomial data, a modification of a two-dimensional distance class analysis computer program (2DCLASS), is proposed as a new method to quantify patterns of disease spread. STCLASS tests the hypothesis that all healthy plants in a population have an equal chance of becoming diseased between disease assessments (e.g., days 7 and 14). If true, then patterns of newly-diseased plants not different from random patterns would be expected. During computer-generated simulations, the spatial locations of diseased plants on day 7 are retained, and the number of newly-diseased plants on day 14 are assigned random positions in each of 400 simulated maps. Then, distance class analysis is used to compare observed with expected data to identify whether the appearance of disease is random or non-random. To illustrate the analysis and to show how attributes of contagious spread can be quantified, an epidemic of papaya ringspot virus on papaya in Hawaii and hypothetical data are presented.

A273

PROGRESS OF SPOTTED WILT IN TOMATOES AND POPULATION DYNAMICS OF THE THRIPS VECTORS. H. Puche, R.D. Berger*, and J.E. Funderburk, Entomology and Nematology, and *Plant Pathology Departments, University of Florida, Gainesville, FL 32611.

The thrips vectors (Frankliniella spp.) of tomato spotted wilt virus were monitored by sticky traps in two tomato fields in each of two years. The curves for the cumulative numbers of trapped thrips over time were asymmetrically sigmoidal. Thrips appeared 10 days earlier in 1990 than in 1989, increased at a 20% faster rate, and reached a two times greater asymptotic level. Three generations of thrips occurred in both years, as detected by waves of increased numbers when the population

curves were linearized. Newly symptomatic plants were tagged each week. Incidence of spotted wilt was less than 9% in both years and the spatially random disease appeared temporally as 1-3 separate monocycles. Cycles of disease were not correlated with the generations of thrips or with specific weather events. We concluded that the increase of spotted wilt in tomato fields of northern Florida was almost exclusively from immigrating viruliferous thrips.

A274

THE USE OF STRAIN-SPECIFIC MONOCLONAL ANTIBODIES TO MODEL THE FIELD SPREAD OF SOYBEAN MOSAIC VIRUS. F. W. Nutter, Jr., J. H. Hill, and P. M. Schultz. Department of Plant Pathology, Iowa State University, Ames, Iowa 50011.

Epidemiological studies will be needed to effectively quantify, integrate, and optimize the beneficial impacts that transgenic-based plant virus resistance may have on closing the gap between actual and attainable yield. For the soybean mosaic virus (SMV)/soybean/aphid pathosystem, quantitative information concerning the spatial and temporal movement of specific strains of SMV is lacking. In 1991, we successfully tracked the spatial and temporal spread of SMV strain G-5 in 'Corsoy 79' soybean from a point source using strain-specific monoclonal antibodies (McAbs). McAb S4 reacts with all SMV strains tested, while McAb S2 reacts with all SMV strains except G-5. Each week, plants in 30 cm sections of rows were sampled and tested for the presence of SMV strain G-5. A computer program (Epivirus) was used to plot SMV incidence over time and to calculate the area under disease progress curves. In 1991, the rate of SMV spread ranged from 0.12 to 0.14 units per day. Spatial analyses were also performed to determine if SMV spread was random or clustered for each sampling date. In 1991, spread by aphid vectors was random throughout the season.

A275

INFECTION GRADIENT OF ASPERGILLUS FLAVUS ON CORN PLANTS IN RELATION TO WASTE CORN INOCULUM SOURCES. O.M. Olanya, D.C. McGee and L.H. Tiffany, Dept. of Plant Pathology and Seed Science Center and Dept. of Botany, Iowa State University, Ames, IA 50011.

Previous research has established waste corn in the vicinity of storage bins and cribs as a significant source of inoculum of Aspergillus flavus. In 1992, an intensive spore-trapping study was made at three locations in Iowa to determine dispersal of A. flavus inoculum by wind and nitidulid beetles from waste corn and infection of corn ears in nearby corn fields. At each of the three locations, a distinct gradient of spore concentration was observed in relation to distances from waste corn inoculum sources. Concentration of A. flavus conidia was significantly correlated ($P=0.05$) with leaf and silk colonization as well as kernel infection at the same distances.

A276

EVALUATION OF THREE QUANTITATIVE ASSAYS FOR *SCLEROTINIA MINOR*. K.V. Subbarao, S. Dacuyan, J.C. Hubbard, S.T. Koike, and L.E. Jackson. University of California, Davis, c/o U. S. Agricultural Research Station, 1636 E. Alisal St., Salinas, CA 93905.

Three techniques to quantitatively assay field soil for *Sclerotinia minor* were compared for precision, bias, and time required to assay a sample. The techniques compared were wet sieving, wet sieving with Calgon, and a hydropneumatic root elutriation technique. Precision was measured by the standard error of the mean for repeated assays of forty 100 g samples each of clayey, loamy, and sandy soils artificially infested with 15 viable sclerotia. Bias was measured by the deviation between the number of sclerotia recovered with each of the three techniques and the true number of *S. minor* sclerotia introduced into the above autoclaved soil samples. Regardless of the soil type, the root elutriation technique was the most precise, most unbiased, and also the most efficient method to assay soil for *S. minor*. In general, 90 - 93% of the sclerotia added to soils were recovered with the root elutriation technique. Wet sieving was the least precise, most biased, least efficient, and gave the lowest recovery of sclerotia from a naturally-infested clayey soil. Efficiency of the wet sieving technique was primarily dependent on soil type. Adding Calgon to soil samples before wet sieving increased the precision, reduced bias, and improved the efficiency of wet sieving for all soil types. None of the techniques substantially reduced viability of sclerotia. Root elutriation technique is a precise and efficient method to process large numbers of samples for *S. minor*.

A277

ASSOCIATION OF *FRANKLINIELLA FUSCA* WITH MOVEMENT OF TOMATO SPOTTED WILT VIRUS IN TOMATOES IN LOUISIANA. R.R. Johnson, L.L. Black, H.A. Hobbs, and R.A. Valverde; Dept. Plant Path. and Crop Physiol., La. Agric. Expt. Sta., La. State Univ. Agric. Ctr., Baton Rouge 70803

Seasonal movement of thrips in tomato production areas of Louisiana determined by use of yellow water pan traps and plant washings showed a close association between the movement of *Frankliniella fusca* and infection of tomato plants by TSWV. Recovery of thrips by washing plants of TSWV weed hosts; *Ranunculus sardous* , *R. muricatus* , *Lactuca floridana* , and *Sonchus asper* ; showed all developmental stages of *F. fusca* to be present and the thrips species most commonly associated with these weeds during the winter and spring. *F. fusca* collected from *Ranunculus* spp. from three areas of the state transmitted TSWV to tomato when they were caged with the tomato plants in the laboratory. *F. occidentalis* was found, but sporadically and in numbers much lower than *F. fusca*. This study suggests that *F. fusca* is the vector responsible for TSWV infections in the spring tomato crop in Louisiana.

A278

TIME OF *STRIGA HERMONTHICA* INFECTION IN RELATION TO PARASITE EMERGENCE AND YIELD OF SORGHUM AND MAIZE. D. K. Bemer, E. I. Aigbokhan, and F. O. Ikie. International Institute of Tropical Agriculture, PMB 5320, Oyo Road, Ibadan, Nigeria.

This study examined time of infection of host roots by *Striga hermonthica* in relation to numbers of emerged parasites and host yield. Three sets of experiments were conducted. One set on maize in pots in a water-excluding screenhouse at the International Institute of Tropical Agriculture, Ibadan, Nigeria (IITA). Another on two cultivars of maize in the field during the 1992 rainy season in Abuja, Nigeria. The third on two cultivars of sorghum grown on the soil floor of a near-natural-light screenhouse at IITA during the dry season of 1993. The soil around individual plants was infested with 3,000 germinable *S. hermonthica* seeds by placing seeds 0-4 cm deep in concentric rings around each plant. Infestations were at the time of host planting, or 1, 2, 3, 4, 5, or 6 wk later. Results in all tests showed significant decline in numbers of emerged *S. hermonthica* plants when infestation was delayed at least 4 wk. Host yield significantly increased beginning at this infestation time. Results suggest that protection of the host from *S. hermonthica* parasitism for 4 wk may be adequate to reduce yield loss and parasite reproduction.

A279

EVALUATION OF STREPTOMYCIN-RESISTANT STRAINS OF *ERWINIA AMYLOVORA* FROM MICHIGAN. P. McManus, C.-S. Chiou and A.L. Jones, Department of Botany and Plant Pathology and the Pesticide Research Center, Michigan State University, East Lansing, 48824.

Streptomycin-resistant strains of *Erwinia amylovora* were detected in one Van Buren County, Michigan apple orchard in 1990. A 1991 survey of Michigan apple orchards revealed resistant strains from four and two orchards in Van Buren and Kent Counties, respectively. In 1992 resistance was detected in six Van Buren County orchards, two Kent County orchards, and two orchards in Newaygo County. Two genetically distinct mechanisms of streptomycin resistance were found to function in Michigan isolates of *E. amylovora*. Two genes encoding phosphotransferase-mediated resistance, *str*A, and *str*B, were usually carried by transposon Tn*5393* inserted on the conjugative plasmid pEa34; however, in three strains from one orchard, Tn*5393* with *str*A/*str*B was inserted on pEa29, a non-conjugative plasmid unique to and ubiquitous in *E. amylovora*. In streptomycin-resistant strains lacking *str*A and *str*B, a chromosomal determinant conferred resistance.

A280

DYNAMICS OF CLAVIBACTER MICHIGANENSIS SUBSP. SEPEDONICUS POPULATION IN POTATO PLANTS UNDER HIGH LATITUDE ENVIRONMENTAL CONDITIONS. M. Di and J. H. McBeath. Department of Plant, Animal and Soil Sciences, SALRM, University of Alaska Fairbanks, Fairbanks, AK 99775-0080.

Potato cv. BakeKing seed pieces were inoculated (10^9 cfu/tuber) with Clavibacter michiganensis subsp. sepedonicus (CMS), the cause of bacterial ring rot, and planted at Fairbanks, Alaska (65°N latitude). Plants were sampled intensively throughout the growing season. After surface sterilization, samples were cut to sections, weighed, macerated and diluted with 0.05 M phosphate buffer and plated on NCP-88 semiselective medium and nutrient broth yeast medium. CMS was detectable in stolons and lower stems 45 days after planting. The viable CMS ranged from 10^{9-11} cfu/g of stem tissue to 10^{3-6} cfu/g of stolon tissue. After the symptoms appeared in 65-70 days, CMS was detected systemically. but it seemed to be confined to stem and stolon. No detectable CMS was found in the mesophyll and roots. Disease development seems to be closely correlated to environmental conditions.

A281

INFECTION DYNAMICS OF HETEROBASIDION ANNOSUM (FR.) BREF. IN PINE IN NORTHEASTERN CALIFORNIA. A.W. Ratcliff, F.W. Cobb Jr.

and W.J. Otrosina. U.S.D.A. Forest Service, P.O. Box 245, Berkeley, CA 94701, and Dept. of Environmental Sciences, Policy and Management, Univ. of Calif., Berkeley CA 94720.

We report preliminary results of a study to test the commonly-held hypothesis that H. annosum initially infects pine through cut stumps, and to determine the dynamics of its spread and genetic structure in pine centers. Snags, stumps and living and dead trees were sampled. Molecular markers and mating techniques are being used to identify clones. Vegetative compatibility tests indicate that 2 to 10 clones were present per infection center. Some stumps and adjacent trees contained the same clones indicating that these stumps could have served as primary infection courts, and root contacts could have facilitated infection of adjacent trees. Factors influencing the observed clonal distribution may include differences in competitive ability among clones, substrate availability and infection courts at sites other than stump tops, and root contacts.

A282

A AND B MORPHOTYPES OF *SPHAEROPSIS SAPINEA* DIFFERENTIATED USING RAPDS. D.R. Smith and G.R. Stanosz. Dept. Plant Pathology, University of Wisconsin-Madison, Madison, WI 53706.

Sphaeropsis sapinea (Fr.:Fr.) Dyko & Sutton in Sutton causes shoot blight and cankers on many conifers, especially *Pinus* spp. A and B morphotypes, which have been suggested to vary in virulence and geographic distribution, are differentiated by culture morphology, spore characteristics, and isozymes. Relationships among 18 isolates were studied by analyzing random amplified polymorphic DNA markers (RAPDs). Fifteen isolates included nine A morphotype isolates collected in Hawaii, Michigan, Minnesota, Wisconsin, New Zealand, or South Africa from *P. nigra*, *P. radiata*, *P. resinosa*, or *Larix decidua*, and six B morphotype isolates collected in Michigan or Wisconsin from *P. banksiana* or *P. resinosa*. One unidentified *Sphaeropsis* isolate from each of the following hosts (locations) also were used: *P. monticola* (Pennsylvania), *Malus* (Wisconsin), and *Ulmus* (China). Phenetic and cladistic analyses (the Weighted Pair Group Method using Averages (WPGMA) and maximum parsimony in the computer program Phylogenetic Analysis Using Parsimony (PAUP), respectively) of DNA fragments support maintaining the A and B morphotypes as separate entities. Unidentified isolates do not appear to be related closely to either isolate morphotype. Information will be used in further taxonomic and pathological studies, including development of a rapid method of morphotype identification.

A283

ABILITY OF LOBLOLLY PINES TO LIMIT INFECTIONS BY CRONARTIUM QUERCUUM F. SP. FUSIFORME. C. H. Walkinshaw, and J. P. Barnett. USDA Forest Service, Box 5500, Pineville, LA 71361.

Loblolly pine (Pinus taeda L.), the most frequently planted tree in the United States, grows rapidly on a wide variety of sites and tolerates repeated attacks by fusiform rust. To test the hypothesis that host tissue isolates the pathogen through compartmentalization, tree discs and microscopic sections of loblolly pines with rust were observed. We found that internal stems had less disease than suggested by external observations. Galls that entered the stem from infected branches were confined in the outer stem. Many of these galls appeared dead but a significant number were observed to sporulate when approximately 100 years old. These observations help explain why loblolly pines can tolerate multiple stem invasions by the rust fungus. Our hypothesis appears to be correct for trees in the field and is substantiated by prior studies in the greenhouse.

A284

HYPERSENSITIVE REACTION ZONE OF WHITE, SITKA AND LUTZ SPRUCE INDUCED BY BARK BEETLE-ASSOCIATED LEPTOGRAPHIUM ABIETINUM (Peck). *Barbara L. Illman, Forest Products Laboratory, Madison, WI 53705, and Richard A. Werner, Institute of Northern Forestry, USDA/FS, Fairbanks, AK 99775.*

White, Sitka and Lutz spruce at four locations on the Kenai Peninsula, AK, responded with a hypersensitive reaction (HR) following inoculation with the bark beetle-associated fungus Leptographium abietinum (Peck). The HR lesion length was greater in wounds associated wtih fungi than in mechanical wounds. Phloem samples from within and outside the lesion area were analyzed by electron spin resonance for manganese and iron and by gas chromatography and mass spectroscopy (GC/MS) for stilbenes. The GC/MS samples were extracted with methanol and derivatized by trimethylsilyl. No changes were detected in iron and manganese. Two unknown chemicals were in the non-reaction zone and a low molecular weight unknown was found only in the HR zones. Unknowns were not identified using hydroxystilbene standards. Unknowns will be analyzed by NMR.

A285

USE OF A GEOGRAPHIC INFORMATION SYSTEM TO STUDY THE INCIDENCE OF ANNUAL CANKER ON SUGAR MAPLES. D.R. Bergdahl, *L.M. Tritton, *P.E. Sendak, and D.R. Tobi. University of Vermont, Burlington, VT 05405 and *Northeastern Forest Experiment Station, Burlington, VT 05402.

Annual canker of sugar maple (Acer saccharum Marsh.) is caused primarily by Fusarium solani and produces visible stem defect in sapling and pole-sized trees. Sites with shallow, poorly-drained, or droughty soils are believed to stress trees, predisposing them to infection. The objective of this study was to assess the incidence of annual canker on a 600-acre northern hardwood site in northcentral Vermont for which extensive ecological data were available. A total of 1881 trees were evaluated on 73, fifth-acre plots. The spatial distribution of annual canker incidence was entered into a Geographic Information System and compared with data layers on soils, cover types, habitats, forest management areas, and topography. Over 90% of the trees with annual canker were in the 2- to 9-inch diameter class. Statistical analysis suggested that annual canker was most frequently found on steep, south-facing slopes associated with very shallow soils on either fine till or bedrock.

A286

ERRORS IN WOOD DECAY STUDIES ATTRIBUTABLE TO FUNGAL BIOMASS. H.L. Jones and J.J. Worrall, State University of New York College of Env. Sci. & Forestry, Syracuse, NY 13210

Fungal biomass in decayed wood was measured (using glucosamine as an indicator) in order to assess errors in wood weight loss, wood sugar, and lignin measurements attributable to fungal mass. Lignin, wood sugars, glucosamine content, and apparent weight loss of wood blocks decayed by *Trametes versicolor, Oligoporus placentus, Bjerkandera adusta, Gloeophyllum trabeum,* and *Phialocephala dimorphospora* were measured at selected intervals. Glucosamine content of the 5 species tested ranged from 1.29 to 3.11%. Birch decayed by *T. versicolor* contained more fungal biomass than any other fungus-wood combination. After 12 wks of decay by *T. versicolor,* birch blocks contained 31.3% fungus. Fungal biomass measurements in birch decayed by *B. adusta* and *G. trabeum* exceeded 9.0%. Percent error in apparent weight loss measurements was greatest at lower weight losses. The overall contribution of fungal components to wood component analysis was relaltively small.

A287

Preliminary screening of Botryosphaeria ribis isolates for pathogenicity to Melaleuca quinquenervia. M.B. Rayachhetry, and R.S. Webb, Dept. of Forestry, University of Florida, Gainesville, 32611.

Six isolates of Botryosphaeria ribis were obtained from cankered Melaleuca quinquenervia trees in South Florida. Single-spored cultures (SSCs) within isolates revealed hyphal color variation on PDA. Variations in hyphal color and pycnidial production among SSCs under similar growth conditions suggested possible differences in putative pathogenicity towards M. quinquenervia. Bioassays of SSCs using detached sterile leaves from four tree clones were used to evaluate relative pathogenicity on the basis of mean necrotic leaf area. No significant differences in pathogenicity among SSCs were observed within or among isolates of the pathogen. Bioassay results will be corroborated with results from more intensive clonal stem inoculation trials using SSC isolates.

A288

INFLUENCE OF TIMBER HARVESTING METHODS ON TRICHODERMA SPP. IN A SOUTH ALABAMA BOTTOMLAND HARDWOOD FOREST. D.A. Brown, B.G. Lockaby, and W.D. Kelley. School of Forestry, Auburn University, AL 36849-5418.

Environmental impacts of timber harvesting on forested wetlands in the southeastern United States have not been adequately described. In 1991, a study was initiated to identify effects of logging practices on wetland quality and function. Impacts of handfell/helicopter and feller-buncher/skidder harvesting, representing minimum and maximum disturbance, respectively, on populations of the important litter decomposers, Trichoderma spp., were examined. Significant decreases in Trichoderma populations in the order maximum disturbance and minimum disturbance > undisturbed control were observed following timber harvest. Currently, alterations in hydroperiod, soil moisture status, temperature, and floodwater nutrient status are being

examined as factors affected by logging that may influence Trichoderma populations. Also, the possible role of these fungi as bioindicators of ecosystem stress is being considered.

A289

PATHOGENICITY AND VIRULENCE OF Armillaria ostoyae ON EIGHT FOREST TREE SPECIES. D.W. Omdal[1], C.G. Shaw, III[2], W.R. Jacobi[1], and T.C. Wager[1], [1]Depart. of Plant Pathology and Weed Science, Colorado State University and [2]USDA Forest Service, Rocky Mountain Research Station, Fort Collins, CO, 80523.

Ten clones of Armillaria ostoyae obtained from white fir, Douglas-fir, southwestern white pine, ponderosa pine, blue spruce, and aspen in northern New Mexico were used to inoculate seedlings of these hosts and lodgepole pine and western larch. At 18 months there were no significant differences ($p < 0.05$) in mortality among the 8 hosts or in virulence across all clones. After 30 months, which covers 3 growing seasons, lodgepole pine was significantly ($p < 0.05$) more susceptible to infection than either white fir or Douglas-fir. Ponderosa pine, the dominant species in this region, did not differ significantly from either of the exotics (western larch and lodgepole pine) in susceptibility to infection or mortality. Aspen was significantly more tolerant of disease than conifers, being frequently infected but rarely killed. A clone's ability to incite disease was highly correlated with its production of rhizomorphs ($r = .94$, $p < .01$). Across all clones and hosts save aspen, the order of the clones' ability to incite disease matched their order in killing hosts. One clone, isolated from blue spruce, failed to infect any trees.

A290

RFLP ANALYSIS OF CERATOCYSTIS FAGACEARUM ISOLATES FROM TEXAS, WEST VIRGINIA, AND WISCONSIN. T. Kurdyla, P. Guthrie and D. Appel. Dept. Plant Pathology and Microbiology, Texas A&M University, College Station, TX 77843.

Mitochondrial DNA (mtDNA) of Ceratocystis fagacearum from 6 Texas, 2 Wisconsin, and 2 West Virginia isolates was digested with 4 restriction enzymes. No variation in the mtDNA haplotype was observed among isolates with any enzyme. The mitochondrial genome was approximately 144 kb in size and was restricted into an average of 16 fragments per enzyme. A library of random DNA fragments was constructed from C. fagacearum nuclear DNA (nuDNA). A total of 104 clones from the library was screened against the total DNA from 5 Texas, 2 West Virginia, and 2 Wisconsin isolates. Southern blot hybridizations revealed a low level of variability for nuDNA digested with 6 restriction enzymes. Eight probes hybridized to variable single loci. Twenty one probes detected low-copy or moderately repetitive DNA sequences. Results suggest that the North American population of C. fagacearum has very limited genetic diversity.

A291

SUSTAINED SUPPRESSION OF PYTHIUM ROOT ROT: A FUNCTION OF MICROBIAL CARRYING CAPACITY AND BACTERIAL SPECIES COMPOSITION. T. Wu, M.J. Boehm, L.V. Madden, and H.A.J. Hoitink, Dept. of Plant Pathology, OARDC/The Ohio State Univ., Wooster, OH 44691

A direct relationship was revealed between decreased suppressiveness to Pythium root rot, the rate of hydrolysis of fluorescein diacetate (FDA) and the carbohydrate content of a soilless potting mix monitored directly with CPMAS ^{13}C-NMR spectroscopy. Total microbial biomass and bacterial species diversity did not change as suppressiveness, carbohydrate content, and rate of FDA hydrolysis declined. A major shift in the composition of bacterial species present within the mix occurred. Populations of Pseudomonads and other microorganisms capable of inducing biological control declined. Populations of *Arthrobacter*, *Bacillus*, other Gram positives and putative oligotrophs typically associated with more highly mineralized soil fractions inreased as the microbial carrying capacity declined. In conclusion, this general suppression phenomenon was a function of organic matter decomposition level and the composition of bacterial species supported within the mix.

A292

EFFECT OF SOURCE AND CONCENTRATION OF CARBON AND NITROGEN ON BIOMASS AND ASCOSPORE PRODUCTION OF THE BIOCONTROL AGENT TALAROMYCES FLAVUS. Cheryl Ann Engelkes, R. L. Nuclo and D. R. Fravel, Biocontrol of Plant Diseases Laboratory, USDA, ARS, Beltsville, MD 20705.

Talaromyces flavus, applied as ascospores, suppresses Verticillium wilt. Hyphal growth and ascospore production of T. flavus were measured in Neurospora minimal medium, pH 5.5, containing each of 37 carbon (C) and 42 nitrogen (N) sources and at six C:N ratios of 5-30:1 for two of the C and two of the N sources. Biomass production in 5 days and sporulation in 6 wk depended more on the C than N sources and were inversely related for each C source and C:N. Mean hyphal dry weights were highest in polysaccharides and 5:1 C:N and lowest in monosaccharides and at 30:1 C:N. Ascospore numbers were highest on oligosaccharides and lowest on mono- and polysaccharides and at 5:1 C:N. Verticillium wilt incidence was 50% lower for eggplants drenched with ascospores grown on potato-dextrose agar compared with eggplants either nondrenched or drenched with ascospores grown on media with hypoxanthine plus lactose or maltose. Thus, biomass and ascospore production were not related to biocontrol ability.

A293

INHIBITION OF SCLEROTINIA MINOR AND SCLEROTIUM ROLFSII BY MICROORGANISMS IN SOIL AMENDED WITH RAPESEED MEAL. Xin Li, H. A. Melouk, J. P. Damicone, and K. E. Jackson. Department of Plant Pathology and USDA-ARS, Oklahoma State University, Stillwater, OK 74078-9947.

Sclerotia of S. minor and S. rolfsii placed in cloth pouches were retrieved from soil amended with rapeseed meal (RSM), containing 30 μM glucosinolates/g meal, at varying concentrations of 10-40 g RSM/Kg soil, and plated on potato-dextrose-agar (PDA) after 5 to 30 days of incubation at 22 C. Several microorganisms colonized the sclerotia of one or both S. minor and S. rolfsii at various incubation periods, which included Mucor spp., Trichoderma spp., Penicillium spp., Bacillus spp., Fusarium spp., and Erwinia spp. One Trichoderma sp. and one Bacillus sp. excreted substances that inhibited the mycelial growth of both S. minor and S. rolfsii on PDA as determined by the minimal inhibitory concentration (MIC) procedure. Cell free culture filtrates of the Trichoderma sp. and the Bacillus sp. at 1:2 and 1:4 (V/V) dilutions, respectively, were the MICs.

A294

THE USE OF BACTERIAL MIXTURES TO IMPROVE THE BIOLOGICAL CONTROL OF TAKE-ALL OF WHEAT. E. A. Pierson and D. M. Weller. Department of Plant Pathology, University of Arizona, Tucson, AZ 85721 and Agricultural Research Service, U. S. Department of Agriculture, Pullman, WA 99164.

Fluorescent *Pseudomonas* strains including Q2-87, Q1c-80, Q8d-80, Q65c-80, and Q69c-80 were tested for the ability to suppress take-all of wheat caused by *Gaeumannomyces graminis* var. *tritici*. In field and greenhouse tests, combinations of strains generally provided better disease control than the strains used individually. For example, in a field test at Pullman, WA on spring wheat the treatment Q2-87 + Q1c-80 + Q8d-80 + Q65c-80 significantly ($P = 0.05$) increased yield (20.4%) compared to a nontreated control. However, each strain used individually had no significant effect on yield. Our field and laboratory tests demonstrate the potential benefits of using bacterial mixtures to control take-all and suggest the importance of additive and interactive effects among rhizosphere microorganisms in disease control.

A295

Isolation of Bacteria Antagonistic to *Magnaporthe poae* Using Enrichment Cultures. D. Kobayashi, N. El-Barrad, D. Thompson and B. Clarke. Rutgers University, Dept. Plant Pathology, New Brunswick, NJ 08903

Continuous enrichment cultures consisting of a minimal medium supplemented with 0.1% fungal mycelium as a sole carbon source were used to isolate bacteria antagonistic to *Magnaporthe poae*, the causal agent of summer patch disease of turfgrass. Several different soil sources were baited with fungal mycelium grown on cellophane. After incubation in the soil, mycelia were recovered, vigorously washed in distilled water, and transferred to a minimal medium broth supplemented with *M. poae* mycelium. Cultures were incubated for two weeks, then subsequently subcultured to fresh media supplemented with fungal mycelium. These transfers were repeated several times. The percentage of chitinase-producing bacteria in cultures increased with successive transfers, suggesting a selection for bacterial populations with the potential to parasitize *M. poae*. Selected isolates obtained from these enrichment cultures effectively suppressed disease symptoms in growth chamber assays.

A297

SUPPRESSION OF *PYTHIUM GRAMINICOLA* ROOT ROT AND DAMPING OFF OF CREEPING BENTGRASS WITH COMPOSTS OF DIFFERENT ORIGINS AND LEVELS OF MATURITY. E. B. Nelson, C. M. Craft, Plant Pathology, Cornell University, Ithaca NY, and K. A. Feldman, Earthgro Inc., Lebanon CT.

Lab and field studies with a variety of composts have been conducted over the past several years. In the field, some compost-amended sand topdressings have suppressed Pythium root rot symptoms as well as metalaxyl. In lab bioassays with selected sterile vs. nonsterile composts, biotic suppression of damping off of bentgrass seedlings was demonstrated. However, in certain materials, disease suppression was a result of a combination of biotic and abiotic components. In 1992, a study was conducted to examine effects of compost maturity on microbial populations and Pythium root rot suppression. Starting materials included yard waste, food waste and animal manures. No apparent effects of compost maturity on Pythium root rot suppression were observed during the 6-month experiment, although levels of suppression differed among different composts. No relationships between fungal, bacterial, and actinomycete populations and *P. graminicola* suppression were apparent.

A298

THE ROLE OF PYRROLNITRIN IN THE SUPPRESSION OF DAMPING-OFF OF PEA BY *PSEUDOMONAS CEPACIA* STRAIN AMMD. K. M. Regner and J. L. Parke, Dept. of Plant Pathology, University of Wisconsin-Madison, Madison, WI 53706.

Pseudomonas cepacia strain AMMD applied as a seed treatment suppresses damping-off of pea caused by *Pythium* spp. However, the mechanisms of biological control are unknown. Strain AMMD inhibits the mycelial growth of *Pythium* spp. on minimal salts glycerol media (MNG). Additionally, broth cultures of strain AMMD cause zoospores of *P. aphanidermatum* to encyst and lyse *in vitro*. An antifungal compound, pyrrolnitrin, was detected in extracts of broth cultures of strain AMMD using TLC, HPLC, and mass spectral analysis. Pyrrolnitrin (Eli Lilly and Co.) inhibits the mycelial growth of *P. aphanidermatum* on corn meal agar and also causes zoospores to encyst and lyse. A pyrrolnitrin seed treatment (100μ g/seed) did not protect peas from damping-off. A Tn5 mutant of strain AMMD that did not suppress damping-off, inhibit the mycelial growth of *Pythium* spp., or lyse zoospores still produced pyrrolnitrin in MNG broth. This indicates that pyrrolnitrin may not be involved, or at least is not the only mechanism in the suppression of damping-off of pea.

A299

MUTATIONAL ANALYSIS DEMONSTRATES THAT GLIOTOXIN HAS A MAJOR ROLE IN BIOCONTROL OF *PYTHIUM ULTIMUM* BY *GLIOCLADIUM VIRENS*. D.C. Straney, S.E. Wilhite, R.D. Lumsden and C. Ding. Department of Botany, University of Maryland, College Park, Maryland 20742, and USDA/ARS BPDL, Beltsville, Maryland 20705.

Gliocladium virens strain G20 is an effective fungal biocontrol agent of pathogens which cause damping off. This strain produces the antibiotic gliotoxin and production of this antibiotic has been correlated with the biocontrol ability of this fungus. To critically test this role and eventually clone the genes required for gliotoxin biosynthesis, we have isolated seven independent UV-induced mutants of *G. virens* which lack the ability to produce gliotoxin. When these gliotoxin non-producing mutants were compared to wildtype *G. virens* in a biocontrol assay with *Pythium ultimum*, all seven mutants displayed only about half the biocontrol ability of the wildtype. This provides genetic evidence that gliotoxin plays a major role in its biocontrol ability against *P. ultimum*.

Maryland Agriculture Experiment Station Contribution Number 8678

A300

BOTRYTIS CINEREA AND *ALTERNARIA ALTERNATA* AS POTENTIAL MYCOHERBICIDES OF PURPLE LOOSESTRIFE. R. F. Nyvall and A. Hu, University of Minnesota, North Central Experiment Station, 1861 Hwy 169 East, Grand Rapids, MN 55744.

Purple loosestrife (*Lythrum salicaria*) an introduced perennial, is crowding out native vegetation in wetlands throughout Minnesota and diminishing their value for wildlife. Mycoherbicides may be an alternative to chemical weed control in these environmentally sensitive sites. Fifty of 2,868 fungal cultures, representing 20 fungal genera, were gathered from 16 sites throughout Minnesota and tested for pathogenicity. Six-wk-old loosestrife plants were misted with a spore suspension (10^6-10^7 spores/ml, depending on spore size) containing 1% spreader sticker and placed at 100% RH for 48 hr. Disease was evaluated in 7-14 days on a scale of 0-4 (0=no disease, 4=dead plant). Two fungi, tentatively identified as *Alternaria alternata* and *Botrytis cinera*, consistently caused disease ratings on plants averaging 2.4 and 2.7 respectively. Plants are either killed, had numerous leaf spots, or stunted due to necrosis of apical growing points.

A301

BIOCONTROL OF SICKLEPOD BY PREEMERGENT SOIL-INCORPORATION OF *ALTERNARIA CASSIAE* SPORES. R.A. Pitelli, R. Charudattan, and J. DeValerio. FCAVJ/UNESP, 14870, Jaboticabal, Brazil and Univ. of Florida, Plant Pathology Dept., Gainesville, FL 32611.

The feasibility of controlling sicklepod (*Cassia obtusifolia*) by preemergent soil-incorporation of *A. cassiae* was studied in the greenhouse. A 5 x 3 factorial design was used with five weekly intervals (0-28 days) between spore-incorporation and seeding, with three spore concentrations (10^4, 10^6, and 10^8 spores/cm^2), and four replications. Control pots received no *A. cassiae*. Conidia were incorporated in the top 1-cm of soil and ten seeds were sowed per 500 ml pots. Necrotic lesions developed along the hypocotyl and on the cotyledonary leaf base, causing early abscission of these leaves and subsequent decrease in plant growth. Eventually, the necrosis expanded to the radicle. After 35 days, the spore-incorporation interval, but not spore dose, had a significant effect in reducing dry matter accumulation (by 57-79% compared to the control). Thus, preemergent incorporation of *A. cassiae* in soil had a residual effect in controlling sicklepod.

A301a

OXALIC ACID PRODUCTION AND MYCELIAL BIOMASS YIELD OF *Sclerotinia minor* FOR THE FORMULATION ENHANCEMENT OF A GRANULAR TURF BIOHERBICIDE. S. C. Brière, S. G. Hallett, and A. K. Watson. Plant Science Department, Macdonald Campus of McGill University, Ste-Anne-de-Bellevue, Québec, Canada, H9X 3V9.

The importance of oxalic acid in the pathogenicity of many species of *Sclerotinia* fungi has been demonstrated in the past by de Bary (1886), Maxwell and Lumsden (1970) and Malgro *et al.*(1984, 1987). During the development of a broadleaf granular turf bioherbicide with the fungus *Sclerotinia minor*, the beneficial effect of oxalic acid to enhance the virulence of the pathogen was investigated. Both the yield of mycelial biomass produced in shake flasks and the oxalic acid content of the formulated mycelium were maximized. *S. minor* was grown in 125 ml of eight different liquid culture media in shake flasks incubated at 20C for 7 days. After which the final pH, mycelial dry weight and oxalic acid production were determined. The virulence of *S. minor* grown on each culture media was also screened on detached dandelion leaves and the necrotic area was measured by image analysis. A 330% increase in oxalic acid was obtained with Modified Richard's Solution (MRS) with the addition of 56 mM of sodium succinate as compared to MRS alone.

A309

EXPRESSION OF ESOPHAGEAL GLAND ANTIGENS DURING PLANT PARASITISM BY MELOIDOGYNE INCOGNITA. E. L. Davis and R. S. Hussey, Department of Plant Pathology, University of Georgia, Athens, GA 30602-7274.

Monoclonal antibodies (MAb) have been used to demonstrate developmentally-regulated synthesis of esophageal gland antigens in M. incognita during parasitism of plants. Slightly swollen, "early" second-stage juveniles (J2), swollen, sexually-

differentiated "late" J2, and "early" adult females (without eggs) of M. incognita were collected from tomato roots and used for indirect immunofluorescence with MAb that bind to secretory granules within the esophageal glands. A MAb ($3H_{11}$) that binds to the subventral glands of preparasitic juveniles did not bind to early J2 or any later parasitic stage. A second MAb ($7A_9$) bound only to somatic muscles of preparasitic and early J2, but labeled only the subventral glands in late J2 and later parasitic stages. A third MAb ($12H_7$) bound to the lateral hypodermal chords in juvenile parasitic stages, but in early and mature females it labeled only the dorsal gland. A fourth MAb ($4B_6$) bound to the dorsal gland of early and mature females, but labeled no other parasitic stage. Two other MAb bound to the dorsal gland ($6D_4$) and subventral glands ($3F_4$), respectively, of all nematode parasitic stages. The developmentally-regulated expression of esophageal gland antigens indicates different roles for the subventral and dorsal esophageal glands during parasitism of plants by root-knot nematodes.

A311

INVOLVEMENT OF MELOIDOGYNE ARENARIA AND *RHIZOCTONIA SOLANI* IN TARO ROOT ROT. M. P. Ko, J. Y. Uchida, J. J. Ooka, and D. P. Schmitt. Department of Plant Pathology, University of Hawaii, Honolulu, HI 96822.

A population of *Meloidogyne arenaria* (*Ma*) and a isolate of *Rhizoctonia solani* (*Rs*) obtained from taro plants on the island of Kauai were evaluated for their roles in taro root rot. Taro plants (*Colocasia esculenta* 'Bun Long') established in 15-cm diam pots containing fumigated soil were inoculated with 0, 200, 2,000, or 20,000 eggs of *Ma* for low, medium, and high Pi respectively. Half of these plants were also inoculated with mycelia of *Rs*. The treatments, each with 5 replicates, were arranged in a randomized complete block design. At 12 and 16 weeks after inoculation, seedlings were harvested and root necrosis was estimated (necrosis index (NI) = % of root area showing necrosis). NI increased over time and correlated more positively with *Ma* levels than with *Rs* levels. Root rot was absent in roots with 0 Pi at both harvest times, even in the presence of *Rs*, but appeared in 8 to 95% of root systems with increasing Pi. *Rs* enhanced root necrosis at all *Ma* levels, though the interaction was significant only at low Pi. *Ma* population densities around roots at high Pi were less than at low Pi, and at week 16 were less than at week 12, possibly because of increased root rot.

A312

ENHANCED COMPETITIVE ABILITY OF A *lacZY* MODIFIED *PSEUDOMONAS* sp. T. G. Lamb[1], D. A. Kluepfel[2], and D. W. Tonkyn[1]. 1 Department of Biological Sciences, 2 Department of Plant Pathology and Physiology, Clemson University, Clemson, SC 29634.

The population dynamics of a *lacZY* modified strain of *P. aureofaciens* was compared with its nonengineered parent under a variety of environmental conditions. The two strains were grown individually and together on the roots of corn, wheat, and broccoli. Combinations of these plants along with four different soil types at five different water potentials were tested. When grown individually on wheat the modified strain achieved a higher density than the parent strain. This occurred only under low water potential in sand and clay soils. This difference was accentuated when the two strains were introduced together at a 1:1 ratio.

A313

CHARACTERIZATION OF THE MICROBIAL COMMUNITY IN SOILS SUPPRESSIVE AND CONDUCIVE TO *CRICONEMELLA XENOPLAX*, USING FATTY ACID ANALYSIS. O.U. Presting, D.A. Kluepfel and J. Lawrence. Department of Plant Pathology and Physiology, Clemson University, Clemson, SC 29634.

Peach orchard sites suppressive to *C. xenoplax* multiplication have been identified and result from the unique microflora present. Fatty acids extracted directly from suppressive (S) and non-suppressive (NS) soil and the rhizosphere, were analyzed on a gas chromatograph. The resulting retention time (RT) profiles then were compared using an unweighted pair-group method utilizing arithmetric means. RT profiles from NS soils clustered more closely with each other than with RT profiles from S soils. This study demonstrates that fatty acids are valid biomarkers, useful in the characterization of complex soil-borne microbial communities.

A314

A MODEL FOR DECOMPOSITION OF ORGANIC MATTER IN SOIL AND ASSOCIATED MICROFLORA AND MICROFAUNA. A.H.C. van Bruggen, Department of Plant Pathology, University of California, Davis, CA95616, A.J. Termorshuizen, and G.J. Bollen, Department of Phytopathology, Agricultural University, Wageningen, the Netherlands.

A computer simulation model was developed for decomposition of organic matter in soil using literature data. Three components of fresh and old organic matter were distinguished: soluble carbohydrates and proteins; cellulose- or chitin-containing material; and lignin-containing or humified material. The decomposing microflora consisted of sugar fungi and bacteria and cellulolytic, chitinolytic and ligninolytic fungi and actinomycetes. A simplified food web was constructed consisting of the microflora, protozoa, bacterivorous nematodes, fungivorous nematodes, springtails, fungivorous mites, and predatory mites. A simulated reduction in microfauna, as occurs after soil treatment with insecticide-nematicides, resulted in a surge in cellulolytic fungi, including Rhizoctonia solani, due to a very slow recovery of springtails. This result is consistent with the association of severe Rhizoctonia canker with strongly reduced populations of springtails in Dutch potato fields treated with nematicides of this type.

A315

CONTROL OF SOILBORNE FUNGI AND ROOT DISEASES IN SWEET ONION IN GEORGIA. D. R. Sumner[1], R. D. Gitaitis[1], J. D. Gay[1], D. A. Smittle[1], B. W. Maw[1], E. W. Tollner[2], and Y. C. Hung[3], University of Georgia, Athens[2], Griffin[3], and Tifton[1], GA 31793-0748.

Field plots in two different counties in 1992 and 1993 were fumigated in October with chloropicrin (C, 123 kg/ha), methyl bromide (MB, 98%: C, 2%; 258 kg/ha), MBC (67%-33%, 127 kg/ha), 1,3-dicloropropene (DP) + 17% C (141 or 177 kg/ha) or nonfumigated. In 1993, treatments with metam-sodium (MS, 236 kg/ha) and DP (113 kg/ha) were included. The soil surface was sealed with overhead irrigation immediately after treatments were applied. Onion was direct-seeded or transplanted in the fall and harvested in the spring. MBC and C were effective in reducing populations of Phoma terristris, Pythium spp., Fusarium spp., and Rhizoctonia solani AG-4. Metam-sodium and DP + 17% C were less efficacious and MB and DP were ineffective. Yield of marketable onion bulbs was increased an average of 8, 6, 4, 3.5, 3, and 1.8 Mg/ha in plots treated with MS, DP, MBC, C, DP + 17% C, and MB, respectively, over the nonfumigated plots.

A316

THE EFFECT OF SOIL PH ON INFECTION OF WINTER WHEAT BY *CEPHALOSPORIUM GRAMINEUM*. C.M. STILES AND T.D. MURRAY. DEPARTMENT OF PLANT PATHOLOGY, WASHINGTON STATE UNIVERSITY, PULLMAN, WA 99164-6430.

Cephalosporium gramineum was isolated from roots, subcrown internodes, and stems of winter wheat plants grown in field plots where soil pH varied from 4.7 to 7.2. Root infection occurred in the fall in both the 1992 and 1993 crop years, however, differences in percent infected roots among soil pH were not apparent until late winter. In February, 1992, the percent infected crown roots was higher at pH 5.5 (15%) and 5.9 (18%) than at pH 6.7 (4%). In March, 1993, the percent infected crown roots was highest at pH 4.7 (42%), intermediate at pH 5.4 (29%) and lowest at pH 7.2 (15%). The percent infected stems did not differ significantly among soil pH levels from October to February, nor did final disease incidence vary among pH in 1992. A higher percentage of infected crown roots at low soil pH may account for observed increases in Cephalosporium stripe in acidic soils in some years.

A317

ASSOCIATION OF SOIL MICROORGANISMS WITH SUPPRESSION OF CORKY ROOT OF TOMATOES IN ORGANICALLY MANAGED SOIL. F. Workneh and A.H.C. van Bruggen, Department of Plant Pathology, University of California, Davis, CA 95616.

In previous field studies, suppression of corky root of tomato (Pyrenochaeta lycopersici) was associated with higher microbial activity in organic compared to conventional farms. To confirm this observation in controlled experiments, tomato seeds were planted into irradiated and non-irradiated soil samples from 5 organic and 5 conventional farms. One half of the samples were infested with 10^4 microsclerotia of P. lycopersici per ml of soil. The difference in disease severity between irradiated and non-irradiated soil was significantly larger for soils from organic farms than for those from conventional farms, indicating that a biological entity was involved in disease suppression. Fungi, bacteria, and actinomycetes were isolated from the rhizosphere of tomato plants grown in soil from 3 organic and 3 conventional farms. Fifty random colonies from each group of microorganisms were tested for antagonism to P. lycopersici and hydrolysis of five substrates. Resemblance functions, calculated for the combined physiological test results for each farm, showed that bacteria and actinomycetes were more similar among organic or conventional farms than between organic and conventional farms. There was no similarity of fungal isolates among farms.

A318

RELATIONSHIP OF BACTERIAL ACTIVITY IN REWETTED RHIZOSPHERE SOIL TO SUPPRESSION OF HOST COLONIZATION BY *PYTHIUM APHANIDERMATUM*. T. Isakeit[1], M.E. Stanghellini[2], and R.G. McDaniel[3]. [1]Dept. Plant Pathology and Microbiology, Texas A & M University, Weslaco 78596 and Departments of Plant Pathology[2] and Plant Science[3], University of Arizona, Tucson 85721.

The effects of antibiotics on metabolic activities and populations of resident rhizobacteria were measured within the first four hours after rewetting an air-dried sugarbeet rhizosphere soil in order to determine the mechanism by which these organisms normally inhibit host colonization by *Pythium aphanidermatum* (*Pa*) at 20 C. Vancomycin and streptomycin treatments of soil resulted in host colonization by *Pa* at 20 C, but did not affect populations of aerobic bacteria. However, during this time, the antibiotic treatments increased the rate of O_2 consumption by bacteria. Prior to rewetting, rhizosphere soil contained 178 μg of water-soluble organic carbon / g soil, a quantity sufficient to support microbial activity prior to an influx of host nutrients. We conclude that competition for these readily available nutrients may not be the sole mechanism for microbial suppression of *Pa* at 20 C, since antibiotics did not inhibit the ability of resident rhizobacteria to utilize nutrients.

A319

CHARACTERIZATION OF A SEEDLING ROOT ROT OF AMERICAN GINSENG. B. D. Hudelson and J. L. Parke. Department of Plant Pathology, University of Wisconsin-Madison, Madison, WI 53706-1598.

A factor limiting production of American ginseng (*Panax quinquefolium*) in Wisconsin is a root rot that can reduce seedling stands by 60% or more. Symptoms include reddening of foliage, wilting and death. Roots of affected seedlings exhibit an orange-brown discoloration, and pruning of the tap and lateral roots. Older, surviving plants show a reduction in the number of leaves, and stunted, branched roots with orange-brown discoloration. Fungi isolated from infected two-year-old ginseng plants have been tentatively identified as species of *Pythium, Rhizoctonia, Fusarium, Cylindrocarpon,* and *Septonema.* In an effort to complete Koch's postulates for this disease, healthy ginseng seedlings were transplanted into pasteurized field soil infested with isolates of the fungi that had grown for approximately four weeks in PDA-vermiculite. Isolates of the four fungal genera listed above exhibited a statistically significant increase in wilting and a decrease in root mass compared to non-inoculated controls. Seedlings grown in *Cylindrocarpon*-infested or *Septonema*-infested soil exhibited root symptoms most consistent with those exhibited by field-infected seedlings.

A320

CHEMICAL PROPERTIES OF TEXAS SOILS WITHIN THE PHYMATOTRICHUM ROOT ROT DOMAIN. S. D. Lyda, and J. L. Riggs, Dept. of Plant Pathology and Microbiology, Texas A&M University, College Station, TX 77843.

Soil cores (848) were collected from 21 counties in Texas where Phymatotrichum root rot (PRR) occurs. Samples were taken from soils known to manifest the disease as well as from soils not exhibiting PRR. Each core (5 x 91cm) was separated into three 30-cm segments. Ammonium acetate (0.5M) extracts of subsamples were analyzed for exchangeable Ca^{++}, Mg^{++} and Na^{+} by atomic absorption spectrophotometry. Electrical conductivity and pH measurements were made on aqueous soil suspensions and sodium adsorption ratios were calculated for each sample. Soils were calcareous (50-150 $cmole_c/kg$) and variable in chemical composition with respect to sampling depth and location. Soils with a history of PRR were low in Na^{+} generally < 2 $cmole_cNa^{+}/kg$, whereas, the Na^{+} composition of noninfested soils ranged between 3-10 $cmole_c/kg$.

A321

RANDOMLY AMPLIFIED POLYMORPHIC DNA ANALYSIS OF FUSARIUM OXYSPORUM ASSOCIATED WITH SPINACH. A.B. THORNTON AND J.C. CORRELL. Dept. of Plant Pathology, University of Arkansas, Fayetteville, AR 72701.

Three vegetative compatibility groups (VCGs) have been identified among a worldwide collection of Fusarium oxysporum f.sp. spinaciae isolates. Isolates in the three pathogenic VCGs have an identical mtDNA haplotype while nonpathogenic isolates belong to multiple VCGs and have multiple mtDNA haplotypes. Forty-eight randomly amplified polymorphic DNA (RAPD) primers were screened for their usefulness in differentiating pathogenic from nonpathogenic isolates and for differentiating the three pathogenic VCGs. Four primers were chosen that consistently amplified multiple fragments and distinguished the three pathogenic VCGs. Two primers, 947GT and 883GT, differentiated the pathogenic VCGs. Two other primers, 827GT and 883CA, differentiated VCG 1 and 3 isolates from VCG 2 isolates. Individual polymorphic bands have been examined as VCG specific probes.

A322

EVALUATION AND SELECTION OF ALFALFA FOR RESISTANCE TO PYTHIUM SEEDLING DISEASES USING A CULTURE PLATE METHOD. Altier, N.A. and J. A. Thies. INIA La Estanzuela, Uruguay and USDA, ARS, U.S. Vegetable Laboratory, Charleston, SC 29414.

Resistance of 253 alfalfa cultivars to Pythium seedling diseases (P. ultimum and P. paroecandrum) was evaluated using a culture plate method. Twenty-five seeds of each cultivar were placed on the surface of a 3-day-old Pythium culture growing on water agar and incubated at 18 C for 5 days. Two resistance criteria were used, Average Severity Index (ASI) and Percent Resistant Plants (PRP). The ASI of the tested cultivars ranged from 3.22 to 5.00 (1 = healthy seedling; 5 = dead seed); the PRP ranged from 0 to 25%. The cultivars Florida 77, Indian, and RamRod were among the most resistant; Belmont, Pierce, and Legend were among the most susceptible. Recurrent selection was effective in increasing the frequency of resistant plants. For example, one selection cycle within the Beltsville International Composite-7 alfalfa population using the L4 Pythium isolate increased the PRP from 3.0 to 43.2%.

A323

DETECTION AND QUANTIFICATION OF PHYTOPHTHORA CAPSICI IN SOIL. Robert P. Larkin, Jean B. Ristaino, and C. Lee Campbell, Dept. of Plant Pathology, North Carolina State University, Raleigh 27695.

Several techniques were compared for their effectiveness in quantitatively recovering specific propagule types of Phytophthora capsici from soil. Zoospores, oospores, or sporangia and mycelial fragments were added to microwave-treated field soil at densities ranging from 1 to 1 x 10^4 propagules per g (ppg) soil. Methods tested included soil dilution plating on selective media and a pepper leaf disk baiting assay or soil dilution plating following a sequence of soil saturation, drainage for 3-6 days, and a 24-hr flooding period. Sporangial inoculum was detected at densities as low as 1 ppg soil with soil dilution plating on Masago's selective medium; zoospores were detected at densities of 10 ppg soil or higher. Recovery efficiency of sporangial, zoospore, and oospore inoculum with soil dilution plating averaged 100%, 10%, and 1%, respectively. Although the leaf disk assay improved recovery of oospore inoculum to about 10%, no technique was effective consistently at detecting oospore inoculum at densities less than 100 ppg soil.

A324

NON-DESTRUCTIVE, THREE-DIMENSIONAL STUDY OF ROOT GROWTH WITH MAGNETIC RESONANCE MICROSCOPY (MRM)
L.S. MacFall and G.A. Johnson. Duke University, Durham, NC 27710

Study of intact roots in soil has historically presented challenges to the researcher. MRM is a non-destructive imaging technique allowing repeated viewing of a subject over time. Three-dimensional image acquisition and rendering strategies have been developed which allow pine roots to be visually and digitally distinguished from the surrounding sand potting medium. Using this approach, the root systems of three pine seedlings have been repeatedly imaged over a four month period. Plants were allowed to grow undisturbed in tube containers filled with fine sand. Sequentially acquired, registered image sets of each plant showed the development of primary and secondary lateral roots, including mycorrhizal root types. Disappearance of fine roots was also observed within this period. MRM clearly has potential for the repeated non-destructive investigation of root growth over time.

A325

PARTIAL CHARACTERIZATION OF A VOLATILE FUNGISTATIC COMPOUND FROM SOIL. J.A. Liebman and L. Epstein, University of California, Berkeley, CA 94720.

We are trying to stabilize and identify a previously reported, volatile, water-soluble, germination-inhibitor that is produced by bacteria in a wide range of soils [Phytopathology 82:147-153 (1992)]. After passage through a 0.2 μm filter and collection into air-tight vials, soil extract inhibited germination of *Cochliobolus victoriae* conidia. One-half the fungistatic activity was lost after the soil extract was transferred to a new vial using a gas-tight syringe. Fungistatic activity was lost rapidly in uncapped vials. In capped vials, one-half the activity was lost within 12 h at 24 C, within 5 min at 100 C, and within 48 h at -70 C. Fungistatic activity was not present in material vacuumed from soil into a liquid N_2 cold trap. CO, NO, NO_2, ethylene, and iso-amyl alcohol were not fungistatic at concentrations found in soil. The effects of SO_2 and NH_3 are being investigated.

A326

INCIDENCE OF *APHANOMYCES EUTEICHES* AND *PHYTOPHTHORA MEDICAGINIS* IN KENTUCKY ALFALFA FIELDS. P. C. Vincelli, W. C. Nesmith and B. C. Eshenaur, Dept. of Plant Pathology, Univ. of Kentucky, Lexington 40546

Breeding for resistance to *Phytophthora medicaginis* Hansen et Maxwell and *Aphanomyces euteiches* Drechsler has been a major objective of alfalfa (*Medicago sativa* L.) improvement in North America in recent years. However, published data on the incidence of these pathogens in alfalfa fields are scarce. Soil samples from 121 alfalfa fields in 30 counties in Kentucky were collected (≥5 2-cm soil cores/field). Samples were tested using a baiting technique reported for *P. medicaginis* ("extended bioassay" in Phytopathology 75:1398); each soil sample was tested in both sterile water and in a 5 ug/ml metalaxyl solution to optimize detection of *P. medicaginis* and *A. euteiches*, respectively. Isolates of *A. euteiches* and *P. medicaginis* pathogenic to alfalfa were detected in 57% (95% confidence interval 48-66%) and 10% (95% CI 6-17%) of samples tested, respectively. Given the prevalence of *A. euteiches* in Kentucky soils, studies are needed to determine whether *Aphanomyces*-resistant cultivars will enhance alfalfa yields. Our data suggest that most alfalfa fields in Kentucky do not have detectable levels of *P. medicaginis*.

A327

ASSIGNMENT OF LINKAGE GROUP MARKERS AND OTHER GENES TO CHROMOSOMES OF *ASPERGILLUS FLAVUS*. K. R. Foutz, C. P. Woloshuk, and G. A. Payne. Dept. of Plant Pathology, North Carolina State University, Raleigh, NC 27695-7616.

In *Aspergillus flavus* over 30 genes have been mapped parasexually to eight linkage groups. Among the mapped loci are those for aflatoxin biosynthesis, spore color, and nutrient utilization. By genetic transformation we have complemented nutritional mutations in seven auxotrophic strains that map to six linkage groups. We also have separated the chromosomes of *A. flavus* into seven bands using pulsed-field gel electrophoresis. Specific probes made from the DNA complementing the *thi-1*, *arg-2*, *lys-4*, *pdx-6*, *arg-7*, *leu-7*, and *his-8* mutations were used to assign these linkage group markers to their respective chromosomes. In addition, we have assigned the aflatoxin genes *nor* and *afl-2* to linkage group VII. The assignment of linkage group markers to chromosomes will allow us to establish a physical map for *A. flavus*.

A328

FURTHER EVIDENCE FOR THE ROLE OF HOST DNA STRUCTURE IN THE INDUCTION OF DISEASE RESISTANCE RESPONSE (DRR) GENES AND PISATIN IN PEAS. Lee A. Hadwiger and Angela Parsons, Dept. of Plant Pathology, Washington State University, Pullman, WA 99164.

In pea pods, the accumulation of pisatin and several DRR genes are induced following treatment with the biotic elicitors, chitosan or DNase. These elicitors are naturally released from *Fusarium solani* f.sp. *phaseoli*, enter the plant cell nucleus, and presumably act on DNA. An abiotic elicitor can similarly enter the nucleus, specifically intercalate between bp, cross link DNA, and induce. Following DNase treatment of pea tissue, subsequently extracted host DNA is measurably enhanced as a substrate for N-terminal additions of dideoxy-ATP, in the presence of terminal transferase,indicating that an increase in strand breaks had occurred. The abiotic elicitor, 4' aminomethyl 4, 5', 8 trimethylpsoralen, was linked *in vivo* to host DNA at a rate of 1 molecule to 5 kb of host DNA. These elicitors, which have different modes of action, may induce the host response by altering normal DNA structure and thus transcription assemblies within the host cell.

A329

USE OF RAPD TO DISTINGUISH PHYSIOLOGICAL RACES OF *PHYTOPHTHORA SOJAE*. T.E. Chase and Z. Liu. Plant Science Department, South Dakota State University, Brookings, SD 57007.

The Random Amplified Polymorphic DNA (RAPD) technique was examined for its utility in distinguishing races of *P. sojae* (=*P. megasperma f. sp. glycinea*). Fifteen out of 80 oligonucleotide primers (Operon Inc., sets A,B,C,& D) tested yielded polymorphic DNA among the 14 races studied. Among the 14 races, 269 bands (92%) were monomorphic, and 24 (8%) bands were polymorphic. No single primer was sufficient to distinguish individual races; however, some individual races (e.g., race 25) could be distinguished from all the other races when composite RAPD profiles were considered. In some cases, different races (e.g., races 12 and 19) had identical RAPD profiles. In other cases independent isolates belonging to the same race had slightly different profiles (e.g., race 3), or more pronounced differences (e.g., race 9).

A330

ISOLATION AND CHARACTERIZATION OF GENES ENCODING PECTIN DEGRADING ENZYMES FROM *ASPERGILLUS FLAVUS*. M.P. Whitehead[1], M.C. Ho[1], J. Cary[2], T.E. Cleveland[2] and R.A. Dean[1]. [1]Department of Plant Pathology and Physiology, Clemson University, Clemson, SC 29634, [2] USDA, ARS, Southern Regional Research Center, 1100 Robert E. Lee Blvd., New Orleans, LA 70124.

Aspergillus flavus produces the highly toxic carcinogen, aflatoxin, on a variety of agronomically important crops. The ability of highly aggressive strains to contaminate cotton bolls appears to be linked to the production of a pectinase, P2c, not found in lowly aggressive strains. We have isolated a number of genes encoding pectin degrading enzymes via strategies of heterologous hybridization and PCR amplification. Expression analysis has determined that, unlike the other pectin degrading enzymes, the gene encoding P2c is not repressed by glucose. Data will be presented on characterizing the difference between pectinase expression in the aggressive and non-aggressive strains of *A. flavus*. Strategies to determine the role of P2c in aggressiveness will be discussed.

A331

CHARACTERIZATION OF THE cAMP DEPENDENT PROTEIN KINASE CATALYTIC SUBUNIT GENE IN *MAGNAPORTHE GRISEA*. T. K. Mitchell, Y. H. Lee, and R. A. Dean, Department of Plant Pathology and Physiology, Clemson University, Clemson, SC 29634

Magnaporthe grisea, the causal agent of rice blast disease, gains entry into its host through producing a specialized infection structure called an appressorium. To elucidate the mechanisms underlying the formation of this structure, second messengers involved in signal transduction systems are being investigated. It was found that cyclic adenosine monophosphate, cAMP, can induce the formation of appressoria on non-inductive hydrophilic surfaces. The primary target for cAMP in other organisms is cAMP dependent protein kinase. This in turn activates a phosphorylation / dephosphorylation cascade resulting in developmental changes. To investigate if similar events take place in *M. grisea*, we have isolated the cAMP dependent protein kinase catalytic subunit gene. Sequence analysis indicates extensive amino acid identity between the cloned gene and conserved regions of the corresponding genes in other organisms. Southern analysis indicates this gene exists as a single copy in *M. grisea*. We intend to disrupt this gene to determine its effect on appressorium formation.

A332

EXAMINATION OF A 30 BP CONSENSUS SEQUENCE IN *FUSARIUM OXYSPORUM* f. sp. *CONGLUTINANS* AND *F. OXYSPORUM* f. sp. *RAPHANI* FOR POSSIBLE AUTONOMOUS REPLICATION (AR) ACTIVITY. C.C. Hinkle and W.A. Powell. State University of New York, College of Environmental Science and Forestry, Syracuse, NY, 13210-2788.

Identification of autonomously replicating sequences in *Fusarium* species would advance our abilities to genetically manipulate these organisms. A 30 bp consensus sequence similar to sequences in pFOLT4 (Powell and Kistler. 1990. J. Bact. 172:3163-3171) was found adjacent to telomeres in *Fusarium oxysporum* f. sp. *conglutinans* and *F. oxysporum* f. sp. *raphani* (Powell and Yan. 1992. Phytopathology 82:1077-1078.). This consensus sequence has been cloned into a pBluescript SK (+/-) vector (Stratagene) in various copy numbers and orientations. A Hygromycin B gene was then inserted into these vectors. The constructed plasmid does not contain the repeated fungal telomere sequence, CCCTAA. We hypothesize that these sequences contribute to autonomous replication of the plasmid in *Fusarium* species. We are currently testing our vector for AR activity in these species.

A333

SELFING IN HETEROTHALLIC *PYTHIUM SYLVATICUM*. Pia D. Gavino and Frank N. Martin, Plant Pathology Dept., University of Florida, Gainesville, FL 32611.

Sexual reproduction in the genus *Pythium* is complex. Most of the approximately 125 described species in this genus are homothallic, while seven are heterothallic and a few isolates of these species may behave as both. *Pythium sylvaticum*, a pathogen of tree and vegetable seedlings, is heterothallic; opposite mating types are generally required for sexual reproduction to occur. However, some isolates also exhibit different levels of homothallism and can form oospores in single culture. Although selfing and germination of selfed oospores are rare events in this heterothallic species, we have aquired a few isolates with homothallic behavior from which selfed progeny may be recovered. The selfed oospore progeny had the same mating type as the parental isolate, but differed in their growth morphology. The electrophoretic karyotypes of the progeny were analyzed and variations in inheritance of chromosomal-sized DNA were observed. Analyses of the inheritance of Random Amplified Polymorphic DNA markers,

ribosomal DNA polymorphism, and probing of Clamped Homogeneous Electric Fields (CHEF) blots are being conducted to further examine the progeny and determine the mechanisms contributing to the observed polymorphisms.

A334

A UNIQUE RAPD FRAGMENT FOR VERTICILLIUM DAHLIAE AND ITS APPLICATION TO THE SPECIFIC DETECTION OF THE PATHOGEN. K.-N. Li, T. L. German, and D. I. Rouse. Department of Plant Pathology, University of Wisconsin, 1630 Linden Dr. Madison, WI 53706

V. dahliae is the primary pathogen of Potato Early Dying. Random amplified polymorphic DNA (RAPD) was performed on seventeen isolates of *Verticillium*, including seven *V. dahliae* isolates, six *V. albo-atrum* isolates and three *V. tricorpus* isolates in search of DNA probes specific for *V. dahliae*. One RAPD primer, E20 (AACGGTGACC), yielded a band of about 600 base pairs (bp) uniquely shared only by all *V. dahliae* isolates. The band from isolate V14, was cloned and sequenced. A data base search revealed no significant sequence homology between this locus and any other known sequence. A pair of PCR primers (VSP1 and VSP2) were designed based on the sequence; PCR tests showed that VSP1/2 amplified a fragment of 500 bp from all sixty tested *V. dahliae* isolates from very diverse host and geographical origins but not from any other fungus tested, including closely related species such as *V. albo-atrum*. Research is underway to apply VSP1/2 to the development of a rapid and sensitive technique to detect and quantitate *V. dahliae* propagules in plant and soil samples.

A335

ISOLATION OF THE *nor-1* GENE FROM *Aspergillus flavus*.
C. S. Brown-Jenco, K. R. Foutz, C. P. Woloshuk, and G. A. Payne.
Dept. Plant Pathology, N.C. State University, Raleigh, 27695-7616.

Aflatoxins are toxic and carcinogenic secondary metabolites produced by *Aspergillus flavus* and *A. parasiticus* on several important food sources. In an attempt to study aflatoxin biosynthesis in *A. flavus*, we are isolating genes in the aflatoxin pathway. We report here the isolation of the *nor-1* gene of *A. flavus*. Two overlapping clones with approximately 37-kb inserts were identified from a cosmid genomic DNA library constructed from the wild-type *A. flavus* strain NRRL 3357 by hybridization with the *nor-1* gene of *A. parasiticus* (courtesy of J. E. Linz, Michigan State University). A 2.8-kb fragment was subcloned and further characterized. Partial sequencing indicated high sequence identity to the *nor-1* gene of *A. parasiticus*. Northern analysis of poly(A)+ RNA from *A. flavus* strain NRRL 3357 grown under aflatoxin-inducing conditions revealed hybridization to a 1.1-kb band when probed with the *A. parasiticus nor-1* gene. Subclones of the cosmids are being tested for complementation of a mutant *A. flavus* strain which is blocked in aflatoxin biosynthesis, but accumulates norsolorinic acid, the first stable intermediate in the pathway.

A336

MOLECULAR ANALYSES OF *POLYMYXA BETAE* AND *POLYMYXA GRAMINIS*. A.L. Pilgeram and J.E. Duffus, USDA-ARS, Salinas, CA.

Polymyxa betae, the vector of beet necrotic yellow vein virus and beet soilborne virus, is an intracellular root parasite of plants within the Chenopodiaceae, Amaranthaceae, and Portulacaceae families. *Polymyxa graminis* is parasitic on the roots of several grasses and vectors several devastating viruses (soilborne wheat mosaic virus, barley yellow mosaic virus, peanut clump virus, ect.). Although the host ranges of the two species are distinctive, morphologically they are quite similar. Ribosomal internal transcribed spacer sequences (ITS) from *Polymyxa* -infected root tissues have been amplified, and the products evaluated using restriction and RAPD analyses. ITS products from the two species, from *P. betae* isolates from different host plants, and from viruliferous and aviruliferous isolates of *P.betae* are compared.

A337

COMPARISON OF THE NUCLEAR rDNA SEQUENCES OF *ALTERNARIA* SPECIES PATHOGENIC TO CRUCIFERS. C. Jasalavich, V. Morales, G. Séguin-Swartz, and L. Pelcher. Agriculture Canada Research Station and NRC-PBI, Saskatoon, Saskatchewan, Canada.

Alternaria brassicae, *A. brassicicola*, *A. raphani*, and *A. alternata*, are common pathogens of crucifers. The 18S rDNA, 5.8S rDNA and internal transcribed spacers (ITS) were amplified by PCR and sequenced for one isolate of each of these *Alternaria* spp. and one of *Pleospora herbarum*. The 5.8S rDNA sequences from the four *Alternaria* spp. were identical and differed at only one base pair in *P. herbarum*. The ITS sequences, especially ITS1, were variable among the five species. Alignment of the 18S rDNA sequences with those from other filamentous Ascomycetes show that all four *Alternaria* spp. and *P. herbarum* have very similar sequences; most of the base pair changes from the consensus sequence are the same for all five species. Phylogenetic analyses of the sequence data indicate that these *Alternaria* spp., as well as the two genera *Alternaria* and *Pleospora*, are closely related.

A338

A FATTY ACID-BASED FUNGICIDE FOR CONTROL OF POWDERY MILDEW. R.A. Haygood, Mycogen Corporation, 4980 Carroll Canyon Road. San Diego, CA 92121.

MYX-1446 F is a new, environmentally compatible fungicide which provides curative control of powdery mildew. The formulation is based on potassium salts of naturally derived fatty acids. Thorough coverage of susceptible plant parts is essential for control since this is a non-systemic, eradicant, 'impact' fungicide which offers no residual activity. The fungicide provides effective control of *Uncinula necator* and *Sphaerotheca pannosa* var. *rosa* on grapes and roses, respectively. In small-scale trials, conducted in 1992 and 1993, in vineyards and commercial rose production greenhouses, the fungicide provided control of powdery mildew when applied at 1.5 and 2% v/v solutions. Due to its unique mode of action, this fungicide should prove to be a useful resistance management tool for incorporation into programs with other fungicides which provide residual and/or systemic activity. MYX-1446 F also has utility in management of sulfur residues on grapes at harvest.

A340

INCREASE IN SEED CORN YIELD WITH FOLIAR FUNGICIDE TREATMENTS. J. M. Rivera-C., C. A. Martinson, and F. W. Nutter, Jr. Department of Plant Pathology, Iowa State University, Ames, IA 50011

Chlorothalonil and propiconazole were applied to seed parent plants in commercial seed corn production fields with time and number of applications varied. Comparisons were made with 3-4 sprays of copper thallate or mancozeb. Four-row plots of 0.003 ha were sprayed from a four row (three nozzles/row) boom powered with a motorized backpack sprayer. The dominant, naturally occurring disease was common rust (CR) (Puccinia sorghi), Northern corn leaf blight (NCLB) (Exserohilum turcicum), gray leaf spot (GLS) (Cercospora zeae-maydis), or Northern leaf spot (NLS) (Bipolaris zeicola) during three years and 19 total experiments with four replications. Maximum increases in yield of saleable seed (units of 80,000 seeds) were 19% when CR was controlled, 25% with GLS control, 3% with NCLB control and 6% with NLS control. Fungicide sprays also increased seed size with up to a 2.5 fold increase in saleable units of medium size seed. A spray applied just prior to detasseling was the single spray most beneficial for CR and GLS control and seed yield increase and with another applied after detasseling it provided adequate disease control and near maximum yields.

A341

EVALUATION OF STEROL-INHIBITING FUNGICIDES FOR FOLIAR DISEASE CONTROL IN WHEAT. A. Y. Chambers, Dept. of Entomology and Plant Pathology, Univ. of Tennessee, Jackson, TN 38301-3200.

Three new sterol-inhibiting fungicides - flusilazole, tebuconazole, and fenbuconazole - were evaluated for control of leaf rust (Puccinia recondita f. sp. tritici), glume blotch (Septoria nodorum), and leaf blotch (S. tritici) of soft red winter wheat (Triticum aestivum) during 1990-92. The fungicides were compared to standard, registered fungicides propiconazole (a sterol inhibitor) and mancozeb plus triadimefon in field plots of 'Saluda' wheat at the West Tennessee Experiment Station at Jackson. In 1990-92, treatments of all fungicides significantly reduced severity of the three diseases and significantly increased yields over no treatment. Flusilazole was comparable to the two standard treatments in disease control and yields in 1990 while treatments of tebuconazole and fenbuconazole significantly improved disease control and yields over the standards. Treatments of flusilazole and fenbuconazole were similar to those of propiconazole and mancozeb-triadimefon in 1991. Only treatment with tebuconazole in 1991 significantly improved disease control and yields compared to the standard materials. There were no significant differences in disease control and yields among the five fungicides in 1992.

A342

EVALUATION OF FOLIAR FUNGICIDES FOR CONTROLLING FUSARIUM HEAD BLIGHT (SCAB) OF WHEAT. E. A. Milus and C. E. Parsons. Department of Plant Pathology, University of Arkansas, Fayetteville 72701 and Strawberry Substation, Bald Knob 72010.

Fusarium head blight, incited primarily by Fusarium graminearum, caused severe damage to soft red winter wheat in the lower Mississippi River Valley in 1990, 1991, and 1993. All local

cultivars are susceptible, and head blight has been severe regardless of cropping history. The objective of this study was to determine if fungicides could reduce disease severity. The design was a randomized complete block with 12 treatments and four reps in each of two inoculum levels. Inoculum level was a split-plot factor. Fungicides (including benomyl, fenbuconazole, flusilazole, mancozeb, propiconazole, tebuconazole, thiabendazole, and triadimenol) were applied to cultivar Florida 302 at heading, and plants were inoculated on three consecutive days during flowering. A mist system provided favorable conditions for infection and systemic spread on days 0-6, 14, 16, and 18 after the first inoculation. Severity (% glumes blighted) was rated visually 24 days after the first inoculation. Severity averaged 83% across inoculum levels and treatments. No fungicide was effective, thus prospects for fungicidal control of head blight are poor.

A343

FORMULATION EFFECTS ON IPRODIONE PERFORMANCE IN PEANUT: IMPACT ON DISEASE CONTROL. K.W. Seebold and P.A. Backman, Dept. of Plant Pathology, Alabama Ag. Exp. Station, Auburn University, AL 36849.

The effects of iprodione tank formulations and application method on two soilborne diseases of peanut, Rhizoctonia limb rot (*Rhizoctonia solani*) and southern stem rot (*Sclerotium rolfsii*), were evaluated using six iprodione/adjuvant combinations and three delivery systems. Iprodione alone (1.12 kg/ha), combined with four sticker-type adjuvants, or mixed with a penetrating surfactant was applied to peanut plants with a broadcast boom, a two-nozzle in-canopy applicator, or a canopy-opening sprayer. Southern stem rot was evaluated after digging/inverting by counting the number of disease loci per 60 row feet. Limb samples were taken from each plot and the number of *Rhizoctonia* lesions present in the yield-bearing portion of the limb counted. No significant reductions in number of stem rot loci were observed for any application method or adjuvant combination. Flutolanil (1.12 kg/ha), the positive control, significantly reduced incidence of both diseases. The number of *Rhizoctonia* lesions per limb was not significantly different from control plots for any treatment; however, iprodione + Plyac and iprodione + Soydex treatments had fewer lesions per limb than the other adjuvant combinations. Regardless of method, *Rhizoctonia* control by iprodione may be enhanced by sticker-type adjuvants.

A344

SUSCEPTIBILITY OF SOME PECAN CULTIVARS TO ANTHRACNOSE. A. J. Latham and H. Lee Campbell, Department of Plant Pathology, Auburn University, Auburn, AL 36849.

Pecan anthracnose, caused by *Colletotricum gloeosporioides*, has gained prominence recently as a shuck and kernel rot disease. The causal fungus overwinters on current season peduncles sporulating and infecting juvenile shuck tissues during the following crop season. Anthracnose occurrence was evaluated in an orchard managed with a standard triphenyltin hydroxide spray program. Fifty nuts with anthracnose lesions were picked from five trees from each of 15 cultivars on 18 Sep 1992. Samples were surface disinfested with sodium hypochlorite, rinsed, plated on PDA, and incubated 2 wk under UV lights. Incidences of anthracnose were 72 and 100% on nuts from Cheyenne and Choctaw pecans, respectively, compared to only 4 to 8% on Cape Fear, Forkert, Jackson, Jubilee and Surprize. The use of fungicides along with anthracnose-resistant cultivars is an effective disease management procedure.

A345

CONTROL OF PLANT PATHOGENS WITH BC1000, A FATTY ACID COMPOUND. J. C. Broome, M. R. A. Chehata, and J. J. Marois. Department of Plant Pathology, University of California, Davis, CA 95616.

BC1000 (Chemie Res. & Man. Co., Inc., Casselberry, Florida) is a fatty acid based product extracted from grapefruit seeds that contains biocidal properties. ED50's of the compound when incorporated into agar plates ranged from 50 ppm (Botrytis cinerea Pers. and Monilinia fructicola (Winte.) Honey) to greater than 1000 ppm (Eutypa lata Pers.:Fr. and Fusarium oxysporum Schlect). Spores of B. cinerea treated with 100 ppm of BC1000 showed a rapid increase in electrolyte leakage within 2 hours after exposure. In field tests, BC1000 controlled B. cinerea (bunch rot) and Uncinula necator (powdery mildew) on grapes (Vitis vinifera L.) as well as the standard registered compounds. On Thompson seedless grapes in Davis, BC1000 (1500 ppm) reduced powdery mildew scarring by 63% and number of active colonies by 85%, as compared to the untreated control. Since BC1000 is a natural plant product, it may be possible to register it for use in organic production systems. It is presently listed on the FDA list of Generally Regarded as Safe compounds as a food additive but is not yet registered with the EPA as a pesticide. It was registered in 1992 for Chilean table grape production for control of B. cinerea in dust and liquid formulations.

A346

G.M. Leavitt[1], P.S. Verdegaal[2], T.M. Martin-Duvall[1], C.S. Thomas[3], W.D. Gubler[3]. Control of Phomopsis viticola on Grape (Vitis vinifera) in California. [1]University of California Cooperative Extension, 328 Madera Avenue, Madera, CA 93637. [2]University of California Cooperative Extension, 420 S. Wilson Way, Stockton, CA 95205. [3]Department of Plant Pathology, University of California, Davis, CA 95616.

Field plots were established over two years in three locations for efficacy of various products in controlling Phomopsis viticola. Traditional fungicides as well as products of softer chemistry were evaluated. Commonly used treatments of captan and mancozeb provided excellent control. Maneb, ziram, ziram/sulfur, copper hydroxide, copper hydroxide/sulfur, sulfur, chlorothalonil, and lime sulfur/sulfur were not statistically different from captan or mancozeb. JMS stylet oil, M-Pede, and Super Suffocant were not effective. Bark removal reduced infection in 92 but was not effective in 93. Soap (1% Dreft) reduced infection, but was not as effective as other compounds and resulted in phytotoxicity.

A347

RESISTANCE OF *SCLEROTINIA HOMOEOCARPA* TO DEMETHYLATION INHIBITOR FUNGICIDES. RC Golembiewski, JM Vargas Jr, AL Jones and AR Detweiler, Dept. of Bot. & Plant Path., Mich State Univ, East Lansing, MI 48824.

Fifty isolates of *Sclerotinia homoeocarpa* were collected from golf courses 1-3 where DMI fungicides had been reported to give little or no control, and from golf courses 4-6 where DMI fungicides had never been used. The mean ED_{50} values based on radial mycelial growth were 0.95, 4.53, 4.67, 0.13, 0.09 and 0.14 μg triadimefon/ml for courses 1-6, respectively. The same trends in mean ED_{50} values were also observed for fenarimol and propiconazol. ED_{50} values for isolates from golf courses 1-3 were 10-42X higher than isolates from golf courses 4-6. Isolates with high ED_{50} values for triadimefon also had high ED_{50} values fenarimol and propiconazol. The range of ED_{50} values for course 2 did not overlap those for courses 4-6, while isolates from courses 1 and 3 had either high or low ED_{50} values. Applied label rates of the DMI fungicides failed to control dollar spot on golf course 2. The significant difference in mean ED_{50} values between courses 1-3 and courses 4-6 and the failure of several DMI fungicides to control dollar spot on course 2, indicates that resistance of *S. homoeocarpa* to DMI fungicides has developed on some golf courses.

A348

CHARACTERIZATION AND SEROLOGICAL ANALYSIS OF WHEAT STREAK MOSAIC VIRUS (WSMV) ISOLATES. J.R. Montana, R.M. Hunger, J.L. Sherwood, and M.D. Bandla, Department of Plant Pathology, Oklahoma State University, Stillwater, OK 74078.

Differences in disease expression have been observed between various wheat cultivars and isolates of WSMV (Plt. Dis. 66:916; Phytopath. 78:703). Ten isolates of WSMV and one isolate of agropyron mosaic virus (AgMV) were evaluated by ELISA, western blots, Ouchterlony agar double diffusion, and SDS-PAGE. AgMV was included in this study because it was once considered to be a serotype of WSMV. Differences were found under both native and denaturing conditions in the reactivity of polyclonal and monoclonal antibodies to these isolates. The identification of WSMV serotypes dictates the use of both expression of disease symptoms and serology in the verification of WSMV and evaluation of disease resistance. This in turn could affect epidemiological studies of wheat streak mosaic.

A349

MAIZE VIRUSES ASSOCIATED WITH SEVERE MAIZE CHLOROTIC DWARF-LIKE SYMPTOMS IN INDIANA FIELDS TREATED WITH NICOSULFURON. G. E. Ruhl[1] and D. T. Gordon[2]. [1]Dept. of Botany and Plant Pathology, Purdue University, West Lafayette, IN 47907. [2]Dept. of Plant Pathology, Ohio Agricultural Research and Development Center, The Ohio State University, Wooster, OH 44691

Sixty corn samples submitted to the Purdue Plant and Pest Diagnostic Laboratory during the summer of 1991 displayed various virus-like symptoms, including chlorosis, stunting, and leaf reddening. Corn viruses, insect damage, nicosulfuron injury and environmental stresses were suspected as contributing causal factors. Serological assays of the samples identified maize chlorotic dwarf virus (MCDV) T or M1, maize dwarf mosaic virus (MDMV) A or B, or various combinations of these. However, some symptomatic plants contained no virus. Most of the plants exhibiting severe symptoms were mixed infections of MCDV-T and MCDV-M1. Further samples were collected in 1992 in an effort to establish the etiology of the 1991 corn problem. Even though severe symptoms were absent, the same viruses were identified. Additional studies are necessary to determine interaction effects among corn hybrids, herbicides, insect vectors and virus symptom expression.

A350

GEMINIVIRUS DIVERSITY IN SINALOA, MEXICO. A. O. Loniello[1], R. E. Ford[2], R. A. Salinas[3], F. J. Morales[4], and D. P. Maxwell[1]. [1]Dept. of Plant Pathology, Univ. of Wisconsin, Madison, WI 53706, [2]Dept. of Plant Pathology, Univ. of Illinois, Urbana, IL 61801, [3]Campo Experimental Valle del Fuerte, Los Mochis, Mexico, and [4]CIAT, Cali, Colombia.

Losses in vegetables caused by geminiviruses have increased during the past decade. Sixty samples of vegetables with symptoms typical of a geminivirus infection were collected near Los Mochis, Sinaloa, MX in February, 1993. These samples were tested for geminiviruses by DNA hybridization techniques and by polymerase chain reaction (PCR) methods using degenerate primer pairs specific for the DNA-A or DNA-B genome components of whitefly-transmitted geminiviruses. Bipartite geminiviruses were present in samples of bean, pepper, tomato, and squash. PCR-amplified fragments for the hypervariable region of DNA-B were obtained from squash (325 bp), bean (460 or 520 bp), tomato (540 bp), and pepper (570 bp). Samples from beans infected with bean calico mosaic virus (BCMoV) or bean golden mosaic virus type II yielded fragments of 460 bp and 620 bp, respectively. Our results indicate that beans were infected with a virus similar to BCMoV and another geminivirus, squash was infected with squash leaf curl virus, and the geminiviruses infecting peppers and tomatoes differed from those in squash and bean.

A351

IDENTIFICATION AND DISTRIBUTION OF PEPPER VIRUSES IN NEW MEXICO. G. Rodriguez-Alvarado, and C. M. Liddell. New Mexico State University, Department of Entomology, Plant Pathology and Weed Science, Box 3BE, Las Cruces, New Mexico 88003.

The occurrence of peppers in southern New Mexico showing virus-like symptoms in the foliage and fruit has increased during recent years. Pepper mottle virus (PeMV) was identified in 1978, but additional information was lacking on viruses infecting peppers in this area. An indirect ELISA was used to detect PeMV, tobacco etch virus, potato virus Y (PVY), and cucumber mosaic virus in leaf samples of peppers, and annual and perennial weeds collected from 308 plants in 13 fields from 2 counties during September-November, 1992. PeMV was found to be the most prevalent virus in Luna County infecting 55% of the pepper samples, while in Doña Ana County PVY was the most prevalent with 53%. The perennial weeds, *Solanum elaeagnifolium* and *Convolvulus arvensis*, are the most important overwintering hosts of these four viruses. An early survey during 1993 has confirmed the presence of the four viruses as well as tomato spotted wilt virus and alfalfa mosaic virus in these weeds.

A352

EXAMINATION OF THE LEVEL OF RESISTANCE IN TWO *CAPSICUM* SPECIES TO PEPPER MOTTLE AND TOBACCO ETCH POTYVIRUSES John F. Murphy and Molly M. Kyle, Department of Plant Breeding, Cornell University, Ithaca, N.Y. 14853

We have examined the level of resistance against pepper mottle (PeMV) and tobacco etch potyviruses (TEV) conferred by the three leading sources of resistance used in commercial *Capsicum* breeding, *C. annuum* 'Avelar' and two *C. chinense* accessions PI 152225 and PI 159236. PeMV accumulated in inoculated leaves of Avelar but to lower levels than that observed in the susceptible control *C. annuum* 'NuMex R Naky'. PeMV was detected in the stem of Avelar but not in young uninoculated leaves. TEV infected and accumulated in inoculated and uninoculated leaves of Avelar in amounts similar to that in NuMex R Naky. In contrast, neither PeMV nor TEV was detected in inoculated or uninoculated tissues of PI 152225 and PI 159236. Protoplasts isolated from PI 152225 and PI 159236 leaves and inoculated with PeMV RNA or TEV RNA did not accumulate detectable levels of viral coat protein. Thus, extreme resistance to PeMV and TEV was observed in *C. chinense*. Avelar appears to restrict the movement of PeMV, particularly out of the vascular tissues. Furthermore, Avelar's resistance against PeMV, but not that of PI 152225 or PI 159236, was lost when plants were coinfected with some isolates of cucumber mosaic virus.

A353

DETECTION AND CHARACTERIZATION OF DENDROBIUM MOSAIC VIRUS IN HAWAII. J. S. Hu, S. Ferreira, M. Wang, and M. Q. Xu, Dept. of Plant Pathology, Univ. of Hawaii, Honolulu, HI, 96822.

Dendrobium mosaic virus (DeMV) was detected in *Dendrobium superbum* Reichb.f. plants (known in Hawaii as the honohono orchid) at several nurseries in Hawaii by ELISA tests using a monoclonal antibody against potyviruses (MAb-PTY 1, from R. L. Jordan). Chlorotic mosaic and distortion symptoms were observed on leaves of diseased honohono orchids and symptoms of color breaking and distortion of flowers were also noted. Flexuous rod-shaped particles were observed in leaf dips of diseased orchids examined by electron microscopy. Potyvirus-like particles were purified and polyclonal antisera were produced. Based on serological reactions in ELISA, DeMV is not related to bean yellow mosaic virus, potato virus Y, papaya ringspot virus, watermelon mosaic virus II, tobacco etch virus, zucchini yellow mosaic virus, soybean mosaic virus, turnip mosaic virus, potato virus A, and dasheen mosaic virus. Molecular weight (m.w.) of the DeMV coat protein was about 34 x 10^3 daltons in SDS-PAGE analysis. The DeMV RNA had a m.w. of about 3 x 10^6 daltons in denatured agarose gel electrophoresis.

A354

PARTIAL CHARACTERIZATION OF CITRUS TRISTEZA VIRUS ISOLATES FROM THE CENTRAL VALLEY SUPPRESSION AREA. J. Ghazanfari[1] and J.A. Dodds[2]. [1]California State Dept. Food and Agric., Tulare, CA 93274 and [2]Dept. Plant Pathology, Univ. Calif., Riverside, CA 92521

Citrus tristeza virus has been isolated from some citrus groves in the three county areas under suppression. These counties are Tulare, Kern and Fresno, CA. Isolates were budded into indicator plants, sweet orange, mexican lime, rough lemon, grapefruit, and sour orange. None of the isolates induced symptoms indicative of seedling yellow or stem pitting strains. Double stranded RNA analysis showed different patterns on polyacrylamide gels. Isolates from North East of Visalia, CA showed a band characteristic of severe isolates. Based on the dsRNA profile, isolates from the Lindsay area are different from the Exeter area. Isolates from two adjacent blocks showed different dsRNA profiles. A monoclonal antibody, MCA-13 from Florida detected the isolate which showed the "severe" dsRNA profile, but did not react with any other isolates in ELISA.

A355

OCCURRENCE OF BEAN GOLDEN MOSAIC VIRUS IN FLORIDA. M.W. Blair[1], A. M. Abouzid[2], E. Hiebert[2], R. T. McMillan Jr.[3]., W. Graves[3], and M. Lamberts[3]. Dept. of Horticulture[1], Dept. of Plant Pathology[2], Univ. Florida, Gainesville, FL 32611; Univ. of Florida, Tropical Research and Education Center, Homestead, FL 33031[3].

An epidemic of bean golden mosaic virus (BGMV-H) was observed in the spring of 1993 in south Florida. The disease was found in snap beans, *Phaseolus vulgaris* L., and *P. limensis* Macf. in S.W. Dade County and S.E. Palm Beach County. Of the estimated 9,100 hectares of snap beans in south Florida, approximately 30% of them were affected. In severe infections, yields were 26-87hL/ha as compared to usual yields of 175hL/ha. In some cases fields were completely abandoned or destroyed. Plants with bright golden mosaic symptoms tested positive for geminivirus infection when extracts were probed (dot blots) with A component DNA from a geminivirus infecting the weed *Macroptilium lathyroides* in the Homestead area or with the recently identified tomato mottle geminivirus in Florida. These samples did not react with probes prepared to the B components for these viruses. The nucleotide sequence of the common region of the genomic DNA and the intergenic region for the B component indicated a very high degree of homology with BGMV Guatemala isolate described by Gilbertson et. al.(Phytopathology 81, 980-985). BGMV-H was mechanically transmissible to *P. vulgaris* cv. Topcrop. The sweetpotato whitefly, *Bemisia tabaci*, was an efficient vector of the virus in transmission tests. This is the first report of a BGMV isolate infecting beans in the field in the Continental USA.

A356

PURIFICATION AND PARTIAL CHARACTERIZATION OF A VIRUS ISOLATED FROM CITRUS TREES AFFECTED BY CITRUS BLIGHT. R. H. Brlansky, C. L. Davis, and D. S. Howd. University of Florida, C.R.E.C., Lake Alfred FL

Using transmission electron microscopy, small, 15-17 nm isometric virus particles were found in root tissue from citrus blight-affected trees. The particles were present in trees on rough lemon, Carrizo citrange and Volkmer lemon rootstocks. Partial purification was accomplished by extraction of root tissue in phosphate buffer containing butanol, sodium sulfite and EDTA followed by polyethylene glycol precipitation. Further purification was done using sucrose gradient and cesium sulfate gradient centrifugations. SDS-PAGE revealed a major protein band of approximately 22-23 kd. Polyclonal antibodies prepared to the partially purified virus were reactive in Western blots to proteins of 22 and 29.5 kd. Further analyses revealed a RNA of approximately 3 kb. Virions were not present in healthy citrus root tissue from California where citrus blight has not been reported. Further characterization of this virus, association with citrus blight and host range determination, is ongoing.

A357

PROPERTIES OF AN ISOMETRIC VIRUS ISOLATED FROM Capsicum frutescens. R. A. Valverde and L. L. Black. Dept. of Plant pathology and Crop Physiology, Louisiana Agricultural Experiment Station, Louisiana State University Agricultural Center, Baton Rouge 70803.

A virus isolate, designated tabasco mosaic virus (TaMV), was isolated from tabasco pepper (Capsicum frutescens) in El Negrito, Honduras. TaMV was mechanically inoculated to several C. frutescens, C. chinense, and C. annuum cultivars. Symptoms consisted of mosaic and yellow mottle with C. frutescens and C. chinense, respectively, and mild leaf distortion with C. annuum. TaMV was purified and an antiserum was prepared. Viral ssRNA, dsRNA, and protein coat subunits were analyzed by gel

electrophoresis. Epidermal strips from infected pepper plants were stained and viewed with the light microscope. Results of these tests indicate that TaMV have properties similar to members of the comovirus group. However, serological tests with several comoviruses were negative.

A358

BIOLOGICAL DIVERSITY AMONG EXOTIC AND U.S. ISOLATES OF CITRUS TRISTEZA VIRUS (CTV). S. M. Garnsey, USDA, ARS, Orlando, FL 32803, E. L. Civerolo, USDA, ARS, Beltsville, MD 20705, D. J. Gumpf, Dept. of Plant Pathology, University of California, Riverside, CA 92521, R. F. Lee, University of Florida, CREC, Lake Alfred, FL 33850, and R. K. Yokomi, USDA, ARS, Orlando, FL 32803.

Severity of CTV-induced symptoms differs among isolates and between countries. The severe stem pitting observed in many countries is rare in the U.S. Over 220 CTV isolates from 26 countries have been established under quarantine at Beltsville, MD, and 156 have been tested on a host range of Mexican lime, sour orange, sweet orange, grapefruit, and sweet orange grafted on sour orange. Several reaction patterns have been observed. Forty-five isolates from 12 different countries induced stem pitting in Madam Vinous sweet orange seedlings, and over 70 isolates induced stem pitting and/or severe seedling yellows in Duncan grapefruit. Several grapefruit stem pitting isolates did not stunt grafted combinations of sweet and sour orange. Some isolates may be strain mixtures since subcultures obtained by transmission with *Aphis gossypii* sometimes differed from the parent source. Many exotic isolates of CTV are more severe and pose a greater disease threat than those isolates currently present in the U.S. The library of isolates is valuable for serological and molecular characterization studies and for further development of detection and control procedures for exotic isolates.

A359

THE DISTRIBUTION AND PARTIAL CHARACTERIZATION OF CASSAVA VEIN MOSAIC VIRUS. L.A. Calvert, and M.D. Ospina. Centro Internacional de Agricultura Tropical - CIAT. Apartado Aereo 6713, Cali, Colombia.

Cassava vein mosaic virus (CVMV) is a member of the caulimovirus group. The isometric virions are approximately 50 nm in diameter and the ds-DNA genome is approximately 8000 bases. The symptoms include chlorosis that follows the veins and can coalesce to form a mosaic pattern. There is often leaf distortion and epinasty of the young leaves. In older plants the expression of symptoms is more severe when the temperatures are higher and during cooler seasons the infection can be latent. Surveys were made throughout Brazil to determine the range of the disease caused by CVMV, and the disease is most prevalent in the semiarid region in the northeastern states. It is not unusual to find fields with more than 50% of plants infected with CVMV. A DNA clone to CVMV was kindly provided by Dr. R. Shepherd of the U. of Kentucky. Approximately 5000 bases of the clone have been sequenced and one major ORF of over 3000 bases has been identified. The putative protein encoded by this ORF shares only limited homology with the proteins encoded by CaMV or FMV. A PCR based detection system has been developed and it is being used to explore strain diversity.

A360

USE OF ANTIGEN-COATED MAGNETIC BEADS IN PRODUCTION OF MONOCLONAL ANTIBODIES TO TOMATO SPOTTED WILT VIRUS (TSWV) NON-STRUCTURAL PROTEIN (NSs). M.D. Bandla,[1] J.L. Sherwood,[1] K.D. Chenault[1] and T.L. German,[2] [1]Department of Plant Pathology, Oklahoma State University, Stillwater, OK 74078, and [2]Department of Plant Pathology, University of Wisconsin, Madison, WI 53706.

The use of antigen-coated magnetic beads in production of monoclonal antibodies was investigated using NSs protein of TSWV. Stable hybridoma cell lines were produced by fusing P3-X63-AG8.653 cells with spleen cells from BALB/c mice immunized with NSs protein. One ml containing 4 X 10^4 beads (Dynal Inc.) were coupled to 250 μg of NSs protein. Half of the hybridoma cells were incubated with antigen-coated magnetic beads at a bead to cell ratio of 5:1 and the cells secreting the specific antibody were separated and cloned. The other cells were plated and screened for the secretion of antibody to NSs by ELISA. Positive cell lines were cloned by limited dilution. The affinity of antibodies from these two methods was evaluated by immunoprecipitation and ELISA. Cells secreting high affinity antibody could be selected at less time and cost by using antigen-coated magnetic beads than by the conventional approach.

A361

VARIATION AMONG ISOLATES OF HIRSUTELLA RHOSSILIENSIS. TEDFORD, E. C., B. A. JAFFEE, and A. E. Muldoon. Department of Nematology, University of California, Davis, CA. 95616

Twenty-nine isolates of the nematophagous fungus Hirsutella rhossiliensis were obtained from different hosts and geographical locations. All isolates infected Meloidogyne javanica, Heterodera schachtii (Hs), and Steinernema glaseri in vitro, but isolates from Rotylenchus robustus (Rr) and Hoplolaimus galeatus (Hg) infected more slowly than did those from other nematode species or mites. In soil microcosms, Hs acquired spores of all isolates, but the percentage of Hs that acquired at least one spore in 66 h at 20 C was 19.2 ± 0.9% with isolates from Rr and Hg and 48.6 ± 1.6% with other isolates. Isolates from Rr and Hg produced larger spores (9 x 6 μm vs 7 x 5 μm) and grew slower (0.5 vs 0.9 mm/day at 20 C) on cornmeal agar than did other isolates. The hypothesis that isolates from Rr would infect Rr better than would those from Hs was tested and rejected. RAPD analysis of genetic variability showed clustering based on host nematode but not on geographical location.

A364

SMALL-SCALE FIELD TRIALS WITH NOVEL MANAGEMENT AGENTS FOR SOYBEAN CYST NEMATODE. S. Meyer[1], M. Dimock[2], J. Fahey[2], G. Johnson[2] and R. Huettel[1]. [1]Nematology Lab, USDA, ARS, Beltsville, MD, 20705; [2]Crop Genetics International, Columbia, MD, 21076.

Small-scale field studies were conducted in two Maryland fields during 1992 to determine effects of novel management agents on soybean yields. The target pest was *Heterodera glycines*, the soybean cyst nematode (SCN). Significant yield increases compared to the untreated susceptible cultivar were obtained with three treatments. These treatments were: a) aldicarb (40% yield increase, $P<0.01$); b) methyl vanillate, an analog of the SCN sex pheromone (29% increase, $P<0.05$); and c) the SCN sex pheromone (vanillic acid) combined with the fungus *Verticillium lecanii* (22% increase, $P<0.10$). Application of *V. lecanii* alone, vanillic acid alone, three additional analogs used individually, and another fungus/analog combination resulted in yield increases ranging from 11% to 21%. These increases were not statistically significant, but the trends indicated that several treatments have potential to act as management agents.

A376

CONSIDERATION OF VARIANCE IN MONITORING NEMATODE COMMUNITY STRUCTURE ON A REGIONAL BASIS. Deborah A. Neher and C. Lee Campbell, N.C. State Univ. and USDA/ARS, Raleigh, NC 27695.

Sample size necessary to detect changes in condition of agricultural soils on a regional basis was quantified using nematode communities (plant-parasitic and free-living) as a bioindicator. Nematodes were extracted from composite soil samples (20, 2x20 cm cores per 2-ha) collected from 92, 80, and 195 fields across the Coastal Plain, Piedmont, and Mountain regions of North Carolina in 1990, 1991, and 1992, respectively. Fields of annually harvested crops to be sampled were selected based on probabilities proportional to their hectarage. Components of variation associated with indices of maturity and trophic diversity were quantified among fields, among independent transects within fields, and within composite soil samples. The largest component of variance was among fields and within composite samples for maturity and trophic diversity indices, respectively. Based on power curves, sample sizes required for regional studies would be less if maturity indices were used rather than trophic diversity indices.

A382

TEMPORAL AND SPATIAL DISTRIBUTION OF *MELOIDOGYNE* N. SP. IN A COFFEE FIELD. F. R. Zhang and D. P. Schmitt. Department of Plant Pathology, University of Hawaii, Honolulu, HI 96822.

Temporal changes in population densities of a new species of *Meloidogyne* on coffee were examined from 1991 to 1992 on cultivars Guatemala and 502, and on 4 rootstocks (Purpuree, Congensis, Deweveri, and Kaffe) with Guatemala or 502 as a scion. The spatial pattern was determined on roots of Guatemala and Deweveri. Ten soil cores (2.5 cm diam and 15 cm deep) were collected from each replication of five trees and composited to determine population changes over time. For the spatial pattern study, soil cores (5 cm diam) were collected from 3 positions (20, 40, and 60 cm from the trunk) and 3 depths (0-15, 16-30, and 31-45 cm). Nematodes were extracted from 250 cm^3 soil by a combination of elutriation and centrifugal flotation. The temporal changes were different among rootstocks ($P < 0.05$) and times ($P < 0.01$). The greatest number of second-stage juveniles occurred on Guatemala and the fewest on Deweveri. More nematodes were found in March than in July of both years. The spatial distribution varied by positions on Guatemala. The highest population density was at 60 cm from the base of the tree. More J2 were recovered at the lower depths ($P < 0.1$).

A388

MOLECULAR ANALYSIS OF THE DIVERSITY BETWEEN THE TOMATO SPOTTED WILT TOSPOVIRUS M RNAS. C.M. Hickey Tiani, S. Geske, J.M. Hall, and J.W. Moyer, Dept. of Plant Pathology, North Carolina State University, Raleigh, NC 27695-7616.

Although the diversity of the Tospoviruses is widely known, the cause of this diversity is still unclear. The Tospoviruses are characterized by their enveloped, quasi-spherical particles containing the tripartite ssRNA genome designated Large (L), Middle (M), and Small (S). Variants containing defective interfering particles from the L RNA or N protein epitope variation of the S RNA have been identified, but the M RNA has received less attention. We have detected variants in the MRNA open reading frame coding for the G1 and G2 envelope glycoproteins. Distinct differences were found in the mobility of PCR fragments amplified using primers specific for one region of the G2 protein. In addition, a single isolate was found with a slower migrating G2 protein.

A389

PHYLOGENETIC ANALYSIS OF BEAN COMMON MOSAIC POTYVIRUS STRAINS.
P. H. Berger[1] and S.D. Wyatt[2], [1]Dept. of Plant, Soil and Entomological Sciences, University of Idaho, Moscow, ID 83844 and [2]Dept. of Plant Pathology, Washington State University, Pullman, WA 99164.

Nucleotide sequences of the coat protein cistron and 3'-end of twelve strains representing each of the known pathogroups of bean common mosaic virus (BCMV), were obtained. These data and sequences previously published were used for phylogenetic analysis of nucleotide and amino acid sequences using neighbor-joining, unweighted pair-group method using averages (UPGMA) and parsimony approaches. These three methods gave similar results in terms of their phylogeny reconstructions of BCMV strains, indicating the reliability of these predictions. Phylogenetic analyses were also perfomed with other legume-infecting potyviruses and other members of the *Potyviridae*. The results indicate that serogroup A strains are easily distinguished from serogroup B strains. However, certain unexpected relationships are apparent and will be discussed.

A390

LOCATION AND COMPOSITION OF CYTOPLASMIC INCLUSIONS IN THRIPS CELLS INFECTED WITH TOMATO SPOTTED WILT TOSPOVIRUS (TSWV). Diane E. Ullman[1], John L. Sherwood[2], Thomas L. German[3], Daphne M. Westcot[1], Kelly D. Chenault[2], and Frank A. Cantone[4]. [1]Department of Entomology, University of Hawaii, 3050 Maile Way Rm. 310, Honolulu, HI 96822; [2]Department of Plant Pathology, Oklahoma State University, Stillwater, OK 74078; [3]Department of Plant Pathology, University of Wisconsin, Madison, WI 53706; [4]Boyce Thompson Institute, Cornell University, Ithaca, NY 14853.

Tomato spotted wilt tospovirus (TSWV), type member of the only plant infecting genus in the Bunyaviridae, has been shown to replicate in both plants and its thrips vectors. *In situ* localization of proteins encoded by the TSWV genome with antibodies demonstrates presence of the nucleocapsid (N) and membrane glycoproteins (G1 and G2) in cytoplasmic inclusions and specialized vacuoles in thrips midgut epithelia, muscle and salivary gland cells. In addition, fibrous, paracrystalline inclusions composed of the nonstructural protein encoded by the S RNA (NSs) are present in many cells. A model for TSWV pathogenesis in thrips cells will be proposed.

A391

CHARACTERIZATION OF A SEVERE STRAIN OF PEA STREAK CARLAVIRUS ISOLATED FROM CHICKPEA IN WASHINGTON. R.C. Larsen, USDA-ARS, Prosser, WA 99350, S.D. Wyatt, Dept. of Plant Pathology, Washington State University, Pullman, WA 99165, and W.J. Kaiser, USDA-ARS, Pullman, WA 99165

Pea streak carlavirus is a common pathogen in the Pacific Northwest and is transmitted to leguminous crops by the pea aphid, *Acyrthosiphon pisum*. A strain of the virus (PSV-WW) causing leaf necrosis, severe stunting, or plant death was isolated from chickpea (*Cicer arietinum)* growing near Walla Walla, WA. A single coat protein band of ca. 28,000 daltons was visualized in western blots of total protein preparations; however, capsid protein from purified virions analyzed by SDS-PAGE and western blots appeared as several bands which probably represent degradation products of the 28,000 dalton protein. PSV-WW was serologically indistinguishable from the type strain of PSV or from alfalfa latent virus by indirect ELISA or western blots. PSV-WW yielded three distinct nucleoprotein zones in sucrose density gradients (SDG). RNAs corresponding to the nucleoprotein zones resolved in agarose glyoxal-denaturing gels as 7.5 kb, 1.8 kb, and 1.2 kb. In contrast, the type strain of PSV yielded one SDG band from which only a single genomic RNA of 7.5 kb was resolved. The RNAs have been cloned and are currently being sequenced and compared to other known carlaviruses.

A392

COAT PROTEIN GENE-MEDIATED RESISTANCE TO BARLEY YELLOW DWARF VIRUS IN MAIZE AND BARLEY. S.D. Wyatt[1], P.H. Berger[2], and P.G. Lemaux[3]. [1]Dept. Plant Pathology, Washington State University, Pullman, WA 99164. [2]Dept. Plant, Soil and Entomological Sciences, University of Idaho, Moscow, Id. 83844. [3]USDA Plant Gene Expression Center, Albany, CA 94710.

Maize and barley were for the first time transformed with the barley yellow dwarf virus (BYDV) coat protein gene and then analyzed for susceptibility to BYDV. Transformation of calli was performed using microprojectile bombardment and subsequent selection on media containing the herbicide bialophos. Presence of the coat protein gene in regenerated plants was confirmed by Southern and northern analyses. Little expressed virus coat protein was detected in R_1 or R_2 maize plants by ELISA, but the titer of BYDV following aphid inoculation was suppressed in several lines. Expressed coat protein was detected in transgenic barley by ELISA. Virus titers were significantly suppressed in progeny of two of three independent transformation events tested so far over that found in the nontransformed parent line.

A393

FACTORS CONTRIBUTING TO THE ANOMALOUS ELECTROPHORETIC MOBILITY OF CUCUMOVIRUSES COAT PROTEINS IN SDS/POLYACRYLAMIDE GELS. C. C. Hu and S. A. Ghabrial. Department of Plant Pathology, University of Kentucky, Lexington, KY 40546.

Although the relative molecular mass (M_r) of the coat proteins of several cucumoviruses has been calculated from their deduced amino acid sequences as 24.5-25 x 10^3, the estimated M_r using the Laemli SDS/PAGE system was 30-31 x 10^3. Examination of the amino acid composition revealed that these proteins are neither highly acidic nor highly basic. Posttranslational modification was also ruled out as a contributing factor because the products of in vitro translation of RNA 4 and in vivo bacterial expression of cloned coat protein genes co-migrated with authentic cucumovirus coat proteins. Comparison of the hydropathy profiles of the coat proteins revealed the presence of a conserved highly hydrophilic N-terminal region of 30-32 amino acid residues that contained a cluster of basic amino acids. Selective chemical cleavage was used to generate peptides that contained or lacked the conserved region. The results showed that only peptides containing the conserved region exhibited the anomalous electrophoretic mobility.

A394

TRANSFORMATION OF SOYBEAN WITH BEAN POD MOTTLE VIRUS COAT PROTEINS-PRECURSOR GENE USING THE BIOLISTIC METHOD. R. Di, G. B. Collins, and S. A. Ghabrial. Departments of Plant pathology and Agronomy, University of Kentucky, Lexington, KY 40546

Bean pod mottle comovirus (BPMV) is an economically important pathogen of soybeans in Kentucky. Because soybean cultivars with resistance to BPMV are not commercially available, we have resorted to the strategy of coat protein-mediated protection to produce transgenic resistance to BPMV in soybean. Embryogenic suspension cultures of soybean were generated from immature zygotic cotyledons, and bombarded with tungsten particles coated with plasmid DNA that contained the BPMV coat proteins-precursor (CP-P) gene. After a few cycles in a selection medium, the surviving embryogenic tissues were plated on MSM maturation medium. All the somatic embryos that grew out on the maturation medium contained the CP-P gene, as verified by a PCR assay. These transgenic embryos are being germinated and grown into plants. Our recent studies with tobacco as a model system, have demonstrated that constitutive expression of CP-P conferred resistance to comovirus infection.

A396

ASSOCIATION OF THE COAT AND P3 PROTEINS OF TOBACCO VEIN MOTTLING POTYVIRUS (TVMV) WITH CYLINDRICAL INCLUSIONS IN INFECTED TOBACCO LEAVES AND PROTOPLASTS. E.D. Ammar, E. Rodriguez-Cerezo, J.G. Shaw, and T.P. Pirone, Dept. Plant Pathology, University of Kentucky, Lexington, Ky. 40546.

Immunogold labeling was used to localize the coat protein (CP), the P3 (42 kDa) protein, and the cylindrical inclusion protein (CI) of TVMV in thin sections of tobacco leaves and

protoplasts at various times post-inoculation (p.i.). In leaves fixed 5-21 days p.i., antibodies to P3 or CI labeled only the cylindrical inclusions, whereas antibodies to CP labeled bundles of virus particles and the cylindrical inclusions. In protoplasts fixed 10, 21, or 45 hr p.i., the intensity of labeling of the cylindrical inclusions with P3 or CP antibodies increased with time; antibodies to CP also labeled individual virus particles that were associated with these inclusions as early as 10 hr p.i. Based on these and other results, it is suggested that the cylindrical inclusions may be the site of multiplication and assembly of potyviruses.

A397

COAT PROTEIN AS A DETERMINANT OF VIRUS TRANSMISSION BY LEAF FEEDING BEETLES. T. MAHMOOD, R. C. Gergerich, and K. S. Kim. Department of Plant Pathology, University of Arkansas, Fayetteville, AR 72701.

Specificity in beetle-transmitted viruses is thought to be manifested by interaction between the virus and some plant component(s). To study the role of the viral coat protein in this interaction, heterologous virus particles were assembled *in vitro* from coat protein subunits of the beetle-transmissible cowpea strain of southern bean mosaic virus (CP-SBMV) and the RNA of the non-beetle-transmissible cowpea strain of tobacco mosaic virus (CP-TMV). The reassembled virus particles, which were morphologically indistinguishable from those of the native CP-SBMV, were infectious on both systemic and local lesion hosts for CP-TMV, but not on systemic and local lesion hosts for CP-SBMV. In transmission tests using *Phaseolus vulgaris* 'Black Valentine' bean, the Mexican bean beetle, *Epilachna varivestis*, transmitted the reassembled virus that contained the RNA of CP-TMV within the CP-SBMV capsid. These results suggest that beetle transmission of plant viruses is determined by properties of the coat protein.

A398

AMPLIFICATION OF THE COAT PROTEIN GENE OF CITRUS TRISTEZA VIRUS FROM *Toxoptera citricida*, ITS APHID VECTOR. C.L. Niblett, H.R. Pappu, S.S.Pappu, R.J. Lastra*, and R.F. Lee.**. Plant Pathology Department, University of Florida, Gainesville, FL 32611, *CATIE, Turrialba, Costa Rica, and **CREC, Lake Alfred, FL 33850.

Toxoptera citricida (TC), the most efficient aphid vector of citrus tristeza virus (CTV), has moved rapidly northward from South America to Nicaragua in Central America and to Hispaniola in the Caribbean basin. This poses a serious threat to citrus in these regions, including the United States, especially if TC is carrying the severe quick decline and/or stem pitting strains of CTV known to occur in South America. Using primers from the coat protein gene (CPG) of CTV, reverse transcription and amplification reactions were performed on nucleic acid extracts from groups of three and four TC fed on CTV infected leaves. A single product of ca. 700 bp was obtained. It was confirmed as the CTV CPG by hybridization with a known CPG probe. This approach combined with nucleotide sequencing of the CPG will be useful to identify the strains of CTV carried by TC and for epidemiological studies.

A399

PASSAGE OF INJECTED PLANT VIRUSES FROM THE CIRCULATORY SYSTEM TO THE REGURGITANT OF THE MEXICAN BEAN BEETLE AND SPOTTED CUCUMBER BEETLE. R. Y. Wang, R. C. Gergerich, and K. S. Kim. Department of Plant Pathology, University of Arkansas, Fayetteville, AR 72701.

Injection of plant viruses into the hemocoel of beetles makes them viruliferous. Therefore, it has been suggested that the hemocoel acts as a virus reservoir. The objective of this research is to determine the route of virus movement from the hemocoel to the regurgitant in beetle vectors. The beetle-transmissible southern bean mosaic virus and the non-beetle-transmissible cowpea strain of tobacco mosaic virus were injected into the hemocoel of the Mexican bean beetle and spotted cucumber beetle. Virus was recovered in the regurgitant from 100% of beetles 24 h after virus injection. Examination of immunofluorescence-labeled frozen sections of the fore- and midguts revealed that 24 h after injection, virus had not passed from the hemocoel into the digestive system. Similar experiments demonstrated that virus did not pass through the mandibular and maxillary glands of beetles after injection. No virus particles were observed in epithelial cells of either gut or glands by electron microscopy. Based on these and other tests, it appears that contamination during injection, or reflexive bleeding after injection, was the source of virus for viruliferous beetles.

A401

RESPONSE OF VIRGINIA-TYPE CULTIVARS OF *ARACHIS HYPOGAEA* L. TO PEANUT LEAF SPOTS. B. B. Shew*, J.E. Bailey, and M.K. Beute. Departments of Crop Science* and Plant Pathology, North Carolina State University, Box 7616, Raleigh 27695-7616.

Eight cultivars of virginia-type peanut were planted in field plots in 1992 and 1993. The 3.7 m x 6.9 m plots were bordered on all sides by at least 7.3 m of nonhost cotton. Standardized areas under the disease progress curve were calculated from percent defoliation (AUC-D) data and from estimated incidences of early leaf spot caused by *Cercospora arachidicola* (AUC-E) and late leaf spot caused by *Cercosporidium personatum* (AUC-L). In 1992, AUC-E ranged from 32% for NC 6 to 77% for NC 10C and AUC-L ranged from 3% for VC 1 to 22% for NC 6. Defoliation (AUC-D) varied less, ranging from 19% for NC 6 to 30% for NC 10C. The widely grown cultivar NC 7 had significantly smaller AUC-E and significantly larger AUC-L than Florigiant, the cultivar it replaced. These results and field observations suggest that *C. personatum*, which is the more difficult disease to control, may become increasingly important in the foliar disease complex in North Carolina.

A402

KARNAL BUNT OF WHEAT IN THE YAQUI VALLEY, SONORA, MEXICO. Ruben Garcia-Valle, Mexican Department of Agriculture and Water Resources, Cd. Obregon, Sonora; Guillermo Fuentes-Davila, CIMMYT, Apdo. Postal 6-641, 06600 Mexico, D. F.

Karnal bunt of wheat has been endemic in the valley since 1969-70. However, it did not become important until the early 80's when incidence levels increased. The presence of the disease during the last 12 wheat cycles, including 1992-93, has been erratic. While in 1984-85 the number of wheat samples affected with Karnal bunt reached a record high of 72.5%, in 1986-87 only 0.35% of samples were affected. Then, from 3.1% in 1987-88 it went up to 55.5% the following cycle. The Internal quarantine No. 16 emitted, by the Mexican Department of Agriculture in 1987 contemplates the following objectives: to confine the disease within the original limits avoiding its dissemination to areas free of the pathogen, and to specify cultural and preventive control measures to minimize the impact of the disease in the affected regions. The erratic occurrence of the disease as indicated by results from wheat sampling, has been due to variable weather conditions prevailing during the flowering stage of the plant, the susceptibility of the cultivars used, and the proportion of Triticum aestivum and T. durum cultivated. Research should be reinforced to provide more knowledge about the pathogen in order to be able to adjust legal, cultural, and chemical control measures.

A403

IMPORTANCE OF HUSK COVERING AND INTRA-EAR THRIPS POPULATION ON THE SUSCEPTIBILITY TO FUSARIUM EAR ROT AMONG CORN HYBRIDS. C. Y. Warfield and R. M. Davis. Department of Plant Pathology, University of California, Davis 95616.

The role of the husk covering and the involvement of thrips on the severity of Fusarium ear rot was evaluated. Treatments aimed at either increasing or decreasing thrips colonization within the ear were imposed on six corn hybrids ranked as either susceptible or tolerant to Fusarium ear rot. Splitting open and partially removing the husks, thereby compromising the natural barrier to insects, resulted in an increase in disease severity and a decrease in yield for the tolerant ranked hybrids. Acephate applications reduced disease severity and increased yield among both tolerant and susceptible hybrids. Partial removal of the husks, followed by acephate application, increased the disease severity among the tolerant hybrids, but had no effect on yield. Population counts of intra-ear thrips showed that susceptible ranked hybrids generally supported a larger thrips population at an earlier stage of kernel development compared to the tolerant ranked hybrids.

A404

PATHOGENICITY OF *SORDARIA GUAYULEAE* TO KENAF (*HIBISCUS CANNABINUS*). R. L. Schading, Dept. of Biology, Univ. of Texas Pan-American, Edinburg, TX 78439 and B. A. Mullin, USDA-Agricultural Research Service, Weslaco, TX 78596.

Sordaria guayuleae, a newly described four-spored ascomycete isolated from stem lesions on guayule (*Parathenium argentatum*) from the semi-arid region of South Texas was tested for pathogenicity to kenaf (*Hibiscus cannabinus*), another alternative crop being tested in the region. Three mycelial plugs cut from vigorous cultures of *S. guayuleae* were placed near the roots of three-day-old kenaf seedlings transplanted into culture tubes filled with sterile vermiculite and moistened with .5 strength Gamborg's B5 nutrient solution. Inoculated and control seedlings were evaluated for disease severity every other day for two weeks and rated from 1-9 for presence of stem and root lesions (1 = free from symptoms, 9 = dead). Several inoculated seedlings died and severity ratings ranged from 6 to 8 for the remaining inoculated seedlings. Growth was significantly reduced in the treated plants, with height only 50-65% of control plants. *S. guayuleae* was reisolated from all diseased, inoculated plants, satisfying Koch's postulates for proof of pathogenicity.

A405

REACTIONS OF KENAF GERMPLASM TO RHIZOCTONIA SOLANI, SCLEROTIUM ROLFSII AND MACROPHOMINA PHASEOLINA. B. A. Mullin and C. G. Cook, USDA-Agricultural Research Service, Weslaco, TX, 78596, and R. L. Schading, Biology Dept., University of Texas-Pan American, Edinburg, TX 78539.

Eleven cvs. and selections of kenaf (*Hibiscus cannabinus*) and one of roselle (*H. sabdariffa* cv. Nohnsung 2) were evaluated for reaction to *Rhizoctonia solani* (RS) in the seedling stage using a mixture of infested soil and potatoes as inoculum. Plants were evaluated after 5 and 10 days. Kenaf germplasm included: Everglades 71, Everglades 41, Cubano, Tainung 1, Tainung 2, 15-2, 45-9, 19-117-2, 78-18-RS10, C108, and 7N. All germplasm tested were completely susceptible and failed to emerge when inoculated with RS, whereas control plants emerged in 3 d. In another trial, culture tubes filled with vermiculite were used to assess reaction of 4 kenaf lines to *Sclerotium rolfsii* (SR). Cubano, Tainung 1, BG 53 58-1-907, and 15-2 were all susceptible and died within 2 wk of inoculation with sclerotia of SR. Inoculation techniques using infested soil, toothpicks or mycelial plugs for assessing kenaf reaction to *Macrophomina phaseolina* in the seedling stage were compared.

A406

EVALUATION OF ADVANCED GEORGIA PEANUT BREEDING LINES FOR RESISTANCE TO LATE LEAF SPOT. F.D. Smith, and T.B. Brenneman, Dept. of Plant Pathology; and W.D. Branch, Dept. of Crop & Soil Sciences, University of Georgia, Coastal Plain Experiment Station, Tifton, GA 31793-0748.

Pod yield and resistance to late leaf spot (*Cercosporium personatum*) of 10 advanced Georgia breeding lines and four peanut (*Arachis hypogaea*) cultivars were evaluated during 1987-1988. Peanuts were managed under three leaf spot programs using diniconazole (Spotless 25W) at 0.14 kg/ha: 1) untreated, 2) 28-day, and 3) 14-day spray schedule. Final disease ratings (1 to 10 Fla. scale) were made prior to harvest during the first wk in October. In untreated plots, T-2640 and T-2636 yielded 971 and 807 kg/ha more peanuts than Florunner. T-2640 had a 6% lower disease rating whereas T-2636 did not. Southern Runner yielded only 516 kg/ha more but had a 21% lower rating. In plots treated every 28 days, T-2636 and Southern Runner yielded 1,397 and 616 kg/ha more and had a 6% and 12% lower disease rating than Florunner. In absence of disease pressure provided by the 14-day spray schedule, T-2636, Sunrunner, and Florunner produced yields of 6,314, 6,232, and 5,249 kg/ha, respectively. T-2636, released as Georgia Runner in 1990, appears to have tolerance to late leaf spot.

A408

STATUS OF SOYBEAN PLANT INTRODUCTION 437.654 AS A HOST OF *MELOIDOGYNE HAPLA*. T. L. Niblack and M. J. Kennedy, Department of Plant Pathology, 108 Waters Hall, University of Missouri, Columbia MO 65211.

Cultivars derived from soybean plant introduction (PI) 437.654 are expected to be widely deployed. The purpose of this study was to determine the status of PI 437.654 and the cultivar Hartwig (selected from a PI 437.654 x Forrest cross) as a host for *Meloidogyne hapla*, a root-knot nematode widespread in the North Central U. S. Seedlings of PI 437.654, Forrest, Hartwig, and V85-41 (susceptible check) were transplanted into sterilized field sand infested with *M. hapla* at either 1,000 or 10,000 eggs/plant. Sequential experiments were maintained in water baths at 27 C for ca. 50 days. The effects of cultivar, inoculum level, and cultivar x inoculum level interaction on number of *M. hapla* eggs/plant, plant height, fresh and dry shoot weight, and dry root weight were significant ($P < 0.05$) for all responses but one: no interaction effect was detected for dry shoot weight. PI 437.654 was a highly efficient host of *M. hapla*, similar to V85-41.

A409

***IN VITRO* COLONY GROWTH AND MICROSCLEROTIA PRODUCTION BY *MACROPHOMINA PHASEOLINA* AS INFLUENCED BY PRE-EMERGENCE HERBICIDES FOR GRAIN SORGHUM.** J.S. Russin, C.H. Carter, and J.L. Griffin. Department of Plant Pathology and Crop Physiology, Louisiana State University Agricultural Center, Baton Rouge.

Isolates of *Macrophomina phaseolina* from grain sorghum roots were grown (48 h, 30°C, dark) on potato dextrose agar alone or amended with atrazine or metolachlor (33, 66, 132 µl/l) or alachlor (83, 66, 332 µl/l) as formulated products. These corresponded to 0.5x, 1x, and 2x recommended rates for grain sorghum in Louisiana. Atrazine reduced colony diameter slightly, but only at the 2x rate. Colony diameter was reduced to a greater degree by alachlor at all rates but was reduced by metolachlor at 1x and 2x only. Significant interactions between herbicides showed an antagonistic relationship between atrazine and metolachlor and a synergistic relationship between atrazine and alachlor regarding effects on colony diameter. In a second study, microsclerotia were produced on cellophane-covered water agar (2%) amended with these herbicides at all rates and were counted using an Image I computer scanning system. Microsclerotia production was increased by all three herbicides. Significant interactions between herbicides, however, showed that atrazine at all rates mitigated the effects of metolachlor and alachlor to increase production of microsclerotia *in vitro*.

A410

SEED TRANSMISSION OF CYLINDROCLADIUM CROTALARIAE IN PEANUT (*ARACHIS HYPOGEA L.*). B.L. Randall-Schadel, J.E. Bailey, and M.K. Beute, North Carolina Department of Agriculture Seed Section and Department of Plant Pathology, North Carolina State University, Box 7616, Raleigh, NC 27695-7616.

Cinnamon-brown speckled seed coats are associated with *Cylindrocladium crotalariae (Cc)* in peanut seed. Tests were established in North Carolina fields with no history of peanut. Nonspeckled (NC-V11 in 1992, NC 7 in 1993) and speckled seed (NC 7, NC 10C) of normal shape, size and appearance for seed peanut, were planted. Isolations prior to planting yielded 0% colonization in nonspeckled seed. Percentages from speckled seed were 15 and 10 for NC 10C and NC 7 (1992), and 8 and 10 for NC 10C and NC 7, respectively (1993). Half of the seed were coated with commercial seed treatment and 100 seed were planted for each treatment-seed lot combination with four replications. In 1992, one plant from each speckled seed lot (NC 7 and NC 10C) had disease (transmission rate of 0.25%). No other plants became diseased. As of June 4, 1993, no disease had been detected in plants from nonspeckled seed. Speckled seed of NC 10C had transmission rates of 4.75% and 0.25% from nontreated and treated seed, respectively. Speckled seed from NC 7 had transmission rates of 1.5% and 0.75% from nontreated and treated seed, respectively.

A412

RESPONSE OF ALMOND TREES ON PEACH AND PLUM ROOTSTOCKS GRAFT-INOCULATED WITH TWO MLO BIOTYPES INFECTING PRUNUS SPECIES. J. K. Uyemoto, USDA-ARS, Department of Plant Pathology, Univ. of California, Davis, CA 95616.

Almond scions (Prunus dulcis cvs. Carmel, Peerless, and Price) on plum rootstock, Marianna 2624 (P. cerasifera x P. munsoniana) and Peerless almond on peach seedlings (P. persica cv. Nemaguard) were graft-inoculated with healthy peach and peach with peach yellow leafroll MLO (PYLR-MLO) or X-disease MLO (X-MLO). Budchips were inserted into the almond scions or plum rootstock. In a year, all almond/plum trees that were scion-inoculated, but none of the root-inoculated, with PYLR-MLO exhibited rapid decline and necrotic bark and a pitted woody cylinder at the scion/rootstock junction; trees died. These results suggest that Marianna 2624 is resistant to PYLR-MLO. Trees of the same scion/rootstock combination graft-inoculated with X-MLO appeared healthy during two years of observation. In contrast, symptoms on trees of Peerless/peach incited by PYLR-MLO consisted of an off-green to yellow, small-sized leaves, shortened current season shoots and, at harvest, unfilled and flattened almond kernels; trees did not die. When infected by X-MLO, the canopy was slightly yellow but shoot and kernel development remained normal. As both MLOs cause severe dieback in peach, chronically infected almond/peach trees may decline as they reach full maturity.

A413

TIME OF PEACH FRUIT INFECTION BY *XANTHOMONAS CAMPESTRIS* PV. *PRUNI*. D. F. Ritchie, Dept. Plant Pathology, N.C. State Univ., Raleigh 27695-7616.

Bacterial spot of peach affects leaves, twigs and fruit, and fruit infection can result in significant loss. Incidence and severity of fruit infection varies yearly, and does not necessarily correlate with leaf infection. Based on weather records and occurrence of diseased fruit since 1984, rainfall from late bloom to approximately 3 wk after petalfall appears critical for severe infection of fruit. Fruit symptoms increased rapidly during a 2-3 wk period 5-8 wk after petalfall, followed by little or no increase after this period. Applications of bactericides during a 4-wk period before and after petalfall resulted in significantly less diseased fruit than on the nontreated control. These results suggest there is a period of a few weeks during which peach fruit are very susceptible to infection by *X. c.* pv. *pruni.*

A414

IN VITRO FUNGICIDE EVALUATION AGAINST COLLETOTRICHUM ACUTATUM FROM STRAWBERRY. J.A. LaMondia, CT Agricultural Experiment Station, P.O. Box 248, Windsor, CT 06095.

The effects of benomyl, captan, chlorothalonil, thiram and vinclozolin on conidial germination and colony growth of five Connecticut isolates of C. acutatum were measured in vitro. Captan, thiram, and chlorothalonil-amended disks containing 100, 100 and 500 ug ai/ml fungicide, respectively, resulted in conidial germination inhibition zones for all isolates. Benomyl did not inhibit germination. All fungicides tested reduced colony diameters when C. acutatum was grown on fungicide-amended PDA. Concentrations of 1 ug ai/ml or greater for benomyl, 100 ug ai/ml or greater for captan, thiram and vinclozolin, and greater than 1000 ug ai/ml chlorothalonil reduced colony diameters by 50%. When compared to C. acutatum isolates previously defined as resistant to benomyl, Connecticut isolates ranged from susceptible to resistant. Contact with up to 10,000 ug ai/ml benomyl for 3 days did not kill C. acutatum conidia, which continued growth when transferred to unamended media.

A415

REMOVAL OF TRIPHENYLTIN HYDROXIDE FROM PECAN SEEDLINGS BY SIMULATED RAIN. K. L. Reynolds[1], C. C. Reilly[2], M. Hotchkiss[1,2], and F. F. Hendrix[1], [1]Dept. of Plant Pathology, University of Georgia, Athens, GA 30602, and [2]USDA-ARS SE Fruit and Tree Nut Research Lab., Byron, GA 31008.

A rainfall simulator was used to examine the influence of a synthetic latex spray adjuvant and pecan cultivar on removal of the fungicide triphenyltin hydroxide (TPTH) from pecan foliage. Pecan seedlings were sprayed with TPTH with or without adjuvant, allowed to dry, and exposed to 0 to 5.1 cm of simulated rain. The initial tin concentration on sprayed leaves was normally distributed with greater variation on leaves sprayed with TPTH alone. In most cases fungicide was removed at a constant rate with increasing rainfall. Addition of the adjuvant resulted in significantly greater fungicide tenacity after relatively light rains of 2.5 cm or less, although after 5.1 cm there was no significant difference between fungicide residues on leaves treated with or without adjuvant. The rate of removal of tin from foliage of 13 pecan cultivars after 5.1 cm rain ranged from 0.22 to 0.35 μg tin/cm leaf area/cm rain. The rate of removal was significantly lower in cultivars Moneymaker and Cheyenne than cultivars Mahan and Stuart. Differences in leaf surface characteristics among pecan cultivars have been reported and may be responsible for observed differences in fungicide tenacity.

A416

EVALUATION OF CULTURAL CONTROL STRATEGIES FOR MANAGING BLACK ROT (*GUIGNARDIA BIDWELLII*) OF GRAPES C.M. Becker, and R.C. Pearson, Dept. Plant Pathology, Cornell Univ., NYSAES, Geneva, NY 14456.

Four ground cover management strategies were evaluated in Geneva, NY, for their influence on the release of ascospores of *Guignardia bidwellii* (Ellis) Viala and Ravaz from overwintered mummified berries. During rain events, rotorod spore traps detected significantly fewer ascospores (often none) when mummies were covered with straw mulch or cultivated, compared with mummies on bare ground or grass. Another experiment involved scorching mummies on the ground with a propane-fired weed-burner; however, this did not significantly reduce the numbers of ascospores released from mummies. A third experiment involved removing mummies that were retained within hedged canopies, and dropping them to the ground. Mummy removal significantly reduced the severity of black rot on clusters from 40.4% to 15.4% and 70.4 to 32.9% in 1991 (a dry year), and from 76.5% to 68.9% during 1992 (a wet year), compared to no removal. In the presence of a season-long fungicide program, black rot on clusters was less than 2% in all treatments. Our results indicate that cultural practices may reduce the release of inoculum or the severity of black rot, but fungicide applications during initial inoculum release appear to provide the best control.

A417

EPIDEMIOLOGY OF MUSCADINE GRAPE (*VITIS ROTUNDIFOLIA*) DISEASES IN MISSISSIPPI. N. Kummuang, S.V. Diehl, B.J. Smith, and C.H. Graves, Jr., Mississippi State University, Miss. State, MS 39762, and USDA-ARS, Poplarville, MS 39470.

Fruit rots cause severe losses in muscadine grapes in Mississippi. Epidemiological studies were conducted on 4 cultivars (Doreen, Sterling, Carlos, and Cowart) at 3 locations in southern MS. Muscadines were examined at 2 wk intervals during the 1991 and 1992 growing seasons. Four diseases: black rot (*Guignardia bidwellii f. muscadinii*), bitter rot (*Greeneria uvicola*), Macrophoma rot (*Botryosphaeria dothidia*), and ripe rot (*Colletotrichum sp.*) were present at all locations each year. Bitter rot was the most important disease, followed closely by black rot. Incidence of Macrophoma rot and ripe rot was relatively low. On berries, bitter rot was most severe when berries reached 2-3 mm in size and resulted in a berry drop. Black rot was most severe as the berries began to mature. On leaves, bitter rot was most prevalent on Sterling and was most prominent at Crystal Springs. The greatest severity of bitter rot on leaves occurred following bud break and continued until shoots were up to 12 cm long.

A419

PERFORMANCE OF A PORTABLE SOIL DRENCHING DEVICE TO CONTROL REPLANT PROBLEMS. Michael V. McKenry, 9240 South Riverbend Avenue, Parlier, CA 93648.

A portable soil drenching device was used to apply Metham Sodium (MS) at 732 kg ai/ha rate to peach replant sites, each being 3 m by 100 m in size. Plots were compared to a shanked 336 kg/ha Methyl bromide (MB) and a nontreated check. Plots treated with MS exhibited dead peach roots and *Pratylenchus vulnus* throughout the surface 1.5 m of soil. MB treatments gave kill of old roots and nematodes throughout the surface 1.8 m of soil. Plant growth at 3 months after replanting was notably impaired in the nontreated check. After 5 months the growth and color of replants in sites receiving chemical treatments was identical. Weed control was best following the MS treatment. The MS at 366 kg ai/ha killed nematodes to the 1.5 m depth but live roots were found below 0.6 m depth. MS can be delivered uniformly and with consistency via a portable soil drenching device.

A420

Occurrence of Citrus Tristeza Virus in Commercial Citrus Nurseries and Orchards in the Lower Rio Grande Valley. *Mani Skaria*, Nora Solis-Gracia, and Miao Hongqin, Texas A&I University Citrus Center, Weslaco, TX 78599.

Recent surveys based on ELISA, show that CTV is present in both commercial nurseries and orchards in Texas. In 1991, 22 out of 298 samples from commercial orchards tested CTV positive. In 1992, 46 out of 529 samples were CTV positive in commercial nurseries. In 1993, in two out of 16 locations, CTV detected in trees selected for budding. Also, 9 out of 443 samples of commercial oranges tested CTV positive. To date, none of the CTV positives were a severe strain, based on ELISA and/or virus inclusion body data. The northward movement of the efficient vector aphid, *Toxoptera citricida* from the Central America is an added threat.

A421

EFFECT OF SOIL pH ON Al CONTENT AND ON GROWTH OF SWEET CHERRY TREES AND SEEDLINGS. H. Melakeberhan, G. W. Bird, Department of Entomology, and A. L. Jones, Department of Botany and Plant Pathology, Michigan State University, East Lansing, MI 48824.

The relationship between soil pH and root Al levels in a 16-yr-old declining sweet cherry orchard, and the interaction effect of pH (3.9, 4.7 and 7.0) and 0 to 27 meq Al (applied as aluminum chloride) or calcium chloride (control), on growth of 1-yr-old mazzard seedlings were investigated in greenhouse for 4 wk. The availability of Al incresead below 5.5 soil pH and it was absorbed proportionally ($P = 0.05$) by cherry roots. Seedling mortality was 100% at pH 3.9 with or without addition of Al, and at 0.33 and 1.33 meq Al for pHs 4.7 and 7.0, respectively. Increasing Al treatment resulted in significant ($P = 0.01$) absorption into the shoot system and 2 to 4 times more seedling mortality than Cl. The interaction effect between Al and low soil pH was highly significant ($P = 0.0001$). Calcium chloride had little effect on increasing soil Ca over Al treatments. Overall, the results prove that low soil pH is a major factor in tree decline.

A423

DETECTION OF LATENT INFECTION OF POTATO TUBERS BY PSEUDOMONAS SOLANACEARUM IN BURUNDI. L.G. Skoglund, P.O. Box 1217, Sterling, CO; S.E. Seal, NRI, Chatham Maritime UK; J.G. Elphinstone, Rothamsted Exp. Sta., Harpenden UK; D.E. Berrios, B.P. 75, Bujumbura Burundi.

Pseudomonas solanacearum E.F. Smith, Race 3, is a major constraint to production of potato in Burundi. A major effort in the control strategy has been to minimize latent infect during field multiplication of seed tuber stocks originating from basic materials multiplied in vitro. A collaborative study was undertaken to determine infection frequency in seed stocks by use of NCM-ELISA, a non-radioactive DNA probe, and polymerase chain reaction amplification with P. solanacearum-specific primers. Comparisons of the techniques were made and sampling techniques were evaluated. A preliminary study showed that all three techniques would detect the pathogen under field conditions. Detailed studies indicated that latent infection is highly variable by cultivar, year and season.

A424

Use of Virus Inclusion Bodies for the Detection of Citrus Tristeza Virus in Texas. *Miao Hongqin* and Mani Skaria, Texas A&I University Citrus Center, P.B. 1150, Weslaco, TX 78599.

We attempted the use of virus inclusion bodies as an additional diagnostic tool for the detection of CTV in Texas. Out of 84 petiole sections scanned from a Mexican lime infected with a reference severe strain of CTV, 40 sections showed inclusion bodies. From two mild strain CTV plants, only 2/49 or 2/22 sections, respectively showed inclusion bodies. Twenty one other mild CTVs from field samples showed none or a maximum of 2 inclusion bodies out of 20-46 sections /plant. We are comparing the development of inclusion bodies in Mexican lime plants inoculated with mild or severe CTV. Four weeks after inoculation, only the severe strain showed presence of inclusion bodies.

A425

A RAPID IMMUNOASSAY METHOD FOR QUANTIFYING MOLD IN HARVESTED GRAPES. J. J. Marois, L. H. Kenyon[1], C. D. Lamison[1], and J. P. Smith[1]. Department of Plant Pathology, University of California, Davis, CA 95616 and [1]E. I. Dupont de Nemours, Pencador Corporate Center, 200 Executive Drive, Newark, DE 19702.

A rapid immunoassay specific to Botrytis cinerea was developed to quantify the amount of mold in harvested wine grapes (Vitis vinifera L.). Over 1000 trucks were sampled with the immunoassay and the present visual assay during the 1991 and 1992 harvests from inspection stations in the Central Coast, Livingston, Woodbridge, Ripon and North Coast wine grape production areas in California. The Pearson correlation coefficient (R) of the visual assay and immunoassays was 0.99 and 0.97 for 1991 and 1992, respectively. Analysis of variance of the similarity of the two assays indicated that it was significantly affected by method of harvest (machine or hand) ($P < 0.01$), but not significantly affected by color, site, variety, or time of harvest. The R for hand harvested white and black grapes was 0.72 and 0.73, respectively and 0.73 for machine harvested white grapes. The lowest correlation of the two assays was with machine harvested black grapes (R = 0.23), probably due to the difficulty of detecting diseased berries in machine harvested black grapes. Frequency distribution analyses indicated that the visual assay agreed closely with the immunoassay, usually resulting in a variance to mean ratio of less than one.

A426

RAPID AND SIMPLE PREPARATION OF SAMPLES FOR PCR FROM TISSUE CONTAINING VIRUSES, VIROIDS, AND MLO's. L. Levy and A. Hadidi. USDA-ARS, NGRL, Beltsville, MD 20705.

Previously, nucleic acid sample preparation for PCR analysis required any or all of the following: phenol extraction, column chromatography, methoxy-ethanol treatment, proteinase K digestion, and CTAB or ethanol precipitation. These procedures have complicated the application of PCR to pathogen surveys, germplasm evaluation, epidemiology studies, and evaluation of host-pathogen interactions. With the use of GeneReleaser™ matrix we have successfully prepared samples for PCR analysis from recalcitrant plant tissues. The method requires grinding leaf or midrib tissue in buffer, vortexing 1-2μl of sap with the matrix followed by microwave heating. This preparation can be directly, used without any of the above treatments, for cDNA and PCR for RNA viruses and viroids or for PCR with DNA viruses or MLO's. We have amplified apple scar skin viroid (330 bp) from apple leaf and anthers, potato spindle tuber viroid (359 bp) from potato meristem cultures, and fragments of plum pox potyvirus (220 bp) from prunus, grapevine clostero-virus B (450 bp) and grapevine leaf-roll virus (340 bp) from grape cultivars, and elm yellows MLO (1.1 Kb from rRNA, I-M. Lee) from periwinkle (EY1) and chinese elm (EY1A). This method of sample preparation is simple, inexpensive, rapid and should facilitate the application of PCR technology to large scale studies.

A427

IMPROVED NON-RADIOACTIVE DETECTION OF BYDV-RNA. Antonia R. Figueira[1], L.L. Domier[2] and C. J. D'Arcy[2]. [1]ESAL/Dep. de Fitossanidade. cp 37, 37200000-Lavras, MG. Brazil and [2]Dept. of Plant Pathology, Univ. of Illinois, 1102 S. Goodwin Ave., Urbana, IL, 61801.

The application of non-radioactive viral RNA detection methods can be limited by non-specific hybridization of probes with healthy plant samples and by the length of the protocol. Non-specific background in a BYDV-PAV-IL-RNA detection system was eliminated by homogenization of oat samples in PBS containing 1-3 mg/ml of magnesium-bentonite prepared by the method of Dunn and Hitchborn (Virology 25:171-192, 1965). Extracts were stirred for 5-10 minutes at 22C and centrifuged for 10 minutes at 11000g. The assay was shortened by changing the prehybridization time from 2h to 30 min and by eliminating three of the wash steps. With these modifications the sensitivity and speed of non-radioactive assay were increased, making the technique better suited for large scale applications.

A428

DEVELOPMENT OF MONOCLONAL ANTIBODIES TO CITRUS TRISTEZA VIRUS. M. E. Hooker[1], C. A. Powell[1], R. F. Lee[2], R. H. Brlansky[2], and S. H. Garnsey[3], [1]University of Florida, IFAS, Box 248, Ft. Pierce 34954, [2]University of Florida, IFAS, Lake Alfred 33850, and [3]USDA, ARS, Orlando, Florida 32803.

Citrus tristeza virus (CTV) isolates are highly variable in symptom expression. General and strain selective probes are needed. Monoclonal antibodies were developed that react differently to a selected panel of domestic and exotic CTV isolates which vary in symptom severity. Balb/c mice were immunized with a mixture of two severe CTV isolates, one foreign and one domestic. One cell line, 17G11, reacted with all domestic and nearly all foreign isolates tested. Three other lines, 18H9, 17H9, and 17A4, also

had broad spectrum reactivity. Cell lines 16G11, 2F7, and 1D3 reacted with all severe domestic and most severe exotic CTV isolates tested, but not with domestic mild isolates. Several of the new monoclonals formed, recognize epitopes different from those detected by previously described CTV monoclonals.

A429

A COMBINED FILTER-CENTRIFUGE METHOD FOR EXTRACTION OF TELIOSPORES OF TILLETIA INDICA FROM NATURALLY CONTAMINATED WHEAT SEEDS. C. Castro[1], N. W. Schaad[2], and M. R. Bonde[2]. [1]EMBRAPA, Brasilia, Brazil, and [2]USDA/ARS, Frederick, MD 21702, U.S.A.

A membrane filter and centrifugation (FC) technique was developed for extraction of teliospores of T. indica from wheat seeds. Samples containing 1,000 seeds were shaken 1 min in 200 ml 0.001% Tween 20. The seeds and debris were removed by filtering through a 60-µm pore Millipore membrane and spores collected on a 12-µm pore membrane. The spores were washed from the membrane and centrifuged for 30 sec at 180 x g. Samples of washed and dried seeds to which five teliospores had been added resulted in a positive recovery 100% of the time. A single spore could be recovered from similar samples 25% of the time. Using a naturally contaminated seed lot to compare the new FC method to the ISTA method (Int. Seed Testing Assoc. Handbook on Seed Health Testing, Working Sheet No. 53, 1981) developed for T. controversa gave a mean recovery of 19 by FC and none by the ISTA method. Only when seed was diluted 3X with "clean" seed could teliospores be detected by the ISTA method. The results show that teliospores can be efficiently extracted relatively free of plant debris from wheat seed samples. The FC method should prove useful for extraction of spores for identification by molecular techniques such as PCR.

A430

OCCURRENCES OF *ACREMONIUM* AND NON-*ACREMONIUM* ENDOPHYTES IN SEEDS OF *FESTUCA* AND *LOLIUM* GRASS SPECIES. M. R. Siegel, C. L. Schardl and T. D. Phillips. Plant Pathology Dept., University of Kentucky, Lexington, KY 40546.

The distributions of *Acremonium* (*e*) and non-*Acremonium* (*p*) endophytes were determined in seed from accessions of *Festuca* and *Lolium* grass species in the national plant germplasm collection . Ten seeds of each accession were tested, by tissue print immunoblot assay, for the presence of *e*-endophyte or *p*-endophyte in separate seeds, or for both (*e* + *p*) co-infecting individual seeds. A total of 366 accessions were tested from 36 *Festuca* species and 5 *Lolium* species. The presence of *e*-endophytes was indicated in 24 of the *Festuca* species and in all of the *Lolium* species; while the *p*-endophytes were observed in 8 and 2 species, respectively. The occurrence of *p*-endophytes in accessions of each species was almost always low. For example, among the 72 accessions of *F. arundinacea* 68% had *e*, 4% had *p* and, of these, 1% had *e* + *p*. Among 74 *L. perenne* accessions the distribution was 46% *e*, 3% *p*, and 3% *e* + *p*. This survey did not indicate a general pattern of association between the endophyte types. The infrequent occurrence of *p*-endophytes, in contrast to the *e*-endophytes, indicates that they are probably not ecologically important mutualists.

A431 Withdrawn

A432

ALTERATION OF THE SPHINGOLIPID CONTENT IN DUCKWEED (LEMNA PAUSICOSTATA L.) AND TOMATO VARIETIES BY FUMONISIN B_1 AND AAL-TOXIN. H. K. Abbas[1], T. Tanaka[1], S. O. Duke[1], and R. T. Riley[2], SWSL, USDA-ARS, Stoneville, MS[1]; TMRU, USDA-ARS, Athens, GA[2].

Fumonisin B_1 [FB_1] and AAL-toxin have similar biological activities and have a broad range of phytotoxicity on weed and crop species. Symptoms caused by these toxins on intact plants were correlated with those seen at cellular levels. In duckweed, FB_1 and AAL-toxin at 1 µM caused cellular leakage, chlorophyll loss, and growth inhibition within 72 hr. Phytosphingosine at 50 µM caused similar effects. The toxins also caused the accumulation of free sphingoid bases, tentatively identified as phytosphingosine and sphinganine, after 24 hr of treatment. Surface application of these toxins caused accumulation of the same free sphingoid bases in 1-month-old, intact tomato plants. The toxicity of the fumonisins in animals is closely correlated with disruption of sphingolipid metabolism. Our results indicate that these toxins may have a similar mechanisms of action in plants as in animals.

A433

PEROXIDASE AND CHITINASE AS THEY RELATE TO SUSCEPTIBILITY, RESISTANCE AND INDUCED SYSTEMIC RESISTANCE OF CUCUMBER TO COLLETOTRICHUM LAGENARIUM AND CLADOSPORIUM CUCUMERINUM

Chengsong Xie, Joseph, A. Kuc (1992). Department of Plant Pathology, College of Agriculture, University of Kentucky, Lexington, KY 40546

Four cucumber cultivars, Marketer, Pik Rite, Sweet Slice and Shamrock II, differing in their disease reactions to Colletotrichum lagenarium and Cladosporium cucumerinum, were used in this study. Inoculation of the first true leaf(leaf 1) of the 4 cultivars(induction) resulted in protection of leaf 2 from disease caused by both pathogens. The induced systemic resistance was consistently expressed as a reduction in lesion number and size. The constitutive levels of peroxidase and chitinase activities in leaf 1 and 2 of noninduced plants were not markers for resistance to the pathogens. Activities of peroxidase and chitinase in leaf 1 and leaf 2 of noninduced plants 3-4 days after inoculation also were not markers for resistance to C.lagenarium. Peroxidase and chitinase activities increased in leaf 2 of the 4 induced cultivars, but the level of activity was not correlated with resistance to both pathogens. The levels of peroxidase and chitinase in leaf 2 of induced plants at different time intervals after challenge also were not correlated with resistance to the pathogens. Analyses of isozyme patterns of peroxidase and chitinase indicated that no unique isozymes were associated with either noninduced or induced resistance. These data provide evidence that the activities of peroxidase and chitinase and their isozyme patterns are not markers for resistance or susceptibility and induced systemic resistance. It is unlikely that these enzymes have a direct role in restricting development of C. lagenarium and C. cucumerinum in resistant plants and plants in which resistance has been systemically induced.

A434

THE IMPORTANCE OF BACTERIAL GROWTH PHASE FOR IN PLANTA PATHOGENICITY TESTING. Martin G. Klotz, Biology Dept. and Graduate Program in Molecular Biology, Utah State Univ., Logan, Utah 84322-5305.

Phytopathogenic strains of Pseudomonas syringae have different physiological properties in different growth phases. Screening of several strains at the cellular level revealed that the sensitivity to oxidative stress, heat and selected antibiotics of P. syringae is correlated with the growth phase, properties of enzymes such as catalase, and is affected by the composition of the nutrient environment. The interrelation of responses to different stresses allows the conclusion that coordinated stress response regulation occurs in some strains of P. syringae. Comparison to other fluorescent pseudomonads suggests that this strain specificity is related to the conditions in the natural habitat where the strain has co-evolved with its host plant. This is important for studies that involve plant-bacteria associations where the microbes have to cope with complex stress situations. While the induction of responses to oxidative stress in E. coli occurs at the level of transcription, oxidative stress response in P. syringae seems to be regulated posttranslationally.

A435

TRANSFORMATION OF COLLETOTRICHUM GLOEOSPORIOIDES F.SP. AESCHYNOMENE WITH THE BIALAPHOS RESISTANCE GENE. N.L. Brooker, J. Lydon, and C.F. Mischke. Weed Science Laboratory, BARC-West, ARS, USDA, Beltsville, MD. 20705

Historically, effectiveness of mycoherbicides has been limited by low virulence and narrow host range. One approach to enhancing these characteristics is to co-apply the mycoherbicide with a natural herbicide. *C.gloeosporioides* f.sp. *aeschynomene* (*C.g.a.*) was transformed with pJA4, a fungal expression vector containing the *bar* gene. The *bar* gene codes for an acetyltransferase which can detoxify bialaphos, a natural herbicide. Five *C.g.a.*transformants displayed stable homologous integration of the *bar* gene and the bialaphos-resistant phenotype. Transformants also displayed increased acetyltransferase activity when assayed using acetyl-CoA and glufosinate ammonium as substrates. All transformants retained their virulence on northern jointvetch, and the stability of the *bar* gene integration event in plant-reisolated transformants is currently being determined. Northern jointvetch plants and closely related weed species are being tested with *C.g.a.* transformants and the wild-type, with and without co-applications of bialaphos, to determine the effects on virulence and pathogenicity.

A436

RIBONUCLEASE ACTIVITY IN TOBACCO PLANTS INOCULATED WITH TMV. M. Lusso and J. Kuc, Department of Plant Pathology, University of Kentucky, Lexington, KY 40546, USA.

Ribonuclease activity was measured in tobacco plants (cv. KY-14, N-gene), leaves inoculated with TMV. Control plants were treated with water. Within 3 days of inoculation, a marked increase in ribonuclease activity was observed in the inoculated leaves, and it continued to increase over the next 18 days. Leaves treated with water did not show increases in enzyme activity during this period. Twelve days after inoculation with TMV or treatment with water, leaves above those treated were inoculated with Peronospora tabacina or treated with water. The ribonuclease activity in the leaves inoculated with P. tabacina increased in both treatments, but activity increased more rapidly in the plants that were previously inoculated with TMV than in the plants that were previously treated with water. At 7 days after inoculation this difference was 3 fold. Changes in enzyme activity were not observed in the leaves treated with water. The possible significance of increased ribonuclease and protease activities to induced systemic resistance will be discussed.

A437

ISOLATED MITOCHONDRIA FROM TWO TEXAS MALE STERILE CYTOPLASM INBREDS DIFFER IN THEIR SENSITIVITY TO BMT-TOXIN AND HAVE DIFFERENT RAPD MARKERS. M. O. Garraway[1], T. T. Van

Toai[2], and J. H. Zhang[2], [1]Dept. of Plant Pathology, The Ohio State Univ., and [2]USDA-ARS, Soil Drainage Research Unit, Columbus, OH 43210.

Leaves of the maize inbred B37 in Texas male sterile (T) cytoplasm leaked significantly more electrolytes than did leaves of the T-cytoplasmic Oh43 inbred after they were infiltrated with Bipolaris maydis race T toxin (BmT-toxin) for 24 hr. This differential response to toxin was reversed when these inbreds contained nuclear genes for restoration of fertility (TRf). BmT-toxin was more stimulatory to the respiration of mitochondria isolated from roots of the T-cytoplasmic B37 inbred than to those from the T-cytoplasmic Oh43 inbred, as measured by the relative rates of 2,6-dichlorophenol-indophenol reduction. The mitochondrial DNA from the T-cytoplasmic B37 inbred produced polymorphisms that were distinguishable from those of the T-cytoplasmic Oh43 inbred, as determined by the random amplified polymorphic DNA (RAPD) assay. Also, distinctive polymorphisms were observed in T cytoplasmic mitochondrial DNA when these inbreds contained the TRf genes. Therefore, genetic changes in T-mitochondria may be induced by nuclear genes. Some of these changes could cause differences in the sensitivity to BmT-toxin observed between T-cytomplasmic B37 and Oh43 maize inbreds.

A438

ISOLATION AND CHARACTERIZATION OF CERCOSPORIN-SENSITIVE MUTANTS OF *CERCOSPORA NICOTIANAE*. A.E. Jenns and M.E. Daub. Plant Pathology Dept., North Carolina State University, Raleigh 27695-7616.

Seven mutants of *Cercospora nicotianae* sensitive to the light-activated toxin cercosporin were selected by replica plating of colonies regenerated from UV-irradiated protoplasts on media with and without cercosporin. Exogenous levels of cercosporin ranging from 1-10 μM completely inhibited growth of six of the mutants under high light intensity. One was not completely inhibited by 10 μM cercosporin. All seven mutants sporulated normally and synthesized cercosporin under low light. Mutants were analyzed to test the hypothesis that cercosporin reduction is involved in the defense of *Cercospora* species against the toxin. The six highly sensitive mutants were incapable of chemically reducing cercosporin, whereas the less sensitive mutant was normal in cercosporin-reducing ability. In most cases mutants were protected against cercosporin toxicity by the reducing agents ascorbate, cysteine, and reduced glutathione. Measurement of endogenous levels of these reducing agents is in progress.

A439

A STRATEGY TO REGULATE IAA CONJUGATION IN TRANSGENIC PLANTS. J.B. Szerszen, K. Szczyglowski*, and R.S. Bandurski. Dept. of Botany and Plant Pathology, and *Plant Research Laboratory, Michigan State University, East Lansing, MI 48824.

We have isolated, cloned, and sequenced a 1.7 kb fragment from a cDNA sweet corn endosperm library which encodes the enzyme controlling the ratio of free to conjugated IAA described by the reaction: IAA + UDPG <====> IAGlu + UDP. Since free IAA, in contrast to conjugated IAA, can promote plant growth, we believe that knowledge of the sequence of the gene will allow development of methods for regulation of plant growth by controlling levels of the endogenous auxin. Several attemps have been made to generate transgenic plants overproducing IAA due to expression of the *iaaM* and *iaaH* genes isolated from *Agrobacterium tumefaciens*. However in that case excess IAA was immediately conjugated by the IAGlu synthase, thus making IAA inactive. Our strategy for the first time employs a higher plant gene (from *Zea mays*) and not bacterial genes to manipulate levels of IAA by altering the ability of the plant cell to conjugate free IAA. Possible applications of this new technique in preventing expression of certain plant diseases will be disscused. [Supported by NSF, Integrative Plant Biology, grant 61-2060].

A440

PURIFICATION AND IDENTIFICATION OF THE ACTIVE COMPONENT OF A PAPILLA-REGULATING EXTRACT (PRE) FROM BARLEY. S. Inoue[1], V. Macko[2], and J. R. Aist[1], [1]Department of Plant Pathology and [2]Boyce Thompson Institute, Cornell University, Ithaca, NY 14853.

The active compound in the papilla-regulating extract (PRE) that has been reported to induce both oversize papilla formation and resistance to barley powdery mildew was purified and identified in this study. The crude extract obtained from barley leaves by autoclaving was first passed through a C18 reverse-phase column. Next, the active fractions were loaded onto a gel permeation chromatography column packed with Sephadex LH-20 in 50% methanol, and then chromatographed on a Bio-gel P-2 column in water. Although xylonic acid was a major organic component in the active fractions from the P-2 column, it was not active by itself. After re-chromatography, the active compound was identified as potassium phosphate by Inductively Coupled Argon Plasma Atomic Emission Spectrometry and ion chromatography. Also, the effects of the PRE were found to be Ca^{2+}-mediated.

A441

IDENTIFICATION OF A cDNA INDUCED DURING A HYPERSENSITIVE REACTION WITH CALCIUM-BINDING DOMAINS. P. B. Lindgren, J. A. Smith, X. P. Sun, and J. L. Jakobek, Department of Plant Pathology, North Carolina State University, Raleigh, N.C., 27695

A cDNA library was constructed using poly(A^+) mRNA isolated from bean tissue undergoing a hypersensitive reaction (HR) in response to infiltration with the incompatible bacterium Pseudomonas syringae pv. tabaci Pt11528. Cold-plaque screening was used to identify three novel cDNA clones which were expressed in bean during the HR. Preliminary RNA blot analysis indicated that the transcript corresponding to one of these clones, designated pPm32, did not accumulate in bean plants infiltrated with the P. s. pv. tabaci Hrp$^-$ mutant Pt11528::Hrp1, or in plants infiltrated with Pt11528 cells treated with inhibitors of bacterial protein synthesis; neither treatment induces the HR. In addition, the transcript did not accumulate in plants infiltrated with the chemical elicitor glutathione or plants infiltrated with H_2O. Sequence analysis indicated that the predicted protein product of pPm32 contains two calcium binding domains.

A442

WOUND-ASSOCIATED COMPETENCY FACTORS REGULATE CELLULAR RESPONSE TO GLUCAN ELICITORS IN SOYBEAN. T. L. Graham and M. Y. Graham, Department of Plant Pathology, The Ohio State University, Columbus, OH, 43210.

Wounding of soybean tissues releases factors, which we term elicitation competency factors (CF's), which are required for the proximal cellular defense responses (phenolic polymer and glyceollin acumulation) to the glucan elicitor from *Phytophthora sojae* (PS). Washing of wounded cells removes these factors, which in turn can restore competency to washed cells in a dose dependent manner. Cells treated with the CF's are only transiently competent (3-5 hr) for glyceollin accumulation. Preliminary results suggest the presence of two CF's (CF-1 and 2) which regulate different aspects of response. Of the many factors which we have tested for CF activity, abscisic, 1-aminocyclopropane carboxylic, jasmonic, salicylic, oxalic, and traumatic acids are all inactive. On the other hand, glutathione strongly but partially restores elicitor competency and appears to mimic CF-1. We hypothesize that, in infected tissues, similar competency factors are generated during hypersensitive cell death and program the phenylpropanoid defense responses of surrounding cells to the glucan elicitor.

A443

PHENYLPROPANOID ACCUMULATION AND SYMPTOM EXPRESSION IN THE LETHAL LEAF SPOT MUTANT OF MAIZE. M. Obanni, J. Hipskind, C. Y. Tsai, R. L. Nicholson, and L. D. Dunkle*. Department of Botany and Plant Pathology, and *USDA-ARS, Purdue University, West Lafayette, IN 47907-1155.

Lethal leaf spot is a cell autonomous, developmentally programmed, recessive mutation of maize. During the early stages, symptoms in this disease mimic resemble lesions caused by some fungal pathogens. Homozygous recessive *lls1* plants die usually before pollination. We studied lesion development and compared the phenylpropanoid compounds in and around lesions with those resulting from infection of wild-type sib plants with *Cochliobolus heterostrophus*. Unlike several disease lesion mimics, the *lls* lesions did not develop distinct margins and become delimited. They enlarged at increasing rates until they encountered adjacent necrotic tissue. Two caffeic acid esters accumulated with time in both the genetically induced and pathogen-induced lesions. The data suggested that lesion termination signals proposed as factors that restrict lesion expansion are absent from the *lls* mutant and that the biochemical responses are not consistent with those associated with disease resistance.

A444

IDENTIFICATION AND TOXICITY OF PHYTOALEXINS FROM KENAF (HIBISCUS CANNABINUS L.). A. A. Bell, R. D. Stipanovic, J. Zhang, J. Reibenspies, and M. E. Mace. USDA, ARS, Southern Crops Research Laboratory, Route 5, Box 805, College Station, TX 77845.

Four phytoalexins, designated as A, B, C, and D, were purified from the stem stele of kenaf inoculated with Verticillium dahliae. Phytoalexins A, B, and C were identified as 2,8-dihydroxy-4,7-dimethoxy-6-methyl-1-naphthaldehyde, its anhydroderivative, and 3,8-dimethyl-1,2-naphthoquinone (DMNQ), respectively. Phytoalexin D was not identified. A quantitative turbidimetric bioassay was used to compare toxicity of kenaf, cotton, and elm phytoalexins against both V. dahliae and Fusarium oxysporum f. sp. vasinfectum (F.o.v.). The ED_{50} values of phytoalexin A, B, C, and D against V. dahliae were 25.83, 4.32, 1.18, and 18.01 μg/ml, respectively.

The most toxic kenaf phytoalexin, DMNQ, kills all propagules of V. dahliae and F.o.v. at 8 µg/ml and 12 µg/ml, respectively. DMNQ is more toxic to V. dahliae than desoxyhemigossypol, the most toxic phytoalexin known in cotton and mansonone C, a related phytoalexin in elm. Kenaf also is more resistant than cotton or elm to the wilt pathogen.

A445

MUTANTS OF Sclerotium rolfsii FOR USE AS MYCOHERBICIDES. R. V. Miller, M. K. McCarthy and D.C. Sands. Department of Plant Pathology, Montana State University, Bozeman, MT 59717.

Broad host-range pathogens have been avoided for biocontrol agents of weeds due to concerns over safety. An approach for using such pathogens, first described with Sclerotinia sclerotiorum, has been developed in our laboratory. It involves generating mutants with diminished potential for spread and/or survival. Here we describe the development and use of mutants of Sclerotium rolfsii as potential mycoherbicides. Auxotrophic mutants of this fungus were selected following exposure to N-methyl-N'-nitro-N-nitrosoguanidine. A total of ten virulent auxotrophs were generated, requiring uracil, thiamine, or biotin for growth. Half of the auxotrophs demonstrated virulence comparable to or better than the wild-type.

A446

AN AFFINITY CHROMATOGRAPHY METHOD TO PURIFY CATECHOL SIDEROPHORES. H. H. Barnes and C. A. Ishimaru, Department of Plant Pathology and Weed Science, Colorado State University, Fort Collins, CO, 80523.

Catechols comprise one of the broadest classes of Fe(III)ion-binding siderophores expressed by microorganisms. The catechol functional group is a co-planar cis-diol that is capable of forming a complex with boronate anions under weakly basic conditions. This reaction was utilized to develop a boronate affinity chromatography (BAC) method for the isolation and purification of catechol siderophores. The method was applied to the isolation of chrysobactin, enterobactin, and the catechol siderophore produced by Erwinia carotovora subsp. carotovora W3C105. BAC enabled simultaneous separation of catechol siderophores from hydroxamate siderophores and from rust-colored decomposition products of catechols. BAC provides a simple and rapid means of obtaining high yields of catechol siderophores.

A447

CHARACTERIZATION AND UTILITY OF A PLASMID-ENCODED POLYGALACTURONASE SEQUENCE FROM PSEUDOMONAS CEPACIA. C. F. Gonzalez and V. A. Valadez, Department of Pathology and Microbiology, Texas A&M University, College Station, TX 77843.

A plasmid-encoded endopolygalacturonase (Peh) has been cloned from pPEC320, a resident 200kb plasmid found in *Pseudomonas cepacia* strain PCO25. The limits and transcriptional organization of the structural gene were determined by Tn*3-gus* mutagenesis of a 3.5 kb (pPEC324) fragment that contains the *pehA* gene. A Peh-negative (Peh-) derivative of pPEC324 obtained by Tn*3-gus* mutagenesis, designated pPEC32428, was mobilized into strain PCO25 and three additional phytopathogenic strains of *P. cepacia* that harbor plasmids exhibiting homology to the 3.5 kb fragment. Peh- derivatives were obtained by recombinational exchange. Strains of *P. cepacia* containing the plasmids with the insertionally-inactivated *pehA* gene expressed a Peh- phenotype, were kanamycin resistant, and showed homology to Tn*5*. The Peh- derivatives were used as donors in conjugation experiments to determine transfer proficiency of the Peh-encoding plasmids. However, no evidence of conjugal transfer was observed for the strains tested. The cloned Peh-encoding sequence has been used to determine the location of homologous sequences in *P. cepacia* isolates, in site-directed mutagenesis, and for labeling of Peh-encoding plasmids resident in phytopathogenic strains of *P. cepacia*.

A449

THE ROLE OF WHEAT LEAF EPICUTICULAR WAX COMPONENTS IN LEAF RUST GERM TUBE GROWTH. J. J. Roberts, D. L. Long, R. E. Wilkinson, and G. G. Ahlstrand, USDA/ARS, Cereal Rust Laboratory, St. Paul, MN 55108, The University of Georgia, Griffin, GA 30223, and The University of Minnesota, St. Paul, MN 55108.

Urediniospores of wheat leaf rust (*Puccinia recondita* Roberge ex Desmaz), were germinated in 100 ml Warburg flasks on ^{14}C-labelled components of wheat leaf epicuticular wax. The germ tubes captured radioactive $^{14}CO_2$ indicating metabolic degradation of the wax components by the germinating sporelings. This can be blocked by treating with PCMBS, a sulfhydrl inhibitor. The ^{14}C wax component utilization was measured by the relative amounts of radioactivity transferred. This ranged from zero for alkanes to 92% for Beta diketones. Blocking the degradation with PCMBS resulted in a 27% reduction in infection. Thus, the waxes are implicated in the growth and infection process of wheat leaf rust. Since cultivars differ in composition of epicuticular wax, breeding for higher alkane/diketone ratios may decrease leaf rust infection of wheat.

A451

STIMULATION OF GERMINATION OF TELIOSPORES OF UROMYCES APPENDICULATUS BY SOME VOLATILE ESTERS AND ALDEHYDES. R.C. French, S. E. Nester, and J. R. Stavely. USDA, ARS, Frederick, MD 21702, and Beltsville, MD 20705.

Aroma compounds (106) were tested for activity in stimulating germination of teliospores of Uromyces appendiculatus, the causal agent of bean rust. In preliminary tests, teliospores were exposed briefly to the volatile emanations from 10 µl of known aroma chemicals in petri plates. Compounds inducing a positive germination response were selected for detailed study. Volatile aldehydes, alcohols, esters, ketones, heterocyclics, and isothiocyanates were tested, including compounds active on other species. Several aldehydes and esters were effective. Isobutyraldehyde, isovaleraldehyde, and furfural stimulated teliospore germination 72%, 47%, and 37%, respectively. The esters, furfuryl propionate, propyl propionate, methyl isobutyrate, and allyl butyrate stimulated germination 85%, 76%, 67%, and 44%, respectively; controls averaged 2.1% ± 0.5 S.E. The most effective compounds showed maximum activity over a concentration range of 10 to 250 µl/L in 1% agar, at 14 to 21 days, 24 C, in alternating light and darkness. Chemical stimulators may facilitate studies of the sexual cycle of the fungus which starts with the germination of the teliospores, and may help in the development of resistant bean varieties or lead to new methods of controlling the disease.

A452

INDUCTION OF SESQUITERPENE CYCLASE ACTIVITY IN COTTON COTYLEDONS FOLLOWING INOCULATION WITH *XANTHOMONAS CAMPESTRIS* PV. *MALVACEARUM*. J. Tsuji, M. L. Pierce, and M.

Essenberg. Department of Biochemistry and Molecular Biology, Oklahoma State University, Stillwater, Oklahoma 74078-0454.

The sesquiterpene cyclase, δ-cadinene synthase, is probably the first committed enzyme in the biosynthetic pathway of sesquiterpenoid defense compounds in cotton and is thought to play an important regulatory role in the accumulation of sesquiterpenoid phytoalexins. Inoculation of cotyledons of the glandless cotton line WbM*gl* with *Xanthomonas campestris* pv. *malvacearum* (Xcm) resulted in the induction of sesquiterpene cyclase activity. Sesquiterpene cyclase activity increased after 20 hrs post-inoculation, reached a maximum by 60 hrs post-inoculation, and then declined rapidly afterwards. In contrast, sesquiterpene cyclase activity remained low in mock-inoculated control cotyledons. As a first step towards cloning the sesquiterpene cyclase gene(s) of cotton, we constructed a cDNA library in Uni-ZAP XR (Stratagene) using poly(A)$^+$ RNAs isolated from cotyledons 35-41 hrs after inoculation with Xcm. Probes are currently being devised in order to screen the library. Supported by the NSF EPSCoR, the USDA Competitive Research Grants Program, and the Oklahoma Agricultural Experiment Station.

A453

IN SITU LOCALIZATION OF RESPONSE GENE TRANSCRIPTS IN BARLEY ATTACKED BY *ERYSIPHE GRAMINIS*. T. A. Clark[†], C. Conicella[‡], A. G. Smith[†], R. J. Zeyen[†], [†]Depts. of Plant Pathology and Horticultural Science, University of Minnesota St. Paul, MN 55108, [‡]Center for Vegetable Breeding, C. N. R., Portici, Italy.

Spatial distribution patterns for transcripts of three host response genes, pRP-2, pRP-4, and pRP-5 (Davidson et al. Plant Molecular Biology 8: 77-85, 1987), were determined for two pairs of near-isogenic barley lines, differing at the Mla or mlo loci for resistance, at intervals following inoculation with *Erysiphe graminis* f.sp. *hordei*. Transcript abundance and the spatial distribution of accumulation differed among the three response genes, between the Mla and mlo pairs of isolines, and between the resistant and susceptible members of each pair. Transcripts tended to become increasingly abundant in epidermal cells of both pairs of isolines and were uniformily distributed throughout epidermal tissues of mlo isolines but were restricted to specific epidermal cells, at probable infection sites, and adjacent mesophyll cells in Mla isolines.

A454

LIGHT AND ELECTRON MICROSCOPIC STUDY OF PATHOGENESIS OF *FUSARIUM SAMBUCINUM* IN POTATO TUBER TISSUE. Y. Zeng and R. Hammerschmidt, Department of Botany and Plant Pathology, Michigan State University, East Lansing, MI 48824.

The pathogenesis of potato dry rot fungal pathogen *Fusarium sambucinum* was studied at the light microscopic (LM) and electron microscopic (EM) level. Light and electron micrography revealed that the pathogen was able to infect potato tuber tissue 12 hr after inoculation. The infection process involved enzymic degradation of host middle lamella and cell walls. Lignification of the host cell walls in response to infection was first seen within 12 hr after inoculation and continued to increase throughout the rest of experimental period. However, the lignified cell walls did not restrict further invasion of the fungus.

A456

INVOLVEMENT OF CINNAMYL ALCOHOL DEHYDROGENASE IN INDUCED SYSTEMIC RESISTANCE IN CUCUMBER. P. Yang-Cashman and R. Hammerschmidt. Department of Botany and Plant Pathology, Michigan State University, East Lansing, MI 48824.

To investigate the role of lignification in induced systemic resistance in cucumber (ISR) against *Cladosporium cucumerinum*, cinnamyl alcohol dehydrogenase (CAD) activity was compared in induced and control plants challenged with the fungus. Two peaks of CAD activity were observed in excised petioles and intact young leaves. The first peak of activity appeared 24 hrs after challenge, whereas the second peak occurred 48 hrs after challenge. The earlier peak of activity, which is larger in induced plants compared to control plants, maybe involved in lignification associated with induced resistance. The 48 hr peak of CAD activity appears to be more associated with the magnitude of lesion formation in induced and control plants. These data suggest that regulation of CAD activity is an important component of lignification in induced systemic resistance to the fungus *C. cucumerinum*.

A457

PARTIAL PURIFICATION AND CHARACTERIZATION OF A CUTINASE FROM MONILINIA FRUCTICOLA. R. M. Bostock and S. M. Wilcox, Department of Plant Pathology, University of California, Davis, CA 95616.

Monilinia fructicola, a causal agent of brown rot of peach, secretes a cutinase when grown in a modified Czapek-Dox broth containing purified cutin. The cutinase activity is induced within 8 days after inoculation of the medium, and the activity appears to be repressed when glucose is provided as a carbon source. Inhibitor studies indicate that the *Monilinia* cutinase is a serine esterase, similar to other fungal cutinases. The major cutinolytic activity has a pI of approximately 8.5 as determined by isoelectric focusing. All isolates of *M. fructicola* and *M. laxa* tested produced cutinase in culture, however, isolates varied in the level of cutinase activity. Characteristics of the *M. fructicola* cutinase and preliminary experiments to establish its role in pathogenicity of *M. fructicola* on peach fruit will be presented.

A458

A POSSIBLE ROLE FOR PECTIN LYASE IN PHYTOPHTHORA SOJAE PATHOGENICITY. L.I. Rivera-Vargas and T.L. Graham. Dept. Plant Pathology, Ohio State University, Columbus, OH. 43210

Phytophthora sojae (Ps.) causes Phytophthora root rot and damping off of soybean seedlings. The maceration of tissues that characterizes this disease led us to suspect that an array of cell wall degrading enzymes are produced by this pathogen. Studies were done in planta with two soybean cultivars (Williams and Williams 79) and two Ps. races (3 and 4), to characterize these enzymes. Studies were also done using synthetic media or lima bean broth ammended with different pectic fractions. Four pectolytic enzymes were studied: pectin lyase (PNL), pectatelyase (PL), pectin methyl esterase (PME) and polygalacturonase (PG). PNL was the major pectolytic enzyme produced in culture in response to pectic substrates and also at the infection front in soybean tissues. It is expressed in a non-race specific manner,suggesting an involvement in pathogenicity, but not virulence of Ps. PME, PL and PG did not show significant activity under any conditions in vitro or in vivo. Further studies are in progress.

A459

PARTIAL GENOMIC SEQUENCE OF POTATO SESQUITERPENE CYCLASE. M. Zook, Depatartment of Botany and Plant Pathology, Michigan State University, East Lansing, MI 48824

Three different PCR products were obtained from potato (*Solanum tuberosum*) genomic DNA using primers derived from a genomic clone of the tobacco (*Nicotiana tabacum*) sesquiterpene cyclase. The three PCR products were 1.5, 0.7, and 0.5 kb in length. Approximately 400 bp of each PCR product was sequenced. A comparison of the potato DNA sequence with that of the tobacco sesquiterpene cyclase genomic clone revealed 62 to 73% homology at the nucleotide level. Most the sequence homology was found in exons regions of the tobacco genomic clone. Analysis of the genomic DNA sequences from different solanaceous plants has revealed that there are similarities in the genes which encode sesquiterpene cyclases.

A460

PRODUCTION OF 15-HYDROXYTRICHODIENE BY TOBACCO CELL SUSPENSION CULTURES TRANSFORMED WITH TROCHODIENE SYNTHASE FROM *FUSARIUM SPOROTRICHIOIDES*. M. Zook, T. Hohn, R. Hammerschmidt, Department of Botany and Plant Pathology, Michigan State University, East Lansing, MI 48824

The gene encoding trichodiene synthase, a sesquiterpene cyclase from the fungus *Fusarium sporotrichioides*, was used to transform tobacco. The production of trichodiene in transformant cell lines which possess trichodiene synthase was demonstrated by GC/MS analysis. *In vivo* labeling with [^{3}H]mevalonate and [^{3}H]trichodiene has revealed the presence of a trichodiene metabolite which is produced in the transformant cell lines which have trichodiene synthase activity. Mass spectroscopic analysis revealed that the trichodiene metabolite has a molecular weight of 220. Subsequent NMR analysis confirmed that the transgenic tobacco trichodiene metabolite is 15-hydroxytrichodiene ([S-(R*,R*)]-4-methyl-4-(1-methyl-2-methylenecyclopentyl)-1-cyclohexene-1-methanol). This study demonstrates that a foreign gene can alter plant secondary metabolism.

A461

EFFECTS OF THE ALFALFA PHYTOALEXIN MEDICARPIN AND STRUCTURALLY RELATED COMPOUNDS ON GROWTH OF PHYTOPATHOGENIC FUNGI. J.W. Blount, H.D. VanEtten[1], R.A. Dixon & N.L. Paiva, Noble Foundation, P.O.B. 2180, Ardmore, OK 73402; [1] Univ. of Arizona, Tucson, AZ 85721.

The antimicrobial activities of the alfalfa (*Medicago sativa* L.) phytoalexin (-) medicarpin, its stereoisomer (+) medicarpin from peanut (*Arachis hypogaea*), (-)6a-hydroxymedicarpin, and 3-O-methyl, 6a-hydroxymedicarpin ((-) homopisatin) from lentil (*Lens culinaris*) were tested at concentrations of 0.1 mM and 0.5 mM against a variety of phytopathogenic fungi. Using an agar plate assay, inhibition of linear mycelial growth was measured both as a percent relative to controls and as a rate. The metabolism of these compounds by a variety of phytopathogenic fungi was studied using HPLC. The results from these experiments will be useful in determining future goals of our program involving the genetic manipulation of the alfalfa phytoalexin pathway.

A463

CONJUGATIVE PLASMID TRANSFER IN THE PEA SPERMOSPHERE/RHIZOSPHERE. Padma Sudarshana and G. R. Knudsen. Plant Pathology Division, University of Idaho, Moscow, ID 83844.

Dynamics of conjugative plasmid (R388::*Tn*1721, *inc*W, broad host range) transfer from *Pseudomonas cepacia* to *P. fluorescens* were determined in microcosms containing either a glass bead or a pea seed in either sterile or nonsterile soil (Ψ_m = 100 kPa). Donor and recipient cells were added at 10^4, 10^6 or 10^8 CFU/g of soil. Donors (D), recipients (R) and transconjugants (T) were enumerated on selective media over 7 days. Populations of donors, recipients, and transconjugants all increased on day 1 and declined subsequently. Growth rates for donor and recipient populations were higher for the lower initial cell densities. Plasmid transfer rate constants [$\tau = T \cdot (D \cdot R)^{-1}$] were calculated from mean donor and recipient populations on day 1. Values of τ were not significantly affected by soil vs. spermosphere/rhizosphere or by sterile vs. nonsterile soil. However, τ was inversely related to initial parental density, and directly related to parental growth rates. For initial donor and recipient densities of 10^4, 10^6, or 10^8 CFU/g, τ values averaged 1 x 10^{-9}, 1 x 10^{-12}, and 3 x 10^{-14}. Although τ ideally is independent of parental density, it was indirectly affected by density via parental growth potential in a resource-limited environment.

A464

A laccase-cDNA from *Botrytis cinerea*. Cantone, F.A., and Staples, R.C. Boyce Thompson Institute, Tower Road, Ithaca, N.Y. 14853.

Botrytis cinerea is a necrotrophic fungal pathogen that parasitizes many different species of plants at almost all stages of growth and during storage. When induced by phenolic compounds in culture, *B. cinerea* secretes the enzyme laccase. Fungal laccases are copper-containing phenol oxidases that specifically oxidize *p*-diphenols. When laccase activity is inhibited, the virulence of *B. cinerea* is reduced (Bar-Nun, N. and Mayer, A.M. 1990. Phytochemistry 29:787). We are attempting to evaluate the role of laccase in pathogenicity of *B. cinerea*. To clone laccase cDNA, we designed degenerate primers based on the amino acid translation of the highly conserved copper-binding regions of other fungal laccases. These primers were used with messenger RNA and the reverse polymerase chain reaction to amplify a 1.3 kb fragment of the cDNA. Clones were sequenced and found to have the four copper-binding regions, and had 60% similarity and 44% identity with laccases from *Cryphonectria parasitica* and *Neurospora crassa*. In order to construct a full length cDNA clone, we used RACE (rapid amplification of cDNA ends) techniques to amplify the 5' and 3' ends.

A465

EVIDENCE THAT THE *SYRB* AND *SYRC* GENES OF *PSEUDOMONAS SYRINGAE* PV. *SYRINGAE* ENCODE ENZYMES INVOLVED IN SYRINGOMYCIN SYNTHESIS. J.-H. Zhang, N.B. Quigley, and D.C. Gross. Dept. Plant Pathology, Washington State University, Pullman, WA 99164.

The *syrB* and *syrC* genes of *P. s. syringae* B301D are required for syringomycin production. Analysis of the *syrB* sequence identified a 3,137-bp ORF potentially encoding a protein of 1,046 amino acids with a M_r of 115,647. Database searches revealed that SyrB is related to a family of peptide antibiotic synthetases that employs the multienzyme thiotemplate mechanism. Six core sequences characteristic of thioester-forming domains in peptide synthetases were identified. This suggests that SyrB activates and condenses one of the nine amino acids contained in syringomycin. Analysis of the *syrC* gene identified a 1,299-bp ORF that overlaps the 3' end of *syrB* by 116 bp. The predicted SyrC protein (M_r 47,234) was related to a family of zinc hydrolases. A zinc finger motif exists in the C-terminal of SyrC, and a thioesterase motif is located near the middle of SyrC. Thus, SyrC may serve as a thioesterase that facilitates transpeptidation in syringomycin biosynthesis.

A466

Nucleotide sequence analysis of the *phtE* locus involved in the production of phaseolotoxin. Yuanxin Zhang and Suresh S. Patil*, Department of Plant Pathology and the Biotechnology Program, University of Hawaii, Honolulu, HI 96822.

Previously, we reported that the insert in a cosmid clone, pHK120, contains eight loci (*phtA* through *phtH*) encoding genes involved in the biosynthesis and/or secretion of phaseolotoxin, a phytotoxin produced by *Pseudomonas syringae* pv. *phaseolicola*. The *phtE* locus (6575 bp) was sequenced and revealed five putative open reading frames (ORFs) in the same orientation, preceded by putative ribosomal binding sites. A comparison of the sequences of the putative ORFs with the sequence of known genes revealed that ORF3, encoding a protein of 411 aa, has a 55% homology with the ornithine aminotransferase and acetylornithine aminotransferase genes from *E. coli* and other organisims. This data suggests that the putative gene encodes a protein required for the biosynthesis of ornithine, a constituent of phaseolotoxin. ORF1, ORF2, ORF4, and ORF5 also show some homology with known gene sequences. Two additional ORFs, ORF6 (564 bp) and ORF7 (594 bp), had no discernable Shine-Delgarno sequences, and showed no homologies with gene sequences in the data bases. Results of the sequence comparison and protein expression studies will be presented.

A467

COMPATIBILITY OF BACTERIAL ANTAGONISTS WITH ANTIBIOTICS USED FOR MANAGEMENT OF FIRE BLIGHT. V. O. Stockwell[1], K. B. Johnson[2], and J. E. Loper[1], [1] USDA-ARS, Hort. Crops Research Laboratory, and [2] Dept. of Botany & Plant Pathology, Oregon State Univ., Corvallis, OR, 97331.

Application of the bacterial antagonists, *Pseudomonas fluorescens* A506 and *Erwinia herbicola* C9-1, to pear blossoms reduced incidence of fire blight by 50% in field trials in Oregon in 1991 and 1992. Fire blight of pome fruits is managed commonly with spray applications of streptomycin (Sm) or oxytetracycline (Tc), thus compatibility of bacterial antagonists with antibiotics was tested. In 1992 and 1993, Rome apple trees were sprayed with 10^8 cfu/ml of A506 (Sm^r, Tc^r) or a Sm^r but Tc^s mutant of C9-1 (1993 only). Water, Sm (Agrimycin D, 100 µg/ml) and/or Tc (Mycoshield, 200 µg/ml) were applied 3 and 7 days later. Populations of A506 and C9-1 on stigmas were estimated before and after sprays. Both A506 and C9-1 averaged 10^5 cfu/blossom on water-treated trees. Sm did not affect populations of A506 or C9-1. Tc decreased populations of A506 and C9-1 by 1 log unit in 1993. The number of blossoms that contained A506 was decreased by Tc, but the incidence of C9-1 was similar to controls. Integration of bacterial antagonists with antibiotics may enhance long-term management of fire blight.

A468

POPULATION DYNAMICS OF *PSEUDOMONAS CEPACIA* AND *PAECILOMYCES LILACINUS* IN RELATION TO COLONIZATION OF POLYFOAM ROOTING CUBES BY *RHIZOCTONIA SOLANI*. D. Kelly Cartwright and D. M. Benson. Dept. of Plant Pathology, N. C. State University, Raleigh.

Pseudomonas cepacia (strain 5.5B) and *Paecilomyces lilacinus* (strain 6.2F) effectively control Rhizoctonia stem rot of poinsettia in polyfoam rooting cubes. The population dynamics of these strains and subsequent colonization of rooting cubes by *R. solani* was determined. In cubes treated with *P. cepacia*, population (cfu/ml) declined by 52-73, 82-89, 93-95, and 95-98% of the original population after three, seven, 14, and 21 days while colonization of cubes by *R. solani* ranged from 0, 5-6, 15-26, and 23-36%. After three, seven, 14 and 21 days, population (conidia/ml) of *P. lilacinus* declined by 10-23, 8-11, 10-16, and 22-35% in two tests but increased by 12, 28, 11, and 9% in one test. Colonization of cubes by *R. solani* ranged from 0, 0-5, 0-15, and 13-25%. Colonization of the untreated, infested cubes by *R. solani* was much greater in all tests.

A469

APPLICATION METHODS AND EFFICACY OF *PSEUDOMONAS CEPACIA* (STRAIN 5.5B) AND OTHER *PSEUDOMONAS* ISOLATES TO CONTROL RHIZOCTONIA STEM ROT OF POINSETTIA. D. Kelly Cartwright and D. M. Benson. Dept. of Plant Pathology, N. C. State University, Raleigh.

Pseudomonas cepacia (strain 5.5B) effectively controls Rhizoctonia stem rot of poinsettia caused by *Rhizoctonia solani* in polyfoam rooting cubes. Other isolates of *P. cepacia* as well as isolates of *Pseudomonas fluorescens*, *Pseudomonas aureofaciens*, and *Pseudomonas chlororaphis* were tested in this system. Only some strains of *P. cepacia* were as effective as strain 5.5B for stem rot control. Soaking rooting cubes in a bacterial suspension of strain 5.5B was the most effective application method. Cubes soaked in water followed by a bacterial spray over the top of the cubes gave significant (P=0.05) control of stem rot. Three spray applications of strain 5.5B during a 2-wk period was more effective for stem rot control than one or two sprays.

A470

PREPARATIONS FOR APPLICATION OF *PSEUDOMONAS CEPACIA* (STRAIN 5.5B) AND *PAECILOMYCES LILACINUS* (STRAIN 6.2F) IN CONTROL OF RHIZOCTONIA STEM ROT OF POINSETTIA. D. Kelly Cartwright and D. M. Benson. Dept. of Plant Pathology, N. C. State University, Raleigh.

The effect of antagonist concentration, nutrients, and antagonist-free preparations (culture filtrate) of *Pseudomonas cepacia* and *Paecilomyces lilacinus* on biocontrol of Rhizoctonia stem rot of poinsettia in polyfoam rooting cubes was investigated. Cuttings averaged 0-56% infection in cubes soaked in 2-2.6x10^9 to 2-2.6x10^5 cfu/ml of *P. cepacia*. Infection averaged 27-83% on cuttings in cubes soaked in 2-2.8x10^7 to 2-2.8x10^3 conidia/ml of *P. lilacinus*. Less infection occurred with strain 5.5B (0%) or 6.2F (13%) with nutrients as compared to 13% (5.5B) or 23% (6.2F) without nutrients. No control (100% mortality) of stem rot occurred with sterile, culture filtrate of *P. cepacia* compared to 17% infection of cuttings where the bacterium was present. With culture filtrate of *P. lilacinus*, infection of cuttings averaged 30% compared to 7% infection where the fungus was present.

A471

SECONDARY METABOLITES OF *PSEUDOMONAS CEPACIA*, STRAIN 5.5B, A BIOCONTROL AGENT OF *RHIZOCTONIA SOLANI*. D. Kelly Cartwright, W.S. Chilton and D. M. Benson. Dept. of Plant Pathology and Dept. of Botany, N. C. State University, Raleigh.

Pseudomonas cepacia (strain 5.5B) controls Rhizoctonia stem rot of poinsettia caused by *Rhizoctonia solani* in polyfoam rooting cubes. Experiments were conducted to elucidate and characterize inhibitory compound(s) and a purple pigment produced by strain 5.5B. Compounds inhibitory to *R. solani* and pigment production varied depending on culture medium. The most inhibitory compound to *R. solani* was identified as pyrrolnitrin, a known antifungal compound. The purple pigment was isolated and identified as the phenazine compound, 4,9-dihydroxyphenazine-1,6-dicarboxylic acid dimethyl ester. This compound had activity against *R. solani in vitro*, but was much less inhibitory than pyrrolnitrin. These results indicate that the primary mechanism of antagonism by strain 5.5B may be antibiosis.

A472

EFFICACY OF *PSEUDOMONAS CEPACIA* (STRAIN 5.5B) TO CONTROL RHIZOCTONIA ROOT ROT OF SNAP BEAN ALONE AND IN COMBINATION WITH A BINUCLEATE *RHIZOCTONIA*-LIKE FUNGUS. D. Kelly Cartwright, E. Echandi, and D. M. Benson. Dept. of Plant Pathology, N. C. State University, Raleigh.

In repeated greenhouse trials, *Pseudomonas cepacia* (strain 5.5B) was effective in biocontrol of Rhizoctonia root rot of snap bean caused by *Rhizoctonia solani*. In treatments where *P. cepacia* was used alone, snap bean seedlings had disease ratings of 5.1-9.8, significantly (P=0.05) less than infested controls where disease ratings ranged from 78.1-80.3. The binucleate *Rhizoctonia*-like fungus (BNR, isolate P9023) provided significant (P=0.05) control (ratings from 2.1-8.3) of root rot when used alone. Combining the BNR with strain 5.5B resulted in disease ratings of 0.3-0.6 on seedlings. These results indicate that combining the BNR and *P. cepacia* may enhance biocontrol of Rhizoctonia root rot of snap bean.

A473

OSMOTIC PRESSURE EFFECTS ON GROWTH OF BIOCONTROL AGENTS OF V. DAHLIAE IN VITRO. S. Hussain, M. L. Powelson, and N. W. Christensen. Depts of Bot. and Pl. Path. and Crop and Soil Sci., Oregon State University, Corvallis, OR 97331-2902

Mycelial growth and spore germination of Verticillium dahliae, Gliocladium virens, Trichoderma viridae and Talaromyces flavus were measured on malt yeast peptone medium at osmotic pressures of- 0.12,-0.18,-0.59,-1.10,-2.15,-3.06,-5.12,-6.17,-7.16, and -8.13 MPa. V. dahliae and T. viridae grew at all osmotic pressures whereas G. virens and T. flavus did not grow at -8.13 and below -6.17 MPa, respectively. Mycelial growth of the biocontrol agents decreased linearly as osmotic pressure decreased. In contrast, mycelial growth of V. dahliae showed an increase below -0.59 MPa with maximum growth occurring between -1.10 and -5.12 MPa after which it decreased linearly. The biocontrol agents did not sporulate below -1.10 MPa whereas V. dahliae did not produce microsclerotia below -0.59 MPa when examined after 7 days. Time required for 95% germination increased linearly as osmotic pressure decreased from -0.12 to -8.13 MPa and ranged from 13-60, 16-46 and 16- >72h for V. dahliae T. viridae and G. virens, respectively. Ascospores of T. flavus did not germinate even after a 1 h heat treatment at 70 c. The parameters investigated for all fungi except T. flavus were similar qualitatively but quantitatively different over the osmotic pressures tested.

A475

EVALUATION OF FUNGI FOR THE BIOLOGICAL CONTROL OF SUDDEN DEATH SYNDROME OF SOYBEAN. J. C. Rupe and C.M. Becton, University of Arkansas, Fayetteville.

The effectiveness of 46 fungal isolates collected from the rhizosphere of soybeans was evaluated for the control of SDS. Selected through preliminary greenhouse screenings, the isolates were grown in potato dextrose broth for 2 wks and homogenized in a blender. Roots of 2-wk-old Lee 74 soybean seedlings were dipped in the resulting suspension and incubated in vermiculite. After 4 days, the seedlings were challenged with a 10^7 conidia/ml suspension of the SDS pathogen, *Fusarium solani*, planted in fumigated soil, and observed for 3 wks in the greenhouse. Of the 46 isolates, 9 resulted in significantly less disease than the inoculated control in two experiments. There was no *in vitro* relationship of antibiosis under either high (PDA) or low (root-extract agar) nutrient conditions with the control of SDS. Of the 9 fungi, 5 were *F. solani*, 3 were *F. oxysporum*, and 1 was unidentified.

A476

CHARACTERIZATION OF ACTINOPLANES SPP. BY RESTRICTION ENDONUCLEASE FINGERPRINTING AND CHEMOTAXONOMIC ANALYSIS. Fangli Chen, L. L. Singleton and A. B. Filonow, Oklahoma State University, Stillwater, OK 74078.

Actinoplanes spp. are being studied as potential biological control agents. Cell wall amino acid and whole cell sugar pattern analyses were applied to more than 20 strains of Actinoplanes spp. Clusters were determined based on their chemical relatedness. Total DNAs were prepared from 22 strains; no plasmids were found. Genomic DNAs were compared by restriction endonuclease fingerprinting(REF), using BamHI, PstI, XhoI, KpnI, NotI, HindIII, EcoRI or XbaI. When BamHI fragments

were separated by PAGE and stained with silver nitrate, each strain possessed a reproducible fingerprint, thereby facilitating strain recognition. Two strains of A. missouriensis had identical DNA fingerprints. Some spontaneous color mutants were also analyzed, but no difference was detected in their band patterns. REF profiles were analyzed by densitometer, the degree of similarity between organisms was quantitated and a phylogenetic tree was generated by the Neighbor-joining method. Correlation between chemical and genetic analyses is being evaluated.

A477

GENETIC ANALYSIS OF A LOCUS REQUIRED FOR SYNTHESIS OF 2,4-DIACETYLPHLOROGLUCINOL BY *PSEUDOMONAS AUREOFACIENS* Q2-87. M. Bangera*, H. Hara, D.M. Weller, and L.S. Thomashow. USDA-ARS and *Department of Microbiology, Washington State University, Pullman, WA.

2,4-diacetylphloroglucinol (Phl) is a broad-spectrum antibiotic produced by certain strains of *Pseudomonas* effective in controlling soilborne pathogens. A locus involved in Phl production by *P. aureofaciens* Q2-87 was described previously (Vincent et. al., Appl. Environ. Microbiol. 57:2928). A 7-kb subclone was able to transfer Phl biosynthesis to each of 15 heterologous Phl-nonproducing *Pseudomonas* strains. These strains showed increased inhibition of *Gaeumannomyces graminis* var. *tritici* in vitro and some were more suppressive of take-all than the parental strains on wheat roots. Larger DNA fragments with additional, flanking sequences were not expressed in the heterologous strains. Within the 7-kb fragment 43 unique Tn3HoHo1 (Stachel et. al., EMBO J. 4:891) insertion mutations have been isolated. Based on the expression of the promoterless β-galactosidase gene in Tn3HoHo1, the putative biosynthetic locus has at least one promoter and up to three transcriptional and/or translational units. One of these units corresponds to a locus required for the accumulation of a noninhibitory red pigment which appears to be a derivative of Phl.

A478

ANTHRACNOSE-LIKE SYMPTOMS AND BLIGHT DISEASE ON SOWTHISTLE (SONCHUS ARVENSIS L.) IN MISSISSIPPI. H. K. Abbas and C. D. Boyette, Southern Weed Science Laboratory, USDA-ARS, Stoneville, MS.

Sowthistle is an important annual weed of sorghum, wheat, and soybean crops. In Mississippi during 1991-1993, blight and anthracnose-like diseases on the winter weed sowthistle were observed, but at different sites. Disease severity peaked during seed production. The percentage of diseased plants in infected fields ranged between 80 to 100%. Fungi isolated from infected weeds included Alternaria spp., Fusarium spp., Cladosporium spp., Bipolaris spp., Colletotrichum spp., Ascochyta spp., and Aspergillus spp. and were tested for virulence. Ascochyta sp. caused the anthracnose-like symptoms under growth chamber and field conditions on both small and large plants when sprayed at the rate of 5.4×10^4 sclerotium/mL. The optimum temperature for fungal growth on PDA was 22 - 25 C with a minimum of 15 C and maximum of 28 C. Infection occurred on plants at 24 to 26 C only. No phytotoxins were isolated from this fungus grown on solid or liquid media. Host range experiments showed this fungus to be very specific for sowthistle. None of the fungi isolated caused the blight disease under the conditions tested.

A479

PHYTOTOXICITY OF RIFAMYCINS TO VARIOUS PLANT SPECIES. Robert E. Hoagland; USDA, ARS, SWSL; P.O. Box 350; Stoneville, MS 38776

Rifamycins, natural products with antibiotic acitivty isolated from *Streptomyces mediterrani*, do not effect eukaryotic RNA polymerases at levels 10K-fold higher than required to inhibit bacterial mitochondrial and chloroplast enzymes ($\approx$ 1 μg/ml). Rifamycin B, rifampicin, and rifamycin SV-Na were examined for phytotoxic effects on crop and weed species. Imbibition of seeds of 12 species in solutions of up to 7 mg/ml caused little or no effect on germination in the dark, but some growth inhibition was noted in radish, cucumber, and wheat treated with rifampicin. When root-fed (8×10^{-4} M) to young dark-grown seedlings, significant growth inhibition was caused by rifampicin in coffee senna, okra, cotton, and sicklepod after 72 h; and these plants were dead at 96 h. Leaf disk test showed similar responses. Rifamycin SV-Na and rifamycin B were generally less phytotoxic. Color changes in some chemical treatment solutions after exposure to some species of germinating seeds or seedlings indicated antibiotic metabolism. Preliminary studies indicate these changes are due to enzyme activity. Differential absorption and metabolism may explain the selective phytotoxicity symptoms exhibited by these species. This unique chemistry may offer insight in the development of new herbicides.

A480

PHOMOPSIS AMARANTHICOLA n. sp.: A SPECIES PATHOGENIC TO AMARANTHUS SPP. E.N. Rosskopf, R. Charudattan, and J.T. DeValerio, Plant Pathology Dept., Univ. Florida, Gainesville 32611.

A species of Phomopsis causing leaf and stem blight was isolated from Amaranthus sp. in Florida. Symptoms included brown to black necrotic coalescent lesions, premature leaf abscission, stem girdling, and plant death. Pycnidia appeared in 4-7 days and sporulated after 8-14 days in culture. The fungus produced alpha-, beta-, and C-conidia. Alpha-conidia appeared first and in greatest abundance in culture. All three conidial types were produced within the same pycnidium. Identification of this isolate as a new species (P. amaranthicola) was based on the types of conidia it produced and on measurements of its conidia and conidiophores in comparison with P. amaranthi Ubriszy and Vörös (reported from Hungary on pigweed) and other Phomopsis spp. (Uecker, 1988). Tests indicated that P. amaranthicola was pathogenic to 39 biotypes of Amaranthus spp. belonging to 22 species, including some that are among the world's worst weeds. Thus, P. amaranthicola has potential for use as a broad-spectrum bioherbicide for pigweed species.

A481

BEAN BLOSSOM DEVELOPMENTAL STAGE AND CANOPY TEMPERATURE AFFECT ANTAGONISM OF ERWINIA HERBICOLA STRAINS AGAINST SCLEROTINIA SCLEROTIORUM. G. Y. Yuen, M. L. Craig, E. D. Kerr, and J. R. Steadman. Department of Plant Pathology, University of Nebraska, Lincoln, NE 68583-0722

Erwinia herbicola (Eh) strains isolated from blossoms of dry edible bean inhibited white mold disease, caused by Sclerotinia sclerotiorum, in the laboratory after multiplication on bean blossoms. Multiplication of Eh, however, was restricted to blossoms at the fully-expanded, mature stage, which lasts for only one day under field conditions. During this critical period, reduced Eh multiplication due to unfavorable environmental conditions could result in blossoms being unprotected. Growth of Eh strains on blossoms and antagonism against S. sclerotiorum were reduced at temperatures lower than 20 C. Temperatures less favorable to Eh but more favorable to white mold development were found to occur within bean canopies for over 13 hr each day during the peak white mold infection period. These factors explain why field applications of Eh strains in western Nebraska were ineffective in controlling white mold disease.

A482

BOTRYTIS CINEREA PERS. DICARBOXIMIDE RESISTANCE FREQUENCY IN TWO GREENHOUSES. G. W. Moorman and R. J. Lease. Penn State University, Dept. of Plant Pathology, University Park, PA 16802.

Two commercial greenhouses were visited every 3 months to collect 50 separate samples of Botrytis cinerea spores from each. Spores were plated on potato dextrose agar without and with dicarboximide fungicide (20 µg vinclozolin/ml). Percent spore germination, based on scoring 400 spores/sample, was used as an indication of the frequency of dicarboximide resistance in each population. Grower A never used diacarboximides while B used them at planting. The average frequency of resistance on dates 3/92, 6/92, 9/92, 12/92, 3/93, and 6/93 were 34%, 28%, 27%, 13%, 34%, and 52% for greenhouse A and 76%, 98%, 100%, 89%, 74%, and 49% for B, respectively. It is likely that dicarboximide resistant Botrytis is introduced into A on purchased plants. Grower B used dicarboximides after the 3/92 and 6/92 collection dates, accounting for the dramatic increase in resistance frequency. Resistance frequency has declined since B ceased using dicarboximides after 9/92. When a high level of control is necessary it may be possible to use dicarboximides once on a crop in a mixture with a fungicide to which the fungus is not resistant. The frequency of resistance may gradually decline to allow use the following year on a new crop.

A483

PHYTOTOXICITY CAUSED BY BENLATE^R AND DIFFERENCES AMONG PRODUCTION LOTS. J.Y. Uchida, C.Y. Kadooka, and M. Aragaki. Department of Plant Pathology, University of Hawaii, Honolulu, HI, 96822.

The possibility that plant damage can be caused by Benlate^R has been under investigation in our laboratory for nearly 18 months. Under greenhouse conditions, single applications of Benlate at one-half the maximum label rate for ornamentals caused stunting of marigold, sweet william, and impatiens. Five drench applications of Benlate at biweekly intervals at the maximum ornamental label rate, caused severe interveinal chlorosis and stunting of spathiphyllum. In greenhouse tests, a Benlate WP production lot (K8) was consistently the most phytotoxic among two WP and two DF lots. Exposure of these four Benlate lots to moisture saturation in sealed containers for 35 days resulted in the formation of crystalline dibutyl urea (DBU) in the dish of water serving as the moisture source. The formation of crystalline DBU by K4, a Benlate DF production lot, was markedly slower than by the other three lots. DBU was subsequently shown to cause significant damage to greenhouse grown cucumber seedlings, marigold, and sweet william.

A484

EFFECTS OF SEED COATING AND FUNGICIDE SEED TREATMENT ON STAND COUNT, YIELD AND DISEASE INCIDENCE IN

ALFALFA. D. J. Gallenberg and E. K. Twidwell. Dept. of Plant Science, South Dakota State University, Brookings, SD 57007.

Studies were conducted at two sites in 1991 and 1992 to determine the effects of seed coating (Rhizo-Kote) and fungicide seed treatment on stand count, yield and disease incidence in alfalfa. Noncoated seed plus Apron had the highest initial and final stand counts at both sites in 1991, final stand count at one site in 1992, and total forage yield at one site in 1991. Post emergence damping off was not detected in either season at either site, however Apron fungicide appeared to increase initial and final stand counts on coated and noncoated seed. Seed coating had no apparent effect on stand count, forage yield or disease incidence.

A485

POTENTIAL OF POLYMER COATINGS FOR CONTROL OF CORN SEED DECAY BY SOILBORNE PYTHIUM SPECIES. B. Arias, D. C. McGee, and J. S. Burris, Seed Science Center and Departments of Plant Pathology and Agronomy, Iowa State University, Ames, IA 50011.

Several polymers, applied to corn seeds alone or in combination with captan, were evaluated for their potential for preventing corn seed decay by soilborne Pythium species. Seedling emergence and colonization of ungerminated seeds by Pythium spp. were determined under field conditions or in field soil under greenhouse conditions (7 days at 10 C followed by 7 days at 25 C). Both high and low vigor seeds treated with chitosan, a polymer of B-1,4-D-glucosamine, had higher emergence than untreated seeds in the field in 1993, but had lower emergence than captan-treated seeds. In the greenhouse test, emergence again was lower for chitosan compared to captan-treated seeds, but it was similar to that for untreated seeds. In this test, Pythium colonization of ungerminated seeds was lower for captan and metalaxyl-treated seeds than for chitosan-treated seeds. Light microscope observations of seedcoats showed hyphal swelling and sporangial cell lysis of Pythium spp. in chitosan-treated seeds. Emergence of seeds treated with other polymers, including Sacrust, Sepiret, Certop, and Surelease was either lower than or similar to that for untreated seeds. When captan was combined with each of the polymers, emergence was similar to that of seeds treated with captan alone.

A486

TOLERANCE OF *Venturia inequalis* TO FENARIMOL: BASELINE SENSITIVITY AND SENSITIVITY DISTRIBUTION FOR QUEBEC. Odile Carisse and Jean R. Pelletier. Research Station, Agriculture Canada, 430 Gouin Blvd., Saint-Jean-sur-Richelieu, Québec, Canada J3B 3E6.

This study was initiated to quantify the baseline sensitivity of the apple scab pathogen *Venturia inaequalis* (Cke.) Wint. to fenarimol (Rubigan), an ergosterol synthesis-inhibiting fungicide, prior to its widespread commercial use. During the 1988 season, a series of monoconidial isolates of *Venturia inaequalis* were collected. The field isolates were collected from 26 commercial orchards throughout Quebec, from different leaves and lesions for a total of 552 isolates. Sensitivity to fenarimol was assessed by radial growth inhibition assay. The ED_{50} values for the 26 orchards ranged from 0.0087 to 0.0395 µg ml^{-1} with a mean ED_{50} of 0.0164 µg ml^{-1}. Reduced sensitivity, expressed as ED_{50}, was found in three orchards for an overall frequency of 4.4% of isolates. Sensitive isolates had a mean ED_{50} of 0.006 µg ml^{-1}, yielding a resistance factor of about 67. Four populations were constructed based on the frequency distribution of ED_{50} values for the 26 orchards.

A487

DISEASE PROGRESS OF SPOTTED WILT IN GAT-2741, FLORUNNER, AND SOUTHERN RUNNER PEANUT. A. K. Culbreath, J. W. Todd, W. D. Branch and J. W. Demski, Depts. of Plant Pathology, Entomology, and Crop and Soil Science, University of Georgia, Coastal Plain Experiment Station, Tifton, GA 31793-0748.

Randomized complete block field tests were conducted at Attapulgus, GA in 1990-1992. Apparent incidence of spotted wilt, caused by tomato spotted wilt virus, was determined at two-wk intervals (July-Sept) in Florunner, Southern Runner, and proposed cultivar, GAT-2741, peanut (Arachis hypogaea). Disease incidence increased linearly in all three entries. Across all three years, incidence of symptomatic plants increased more slowly in GAT-2741 and Southern Runner than in Florunner. There was no significant year x cultivar interaction for disease incidence. Three-yr averages for final cumulative incidence of spotted wilt were 5.5, 2.2, and 1.7% (LSD = 2.5%; $P \leq 0.05$), and averages for yield were 3414, 3763, and 3916 kg/ha (LSD = 630 kg/ha; $P \leq 0.05$) for Florunner, Southern Runner, and GAT-2741 respectively.

A488

VIRULENCE OF PHYTOPHTHORA SOJAE ISOLATES FROM IOWA SOYBEAN FIELDS. R. L. Ruff, H. Tachibana, A. H. Epstein, G. L. Tylka, and X. B. Yang, Department of Plant Pathology, Iowa State University, Ames, IA 50011.

In 1991 P. sojae, causal agent of Phytophthora root rot (PRR), was isolated from diseased soybean plants of unknown varieties in growers' fields. In 1992 and 1993, P. sojae isolates were obtained from field soil samples by using PRR-susceptible soybean seedlings as bait. Isolates were grown on a selective medium containing antibiotics and fungicides to obtain pure isolates. Isolates that produced heterogeneous reactions on the soybean race differentials were induced to produce zoospores. Single zoospore cultures were produced and retested on the soybean differentials. Isolates identified thus far are P. sojae races 1, 3 and 4. Isolates have been identified that are virulent on each of the identified P. sojae resistance alleles, but many of these isolates could not be classified as to race. Identification of these virulent P. sojae isolates are discussed in reference to the development of PRR-resistant soybean varieties.

A489

ASCOSPORE INOCULATION OF FIELD-GROWN SOYBEAN WITH *SCLEROTINIA SCLEROTIORUM*. Arthur F. Olah and A. F. Schmitthenner. Dept. of Plant Pathology, OARDC/The Ohio State Univ., Wooster, OH 44691.

Twice-sterilized carrot slices were inoculated with *Sclerotinia sclerotiorum* and incubated without light for 30 days at 24 C. Sclerotia were freed of media using a forceful stream of water and plated on non-sterile water agar. Following 1 wk at 5 C in the dark, sclerotia were moved to 15 C with 350 $\mu E\ m^{-2}\ s^{-1}$ mixed cool white and daylight fluorescent light where apothecia formed within 35 days. Ascospores were collected by vacuum and water trap, air-dried, and stored at 5 C over a dessicant. Spores were applied (± 2 % sucrose) to R2-stage field-grown cultivars at 4.4 x 10^5 spores /plant using a Solo Port 60 high-volume sprayer and plants were kept at 100 % RH for 7 days using overhead irrigation with micromist nozzles. Sucrose was superior to water-only. Correlation of the log percent diseased plants using artificial inoculation to percent diseased plants under natural infestation was 0.69 on 10 cultivars. Thus, artificial field inoculation may be useful for screening for reaction to this pathogen.

A490

THE MECHANISM OF RESISTANCE TO STREPTOMYCIN IN *ERWINIA AMYLOVORA* STRAIN CA11. C.-S. Chiou and A.L. Jones. Department of Botany and Plant Pathology, Michigan State University, East Lansing, MI 48824

Two streptomycin resistance genes, *strA* and *strB*, were cloned from strain CA11 of *Erwinia amylovora* isolated from a Michigan apple orchard. Clones with *strA* were resistant to 100 µg/ml streptomycin, those with *strB* were sensitive, and those with both genes were resistant to 500 µg/ml streptomycin. When *strA* and *strB* were cloned separately on expression vector pTWNHE and induced with 0.1 mM IPTG, the protein produced by *strA*, but not the one produced by *strB*, was overexpressed. Analysis of primary sequence and secondary structure indicated that *strB* lacked an ideal Shine-Dalgarno (SD) sequence and its mRNA formed a stable hairpin structure around the initiation codon. Using the polymerase chain reaction, an ideal SD sequence was introduced upstream of the initiation codon of *strB* and the stability of the secondary structure of the mRNA was reduced. *Escherichia coli* SMB10, harboring the modified *strB*, was resistant to 100 µg/ml streptomycin and overexpressed the StrB protein. Streptomycin was reacted with crude enzyme extracts of StrA and StrB and the respective phosphorylated streptomycins were purified. The proteins produced by *strA* and *strB* were identified as streptomycin-3"-phosphotransferase and streptomycin-6-phosphotransferase respectively, by ^{13}C-NMR analysis of the respective streptomycin products.

A491

SUPPRESSION OF *Stagonospora nodorum* ON WHEAT WITH SYSTEMIC SEED TREATMENTS. Barry M. Cunfer, Department of Plant Pathology, Georgia Station, University of Georgia, Griffin, 30223.

Wheat seed, naturally infected with *Stagonospora nodorum*, was treated with carboxin + thiram or difenoconazole in 1991, and triadimenol or difenoconazole in 1992. Treated seed and nontreated seed were planted in mid-November in the field. Leaves were assayed on a selective medium from January to March. *S. nodorum* was detected in the control on each assay date, but was not detected in difenoconazole-treated plants 81 and 67 days after planting during the two seasons. Carboxin + thiram did not provide adequate control. Leaf colonization on plants treated with difenoconazole and triadimenol was 10-fold (1.3%) and 100-fold less (0.1%), respectively, than the control 125 days after planting (March 23). Difenoconazole reduced glume blotch and seed infection of harvested grain (P=0.05). Seed treatments which control *S. nodorum* from time of planting to early spring in the southeastern U.S. may be an important addition to an integrated disease management system.

A492

FUNGI ASSOCIATED WITH ROOT ROT OF NON-IRRIGATED WHEAT IN THE HIGH VALLEYS OF MEXICO. G. Leyva M. and E. Villaseñor, M. Chapingo University, CEVAMEX INIFAP. Mexico 56230.

Wheat production under non-irrigated conditions has been gradually increased in the high Valleys of Mexico as a good alternative for crop diversification, but disease problems, particularly root and collar rots caused by fungi are important limiting factors. In order to identify the main fungi causing rots 20 fields under different crop rotation systems were sampled; the samples consisted of seedlings, roots with soil and collar rotted plants. The samples were washed off with tap water, sterilized in sodium hypochloride and incubated on PDA and CMA (corn meal agar) media. In this way 7 fungi were isolated, among which the ones associated with more severe damaged substrates were: Pythium spp., Fusarium graminearum, Gaeumannomyces sp. and Rhizoctonia solani. Damage on Temporalera M-87 and Toluca F-73 cultivars were estimated as 36% and 32%, whereas on Mexico M82 and Galvez M87, it was figured out as 3% and 6%, respectively.

A493

EFFECTS OF LEAF DISEASES, OCCURRING DURING THE RAINY SEASON, ON THE YIELD OF WHEAT IN THE HIGH VALLEYS OF MEXICO. G. Leyva M. and. E. Villaseñor M. Chapingo University, CEVAMEX, INIFAP. Mexico. 56230

In the high valleys of Mexico corn is gradually being substituted by wheat because of its higher productivity down there. However, there are some problems that affect its potential yield, among which light rains, early frosts, deficient adaptability of the varieties and diseases are to be mentioned. The most common diseases are those that attack leaves, inciting different types of symptoms and variable damages. To estimate losses due to leaf diseases an experiment was established using a random blocks design with split plots. The big plot was divided in two parts, one of which was protected with fungicides and the other one ramained unprotected. In the small plot three different wheat varieties were evaluated. The main pathogenic fungi inciting leaf diseases were identified as Septoria tritici, Helminthosporium tritici-repentis and Fusarium nivale, with an 8% incidence and 80% severity. Yield loss was 57%.

A494

EFFECTS OF FUNGICIDES, ROW WIDTH, AND SEEDING RATE ON POWDERY MILDEW SEVERITY AND YIELD OF WHEAT. P. E. Lipps and A. L. Johnston; Dept. of Plant Path. OARDC/Ohio State University, Wooster, OH 44691.

Two cultivars (Becker and Cardinal) were used in three field experiments (Hoytville, 1991, and Wooster, 1991 and 1992), to determine the effects of fungicide treatment (Baytan 30 seed treatment at 0.3 g/kg seed, Bayleton foliar treatment at 140 g a.i./ha, and untreated control), row spacings (18, 36 and 54 cm) and seeding rate (40 and 80 seeds/m of row) on severity of powdery mildew, caused by *Blumeria graminis* f. sp. *tritici*, and yield. Seeding rate and row width did not have a consistent significant effect on the area under the powdery mildew disease progress curve (AUDPC) across experiments. Bayleton treated plots had significantly (P= 0.05) lower AUDPC values compared to the control in five, and Baytan in three of six cultivar by experiment comparisons. Generally, highest yield of either cultivar was achieved with 80 seeds/m row in 18 cm row spacing and a foliar application of Bayleton.

A495

INCIDENCE AND IDENTIFICATION OF *PYTHIUM* SPP. PATHOGENIC ON SOFT RED WINTER WHEAT IN ARKANSAS. M. L. Rhoads, C. S. Rothrock and E. A. Milus, Department of Plant Pathology, University of Arkansas, Fayetteville, 72701.

Previous studies determined that Pythium root rot is the most common root disease of soft red winter wheat in Arkansas. Incidence of *Pythium* species was determined to elucidate the role of this genus on wheat production in the southeastern U.S. *Pythium* isolates from Keiser and Kibler in 1990 and 1991 were grouped by growth rate and colony morphology, and representative isolates were selected for pathogenicity tests. Pathogenicity was determined by rating emergence and seedling height in growth chamber assays. Representative isolates from pathogenic groups were identified to species, and incidence of each species was based on the original sample size. *P. irregulare* was the most prevalent species, averaging 45% over three locations-years, but was not found at Keiser in 1991. *P. torulosum* comprised 51% of the pathogenic isolates when *P. irregulare* was absent, but it too was absent in two location-years. Incidence of *P. monospermum*, the most consistently found species, ranged from 12-25% of the pathogenic isolates over four location-years. Other *Pythium* species included *vanterpoolii* (1-20%), *graminicola* (0-30%), *arrhenomanes* (0-20%), *ultimum* (0-18%), *aristosporium* (0-7%), and *sylvaticum* (0-3%). Large variations in incidence of *Pythium* species may help explain differences in root rot severity or efficacy of control measures.

A496

VIRULENCE OF XANTHOMONAS CAMPESTRIS PV. TRANSLUCENS ON SELECTED WHEAT CULTIVARS. E. A. Milus and D. B. Chalkley. Department of Plant Pathology, University of Arkansas, Fayetteville 72701.

Although wheat cultivars resistant to bacterial streak caused by Xanthomonas campestris pv. translucens have been identified, previous reports of strain-cultivar specificity suggested that races capable of attacking resistant cultivars may exist. In this study, strains of X. c. translucens from North and South America were tested for virulence on five susceptible and 14 resistant cultivars. Seedlings were inoculated with 81 strains isolated from wheat, barley, rye, or triticale and rated for watersoaking after 8 days. Averaged over all cultivars, 69 strains caused 14.4-38.1% watersoaking and were classified as virulent, and 12 strains caused 1.1-4.5% watersoaking and were classified as weakly virulent. Among the virulent strains, none were found to be virulent on a resistant cultivar or avirulent on a susceptible cultivar. Thus, there was no evidence for races of X. c. translucens among the strains and cultivars used in this study. Cultivars rated as resistant to bacterial streak in the field are likely to remain resistant relative to susceptible cultivars, although actual disease severities may vary due to differences in inoculum level or environmental conditions.

A497

REACTIONS OF *TRITICUM* SPECIES AND RELATED GENERA TO BARLEY CROWN RUST. Y. Jin[1], B. J. Steffenson[1], D. M. Wesenberg[2], and H. E. Bockelman[2]. [1]Dept. of Plant Pathology, North Dakota State Univ., Fargo, ND 58105, and [2]USDA-ARS, Aberdeen, ID 83210.

Evaluation of species in the tribe Triticeae to the barley crown rust pathogen (a variety of *Puccinia coronata*) revealed that many species in *Triticum*-related genera were susceptible, including those in *Aegilops*, *Agropyron*, *Elymus*, *Elytrigia*, *Hordeum*, and *Secale*. However, most species in *Triticum* were resistant. Six hundred random accessions of *Triticum aestivum* and *T. durum* were evaluated at the seedling stage and 2% were susceptible to the crown rust pathogen. In contrast, 11% of the 549 accessions of hybrids from wheat-*Agropyron*, wheat-*Elymus*, and wheat-*Secale* ("Sando" collection) were susceptible. The higher frequency of susceptibility in the hybrids suggests that interspecific crossing may introduce crown rust susceptibility into wheat. The genetic relation of wheat and wheat relatives in reaction to the crown rust pathogen is being investigated.

A498

RELATIONSHIP AMONG SYMPTOMS OF BACTERIAL STREAK OF WHEAT CAUSED BY *XANTHOMONAS CAMPESTRIS* pv. *TRANSLUSCENS* AND THEIR CORRELATION TO YIELD. B.L. Tillman, J.S. Russin, S.A. Harrison and C.A. Clark. Departments of Agronomy and Plant Pathology & Crop Physiology, LSU Agricultural Center, Baton Rouge, LA 70803.

Three symptoms of bacterial streak have been reported on wheat: leaf streaks, peduncle lesions, and black chaff. However, the relationship of these symptoms to each other and the effect of each symptom on yield are not well documented. In 1992, yield loss in five varieties ranged from about 14% for Florida 304 to 0% for Coker 9227. Mean yield differences between inoculated and control plots were negatively correlated with mean differences in flag leaf ratings at Feekes 10.5.3 to 10.5.4 (-.72, n = 5, P = 0.17), but not with ratings at Feekes 10.5.4 to 11.1 or 11.1 to 11.2. Differences in the number of peduncles with lesions per foot of row were positively correlated with differences in yield (.83, n = 5, P = 0.08). Number of peduncles with lesions per foot of row was not correlated with flag leaf ratings at any growth stage for Coker 9766, Coker 9877, Florida 304 or Terral 101. In contrast, the number of peduncle lesions per foot of row in Coker 9227 was positively correlated to flag leaf ratings at all three growth stages (.76, .77, .94, n = 5, $P \leq 0.03$). These results suggest that flag leaf ratings during Feekes 10.5.3 to 10.5.4 are most related to yield loss and that peduncle lesions have little relationship to yield loss in most cultivars.

A499

OPTIMIZATION OF FUNGICIDE USE FOR THE CONTROL OF LEAF RUST AND POWDERY MILDEW OF WHEAT. Celsa Garcia[1], Steven Leath[1,2], Jack Bailey[1], and Marcia L. Gumpertz[3], Dept. of Plant

Pathology[1], USDA-ARS[2], and Dept. of Statistics[3], N.C. State University, Raleigh, NC 27695

A computer model was constructed as a decision aid for the control of foliar diseases of wheat and to maximize profitability of wheat production in the southeastern United States. An upgraded model, currently near completion, will also optimize fungicide use. The program computes economic returns of applying a fungicide spray by comparing application costs to the projected costs of yield reduction due to uncontrolled disease. Program inputs include cultivar type planted, field history, growth stage of wheat, and amount of leaf rust and/or powdery mildew observed. Advisory outputs are based on computations from a series of linear regression equations that relate amount of disease to predicted yield loss. The program was designed to be used in different environments by including a weather variable which can be adjusted for the circumstances. A menu driven interface, programmed in C language is utilized.

A500

FUNGI ASSOCIATED WITH ROOT AND CROWN ROT OF WINTER WHEAT IN ALABAMA. D. J. Collins, C. Chen, and L. Dalrymple, Department of Plant Pathology, Alabama Cooperative Extension Service and Alabama Agricultural Experiment Station, Auburn University, AL 36849-5409.

Wheat is an important and widely grown winter grain in Alabama, however little is known on the distribution and prevalence of root and crown pathogens of this crop. In 1992 and 1993 over 50 wheat fields throughout the state were surveyed to identify soilborne pathogens that occur on winter wheat in Alabama. Diseased root and crown tissues were washed in tap water, cut in 1 cm sections, surface sterilized, and plated on both PDA amended with antibiotic and water agar. The most prevalent fungi isolated to date were species of *Rhizoctonia*, *Bipolaris*, and *Fusarium*, and *Gaeumannomyces graminis* var. *tritici*.

A501

YIELD LOSSES IN SWEET CORN INOCULATED WITH BARLEY YELLOW DWARF VIRUS. R. L. Itnyre[1], C. J. D'Arcy[1], W. L. Pedersen[1], A. D. Hewings[2], and L. E. Sweets[3], [1]Department of Plant Pathology and [2]USDA ARS, University of Illinois, Urbana, IL 61801 and [3]Pillsbury/Green Giant, Le Sueur, MN 56058.

Five sweet corn hybrids were planted on May 20 and June 20, 1992 and inoculated with BYDV-RMV-IL one month after planting. Visual symptoms, percent infection based on DAS-ELISA, and ear weight were measured. For the early planting date, few plants were symptomatic or positive in DAS-ELISA and ear weights did not differ between inoculated and control plots. For the late planting date, the number of infected plants of two hybrids were significantly greater in inoculated plots than control plots and ear weights of two other inoculated hybrids were about 25% lower than in control plots. Symptom expression did not differ between inoculated and control plots. BYDV-RMV-IL infection caused significant yield loss of 25% and susceptibility was increased in later plantings of some hybrids. Sweet corn ear weight loss due to BYDV-RMV-IL infection could not be predicted from symptomatology or DAS-ELISA results.

A502

EVALUATION OF RANDOM VS. FOCUS INOCULATION ON YIELD AND RELIABILITY OF VISUAL VS. SEROLOGICAL ASSESSMENT OF INCIDENCE OF BARLEY YELLOW DWARF VIRUS IN SPRING OAT. A. D. Hewings[1,2], E. M. Bauske[4], & S.M. Bissonnette[3]. [1]USDA ARS, [2]Dept. Plant Pathology, [3]Coop. Ext. Service, Univ. Illinois, Urbana, IL 61801, & [4]Dept. Plant Pathology, Auburn Univ., Auburn AL 36849.

Using yield plots as experimental units, the relationship between yield and inoculation method was determined for Noble and Ogle spring oat. Plots were inoculated with four levels of barley yellow dwarf luteovirus (BYDV-PAV-IL) using two techniques. Foci of infection were created by placing viruliferous *Rhopalosiphum padi* at 0, 1, 2, or 3 selected points within each plot. Random infections were created by shaking viruliferous vectors over the plots. Similar numbers of vectors were used for each infection level in both focus and random inoculation plots. Preliminary covariate analysis indicated that the two methods are effective in establishing different infection levels and that no inoculation technique by disease incidence interactions were observed. In a second study, disease incidence was determined visually and compared with incidence by ELISA. At 2-wk intervals, 20 leaves from each plot were randomly collected, evaluated for symptoms and analyzed by ELISA. Visual evaluations of individual leaves underestimated incidence; symptom expression was only a fair to good indicator of BYDV-PAV infection. These studies confirmed that visual disease assessment of whole plots at growth stage 10.5 had a significant relationship to yield.

A503

SCAB INCIDENCE, STANDARD GERMINATION AND FIELD EMERGENCE IN VARIOUS SPRING AND WINTER WHEAT SEED LOTS. D. Gallenberg, B. Turnipseed, C. Granier and S. Stein. Dept. of Plant Science, South Dakota State University, Brookings, SD 57007.

During 1992, studies were conducted to determine the relationship between scab incidence, standard germination and field emergence in various wheat seed lots harvested in South Dakota in 1991. A total of 46 seed lots from 5 spring wheat and 2 winter wheat cultivars were initially chosen for testing based on standard germinations which ranged from 46 to 99 %. Scab incidence in these same lots was determined by plating on PDA and ranged from 0 to 25 %. Field emergence was determined in single row, hand planted plots and ranged from 30 to 83.5 %. Correlations between scab, germination and emergence were determined.

A504

FOLIAR SYMPTOMS ASSOCIATED WITH INFECTION OF PINTO BEAN BY APHELENCHOIDES RITZEMABOSI IN WYOMING. Gary D. Franc and Colette M-S. Beaupré, Dept. of Plant, Soil and Insect Sciences, University of Wyoming, Laramie, WY 82071-3354.

Field observations made in north central Wyoming revealed that pinto bean plants (Phaseolus vulgaris L., cultivar Othello) infected by Aphelenchoides ritzemabosi (Schwartz, 1911) Steiner & Buhrer, 1932 had developed numerous dark, angular lesions on leaves and, occasionally, a superficial necrosis on the upper surface of the petiole. Inoculated unifoliate and trifoliate leaves of pinto bean plants (cultivar Othello), grown in a growth chamber, developed angular lesions after ca. 11 days at 22 C that were similar in appearance to those observed in the field. The discoloration associated with angular lesions became more obvious 14 to 20 days after inoculation. Expansion of individual angular lesions was limited by leaf veins with most lesions ranging in size from several millimeters to ca. one centimeter. Occasionally, entire inoculated leaves became chlorotic or necrotic within 24 days after inoculation.

A505

DETECTION OF PHYTOPHTHORA SPP. IN VEGETABLE CROPS BY IMMUNOASSAY. S. A. Miller, R. Bhat and A. F. Schmitthenner. Dept. of Plant Pathology., OARDC/The Ohio State University, Wooster, OH 44691.

Phytophthora spp. were readily detected in naturally infected pepper, summer squash, cantaloupe and pumpkin tissue by using commercial immunoassays (Agri-Diagnostics Assoc., Cinnaminson, NJ). Both the 96-well microtiter plate immunoassay and the 10-minute flow-through assay were effective in detecting Phytophthora spp. in these tissues. Immunoassay results were closely correlated with isolation of Phytophthora spp. on a selective medium. The predominant Phytophthora species isolated from these tissues was P. capsici, although P. cactorum was isolated from one pumpkin fruit sample. Multiple isolates of P. capsici were collected from different fields and the compatibility type of each isolate was determined. Both the A1 and A2 compatibility types were found in all fields sampled. P. capsici was detected both by a pepper seedling bioassay and by immunoassay in soil from three of four sampled fields.

A506

SEVERE OUTBREAK OF PHYTOPHTHORA BLIGHT AND FRUIT ROT OF CUCURBITS IN FLORIDA. R.J. McGovern[1], J.P. Jones[2], D.J. Mitchell[3], R.A. Pluim[1] and P.R. Gilreath[4]. University of Florida, Plant Pathology Department, Immokalee, FL[1], 33934, Bradenton, FL, 34203[2], Gainesville, FL 32611[3], Horticultural Sciences Department, Palmetto, FL, 34221[4].

An unusual and severe blight and fruit rot of cantaloupe, squash and watermelon caused by the fungus *Phytophthora capsici* Leonian occurred in southwest and west central Florida during April, 1993. Symptoms included round to irregular, watersoaked lesions on leaves, and dieback of shoot tips of all 3 cucurbits, wilting and death of squash, and fruit rot of watermelon. Plant death and fruit rot in excess of 90% were observed, respectively, in squash and watermelon fields. In general, zoospore suspensions (10^5/ml) of 3 *P. capsici* isolates from watermelon fruit, and squash and cantaloupe foliage were similarly pathogenic to the foliage and/or fruit of cantaloupe, chayote, cucumber, eggplant, papaya, summer squash, tomato, and wild and cultivated watermelon maintained in humid environments at day/night temperatures of 27/14C. Squash plants were killed and tomato foliage was completely blighted 72 hours after inoculation. These 3 *P. capsici* isolates were nonpathogenic to Madagascar periwinkle and petunia. Variations were noted in the sensitivity of 11 *P. capsici* isolates to metalaxyl (Ridomil 2E) incorporated into corn meal agar and a *Phytophthora*-selective medium at 1, 10, and 100 ppm.

A507

FUNGICIDE RESISTANCE IN CUCURBIT POWDERY MILDEW: EFFECT OF FUNGICIDE USAGE AND IMPACT ON CONTROL. M. T. McGrath and H. Staniszewska, Department of Plant Pathology, Long Island Horticultural Research Laboratory, Cornell University, Riverhead, NY 11901-1098.

Powdery mildew development and fungicide sensitivity of *Sphaerotheca fuliginea* were examined in research plots and production fields of pumpkin on Long Island. Of all 69 isolates collected before fungicide treatment in 1992, 27 were resistant to benomyl and one was moderately resistant to triadimefon. In a commercial field, the proportion of resistant isolates shifted within 2 weeks from 0% to 96% for triadimefon and from 10% to 70% for benomyl following 2 applications of triadimefon (Bayleton 50DF). However, control was commercially acceptable because mildew development was suppressed until Sept. In research plots treated with triadimefon plus chlorothalonil, the proportion of resistant isolates shifted from 3% to 100% for triadimefon and from 10% to 44% for benomyl between 14 Aug and 17 Sept. In these plots, the average powdery mildew severity on upper and on lower leaf surfaces was 0% and 2%, respectively, on 9 Sept and 0% and 35% on 18 Sept; in non-fungicide-treated plots severity was 73% and 83%, respectively, on 9 Sept. Fungicide sensitivity also was determined for isolates collected from 2 triadimefon-treated commercial summer squash plantings in Florida. Only 2 of the 69 isolates were benomyl-resistant; 61 were moderately to highly resistant to triadimefon. Disease control in these fields was unsatisfactory.

A508

SCREENING SWEET CORN FOR REACTIONS TO COMMON SMUT. C. Nankam and J. K. Pataky. Department of Plant Pathology, University of Illinois, Urbana, IL 61801.

Two monosporidial lines of *Ustilago maydis* were used to screen sweet corn for reactions to common smut. One replicate of about 50 plants each of 350 hybrids were inoculated once in 1992 by injecting 8 ml of inocula (ca. 5,000 sporidia/ml) in the silk channel with a hand-held Meterjet Spray Gun 2 to 4 days after the mid-silk growth stage. Incidence of ear galls was about 34% (5,593 of 16,284 plants) and varied among hybrids from 0 to 96%. Incidence was greater than 60% for 36 hybrids and below 10% for 54 hybrids. Several hybrids with less than 10% incidence were previously classified resistant based on natural epidemics. In two non-inoculated replicates, incidence was about 0.5% (174 of 32,533) and varied among hybrids from 0 to 20%. None of the hybrids had a higher incidence of ear galls in non-inoculated replicates than in the inoculated replicate. Three replicates of 380 hybrids and 250 breeding lines are being screened in 1993 to further evaluate the ability of this procedure to differentiate resistant and susceptible genotypes in homogeneous and heterogeneous populations.

A511

FACTORS AFFECTING POTATO SEEDPIECE DECAY CAUSED BY ERWINIA CAROTOVORA SUBSP. ATROSEPTICA AND FUSARIUM SPP. R. V. James and W. R. Stevenson, University of Wisconsin-Madison, Department of Plant Pathology, 1630 Linden Dr., Madison, WI 53706.

Decreased plant stand and vigor, serious problems for Wisconsin's potato growers each year, are usually associated with bacterial soft rot of the seed piece, Erwinia carotovora subsp. atroseptica (Eca), but Fusarium spp. can also be involved. In over a decade of field research, and three years of research in growth chambers, we have evaluated the effect of cultivar, soil type, temperature of potatoes at cutting, healing (temperature and duration), soil temperature and moisture at planting and during emergence, inoculation (Eca and Fusarium spp.), bruising, and chemical treatments on seedpiece decay, emergence and vigor. Chemical treatments generally are effective only for Fusarium. Procedures that allow natural healing of cut seedpieces decrease bacterial decay significantly but do not provide reliable control of Fusarium decay. In general, decay was greatest and stand poorest when soil was saturated after planting, regardless of soil type, pathogen, cultivar, pre-plant treatment, or soil temperature. To use these data for site-specific recommendations, an expert system is being developed. Data from over 25 grower fields and field trials at four locations, representing a diverse array of cultural and environmental factors, are being used to verify the prototype system.

A512

RESPONSE OF TOMATO CULTIVARS TO TWO FRUIT INOCULATION PROCEDURES WITH COLLETOTRICHUM COCCODES AT DIFFERENT STAGES OF FRUIT MATURITY. B. A. Fulling, E. C. Tigchelaar, and R. X. Latin. Purdue University, West Lafayette, IN 47907.

Fruits of three tomato cultivars with different levels of anthracnose resistance were inoculated at various maturity stages with an isolate of Colletotrichum coccodes. A specific site on the side of each fruit was wound inoculated in one set of experiments and surface inoculated in a second set. Fruits remained on the plants during the growing season and were examined every three days for development of anthracnose lesions. In the wound inoculation study, the percentage of sites with anthracnose lesions increased with fruit maturity at the time of inoculation for all three cultivars. The most resistant cultivar had a lower percentage of sites with lesions than the other two cultivars on fruit inoculated at the mature-green or ripe stages. Preliminary results of surface inoculations indicate that fruit maturity at the time of inoculation had no effect on the percentage of sites with anthracnose lesions for the susceptible cultivar. Resistant cultivars had fewer lesions on fruit inoculated at the immature-green stage than at the mature-green or ripe stages. Most lesions developed only after the fruit had ripened regardless of the stage of maturity at which the fruit was inoculated. The surface inoculation procedure may provide a more complete description of the C. coccodes / tomato cultivar interaction over time than the wound inoculation procedure.

A514

IDENTIFICATION OF MATING TYPES AND METALAXYL SENSITIVITY IN NORTH AMERICAN POPULATIONS OF *PHYTOPHTHORA INFESTANS*. K.L. Deahl, S.P. DeMuth, and A. Rivera-Peña, USDA, ARS, Vegetable Laboratory, Beltsville, MD 20705-2350, and Toluca Research Station, Metapec, Mexico.

The A^2 mating type of *P. infestans* was first reported in the United States in 1990. Concurrently, pathogenic strains resistant to metalaxyl were first reported in the Pacific Northwest. Collaborative surveys were undertaken to investigate the occurrence of mating types and sensitivity to metalaxyl in North American populations of *P. infestans* isolated from outbreaks of late blight in potato and tomato crops. Results from *in vitro* tests indicated that most isolates from the northeastern United States and Atlantic Canada were metalaxyl sensitive (MS) and A^1 mating types. Metalaxyl resistance (MR) and A^2 mating types were identified in a few tomato isolates from North Carolina. Seventy-five percent of 87 strains from late blight epidemics in Florida and Texas were both MR and A^2 strains. Although the majority of strains from the Pacific Northwest were MR, only 3 of 134 were A^2 mating types. By contrast, eighty-five percent of isolates from central Mexico were MR and A^2 mating types. The correlation of mating types with resistance to metalaxyl will be discussed.

A515

CLIMATIC FACTORS INFLUENCING HISTORICAL OUTBREAKS OF PUCCINIA GRINDELIAE ON GUTIERREZIA SPP. IN NEW MEXICO AND ARIZONA. C. A. Waddell and C. M. Liddell. New Mexico State University, Department of Entomology, Plant Pathology and Weed Science, Box 3BE, Las Cruces, NM 88003

ABSTRACT: *Puccinia grindeliae* is native to the southwestern United States and, based on recent field surveys, is currently widespread throughout New Mexico and Arizona on *Gutierrezia* spp. We studied the outbreaks of *P. grindeliae* by examining 1,024 dried *Gutierrezia* specimens, collected over the past 100 years. There were 21 diseased specimens of 469 from New Mexico and 10 diseased specimens of 129 from Arizona. Diseased specimens were also collected from California, Colorado, Kansas, Nevada, Texas, Utah, Wyoming and Mexico. Logistic analysis of historical climate data against the proportion of diseased specimens collected from 1891-1991, showed that the probability of collecting a diseased specimen was positively correlated ($\chi^2 = 4.23$, $P < 0.04$) with the product of precipitation in the quarter of collection and mean temperature in the preceding spring quarter. Past epiphytotics of *P. grindeliae* on *G. sarothrae* were during 1906-1907 and 1935-1938, corresponding to conducive climate patterns.

A516

FREQUENCY OF FUNGICIDE RESISTANT STRAINS OF *BOTRYTIS CINEREA* IN WESTERN OREGON SMALL FRUIT AND SNAP BEAN CROPS. K. B. Johnson, T. L. Sawyer, and M. L. Powelson. Dept. of Botany and Plant Pathology, Oregon State University, Corvallis, OR 97331-2902.

In 1990 and 1991, 3496 isolates of *Botrytis cinerea* were collected from grape, raspberry, snap bean, strawberry, and volunteer Himalayan blackberry plantings in Oregon's Willamette Valley. The percentage of isolates resistant to the benzimidazole fungicide, benomyl (5 μg/ml), or the dicarboximide fungicide, vinclozolin (10 μg/ml), averaged 52 and 17% respectively. Frequency of resistance to either fungicide was highest in *B. cinerea* isolates obtained from strawberry. Isolates sampled from nonsprayed, volunteer blackberry that were growing along roadways and fence lines averaged 38% and 6% resistance to benomyl and vinclozolin, respectively. Fungicide application records were obtained for 74 commercial plantings. Frequency of benzimidazole- and dicarboximide-resistant strains of *B. cinerea* in a planting were significantly correlated ($P < 0.05$) with the number of applications of fungicides in each respective class. For strawberry, the frequency of dicarboximide-resistance was estimated to increase by 21% per fungicide application compared to 6% per application in the other crops.

A517

ANALYSIS OF DISEASE CYCLES OF SORGHUM ERGOT IN RELATION TO PLANT FLOWERING. R. Bandyopadhyay, X.B. Yang, and M.V. Satyanarayana. Cereals Pathology, ICRISAT, India, and Dept. of Plant Pathology, Iowa State Univ., Ames, 50011.

Experiments were conducted to quantify the disease cycles of sorghum ergot during flowering seasons. Number of infections per day was determined by counting the individual infected spikelets in relation to date of flowering. Sorghum flowering continued for 24 days with a peak in 2-4 days after initial flowering. The first 10 days of flowering accounted for 60% of total infections. Proportion of infected plants in total flowering plants per day increased from 40% on 7 days to 100% on 20 days after flowering. Number of infected spikelets/plant increased from 40 on 7 days to 180 on 20 days after flowering, indicating an increase of inoculum from secondary disease foci. Disease cycles were identified by peaks of infection curves during flowering. The two years results suggest that there were 2-3 infection cycles, each 6-8 days duration, in a flowering season. From a point source, the disease spread over a field of 33 by 33 m, resulting in more than 25% plant infection.

A519

FORECASTING WHEAT LEAF AND STEM RUST DEVELOPMENT IN EGYPT. M. G. Eversmeyer, C. L. Kramer, and S. Sherif, USDA-ARS, Dept. of Plant Pathology and Division of Biology, KSU, Manhattan, KS 66506.

Wheat leaf and stem rust severities in Egypt were regressed against temperature, relative humidity, precipitation, and deviations of each weather variable from a 10-year average. Variables were averaged over a 2-30 day period prior to the date of prediction. The crop and epidemic year was assumed to begin at physiological maturity of the prior wheat crop. Models were developed for every day of the crop year. However, the daily models which explained the most variation (R^2 = 0.89-0.99) in final severity used data averaged over 10 days prior to prediction date. Models were developed for the oversummering, stand establishment, overwintering, and spring epidemic periods of the crop year. Forecasts of final severity were within 15% of the actual severity.

A520

STRAWBERRY DENSITY EFFECTS ON COLLETOTRICHUM ACUTATUM DISPERSAL BY SIMULATED RAIN. M. A. Boudreau and L. V. Madden, Dept. of Plant Pathology, OARDC/The Ohio State University, Wooster 44691.

Greenhouse-grown strawberry plants were mounted in support structures buried in soil to create artificial two-row canopies at 2, 3, 4, and 8 cm intra-row spacing. Infected fruit placed either within or between the rows provided a spore source for dispersal by simulated rain, generated at constant intensities of 15 or 30 mm/hr. Spore deposition, evaluated on petri plates exposed every 5 min and integrated over a period of 46 min, declined with increasing density ($P<.05$). Largest decreases (>300%) occurred between the two intermediate densities. Density affected deposition on plates both within and between rows. Within-canopy inoculum placement and low rain intensity also reduced deposition, and interaction between these factors occurred for spores trapped within but not between rows ($P<.05$). Deposition gradients on plates between rows were described well by a negative exponential model. Gradient slope was not affected by any factor, but intercept was lowered by increased density, in-canopy inoculum, and less intense rain ($P<.05$). Results suggest canopy density affects spore removal and transport, and can influence dispersal between sources and targets not within the canopy.

A521

DETERMINATION OF THE ENVIRONMENTAL FACTORS REQUIRED FOR SPORULATION OF BOTRYTIS CINEREA ON STRAWBERRY LEAF RESIDUES. M. Sosa-Alvarez, L.V. Madden, and M.A. Ellis. Dept. of Plant Pathology, OARDC/The Ohio State Univ., OARDC, Wooster, OH 44691

Dead strawberry leaf discs were inoculated with a conidial suspension of B. cinerea and incubated at 5, 10, 15, 20, 25 and 30 C. Sporulation (conidia/cm^2) on leaf discs was calculated after exposure to wetness durations of 3, 5, 7, 9, and 11 days. The optimum temperature for sporulation was between 15 and 20 C, with sporulation levels of $1.2x10^6$ and $2.0x10^6$ conidia/cm^2, respectively, after 7 days of wetness duration. As temperatures increased or decreased from the optimum, the rate of sporulation decreased with $3.7x10^2$ and $1.4x10^4$ conidia/cm^2 at 25 and 5 C, respectively, for the same wetness duration. No sporulation was observed at 30 C. Data on sporulation from "in vitro" studies will be used to develop a model for predicting the sporulation of B. cinerea on strawberry leaf residues in the field.

A522

COMPARISON OF CONIDIAL GERMINATION AND STRAWBERRY FRUIT INFECTION BY THREE COLLETOTRICHUM SPECIES. L. L. Wilson, L. V. Madden, M. A. Ellis, Dept. of Plant Pathology, OARDC/The Ohio State Univ., Wooster, OH 44691.

Studies with Colletotrichum acutatum (Ca), C. gloeosporioides (Cg), and C. fragariae (Cf) were conducted to determine why Ca generally has been the most predominate species causing anthracnose fruit rot of strawberry. Conidia germination on a selective medium averaged 93% for Ca, 53% for Cf and 28% for Cg. Immature attached fruit were inoculated with conidial concentrations of each species ranging from 250 to $2.5x10^6$ conidia/ml. Inoculated fruit were incubated at 25 C for 12 h under constant wetness. At a density of $2.5x10^4$ conidia/ml, Ca resulted in ~80% fruit infection. Cg and Cf resulted in only 21 and 16% fruit infection, respectively. Current studies are comparing fruit infection at a range of temperatures, by isolates of the three species from different parts of the U.S. Preliminary results suggest that Ca is the most aggressive of the three species.

A523

COMPUTER ASSISTED EMPIRICAL MODEL FOR PREDICTION OF THE OUTBREAK OF ALTERNARIA BLOTCH OF APPLE. N. Filajdić, and T. B. Sutton. Dept. of Plant Pathology, NC State University, Raleigh, NC 27695.

Empirical models to predict the final defoliation based on disease incidence and the number of days from tight cluster were developed for orchards with high and moderate severity of Alternaria blotch (*Alternaria mali*). Disease incidence, severity, and defoliation were assessed in 2 orchards in western North Carolina from 1991-1992. Percent defoliation before harvest was highly correlated with disease incidence and severity recorded in mid-June. Only disease incidence was used in model development. The model for orchards with a history of severe Alternaria blotch is: defoliation (%) = -63.7 + (0.3 x days from tight cluster) + (0.8 x disease incidence %); whereas for orchards with moderate disease history it is: defoliation (%) = -9.0 + (0.1 x days from tight cluster) + (0.2 x disease incidence %). A menu-driven computer program, written in

TURBO PASCAL for MS DOS, calculates the final percent defoliation based on orchard disease history and current disease incidence and issues recommendations regarding the need for and time of first spray of iprodione.

A524

USE OF PATH COEFFICIENT ANALYSIS TO DETERMINE RELATIVE IMPORTANCE OF ENVIRONMENTAL VARIABLES TO OUTBREAKS OF ALTERNARIA BLOTCH OF APPLE. N. Filajdić, and T. B. Sutton. Dept. of Plant Pathology, N.C. State University, Raleigh, NC 27695.

The correlations between mean temperature (T), relative humidity, leaf wetness duration (LWD), number of rain events, and amount of rain and 3 measures of foliar disease (incidence, severity and defoliation) caused by *Alternaria mali* were examined for data from 2 orchards: McKay in western NC and the Central Crops Research Station (CCRS) in central NC. Cumulative and mean values of each environmental variable were correlated with different aspects of disease and path coefficient analysis was used to calculate direct and indirect effects of each variable on disease intensity. Cumulative daily temperature, cumulative LWD and T had the greatest total effects on all disease measurements at McKay orchard. The T in 1991 and 1992 was not different between the 2 orchards. However, mean LWD from both dew and rain was 11.2 h at McKay and only 4.4 h at CCRS in the 2-wk period before disease outbreak. LWD may be the most important determinant of disease intensity.

A525

SPATIAL PATTERN OF ALTERNARIA BLOTCH OF APPLE. N. Filajdić, and T. B. Sutton. Department of Plant Pathology, North Carolina State University, Raleigh, NC 27695.

The spatial pattern of Red Delicious trees affected with Alternaria blotch within a commercial orchard in western North Carolina was examined in 1993. The orchard was divided into quadrats, each with 40 Red Delicious trees each bordered by rows of Golden Delicious. Every fourth and sixth tree in the second and third row of each quadrat was sampled. One terminal was selected arbitrarily from each tree and severity and incidence of Alternaria blotch were estimated four times during the growing season. Three criteria were used to separate trees into two classes : (1) statistically different disease intensity; (2) an economic threshold; and (3) comparison with a sprayed control. Data were analyzed using two-dimensional-class analysis (2DCLASS). Results were similar for all three criteria used to separate disease classes. Trees with greater disease intensity were concentrated mainly at the edges of the orchard.

A526

SITE AND MODE OF INFECTION OF ANISOGRAMMA ANOMALA IN EUROPEAN HAZELNUT (CORYLUS AVELLANA). J. N. Pinkerton[1], J. K. Stone[2], S. J. Nelson[2], and K. B. Johnson[2],J. [1]USDA ARS Horticultural Crops Research Laboratory, Corvallis, OR 97330, and [2]Department of Botany and Plant Pathology, Oregon State University, 97331-2902

Eastern filbert blight caused by *Anisogramma anomala* is epidemic in Oregon and threatens its hazelnut industry. Experiments have shown that trees are susceptible when juvenile vegetative tissue is present after bud break in spring through shoot elongation. To ascertain the location of infection, ascospores stained with calcofluor were inoculated on terminal buds, the first three internodes, and the four youngest leaves. Under an epifluoresence microscope, spores were observed at the base of trichomes on juvenile stem tissue and to a lesser extent on leaves and bracts. After 5 days, a few spores were observed with germ hyphae <10 µ long; however, no hyphae were observed extending across the plant surface. Germination *in vitro* was via the lateral wall of ascospores with short germ hyphae expanding into 10-20 µ vesicles by 72 hr at 20 C. Hyphae proliferated from vesicles after 120-168 hr. TEM studies of infection events showed that ascospores were attached to the host tissue with an extracellular material. Germ hyphae developed from the lateral walls of ascospores and directly penetrated surface parenchyma cells of very young stem tissue. After 4 days vesicles were observed in these cells. Older stem tissues with a well-developed epidermis and cuticle were not susceptible to infection.

A527

SPATIAL DISTRIBUTION OF POTATO SCAB. D. Liu, L. L. Kinkel, and N. A. Anderson. Department of Plant Pathology, University of Minnesota, St. Paul, MN 55108.

Tubers were sampled from 42 random sites (4 plants/site, cv. Pontiac) in a 2-acre field at the University of Minnesota Sand Plains Experiment Station, Becker, MN in 1991. The average number of 3, 4, and 5 type scab lesions per tuber harvested from each site was determined (3 = lesion breaks periderm, 4 = pit lesion, 5 = deep pit lesion). The Chi-square goodness-of-fit test showed that the observed frequency distribution of number of lesions per tuber was better described by a negative binomial distribution ($p<0.005$) than a Poisson distribution ($p>0.25$), demonstrating that the distribution of potato scab in this field was aggregated. There was an increasing gradient in disease levels from the west to east end of the field presumably due to previous potato cultivation.

A528

DISSEMINATION OF SEPTORIA LYCOPERSICI SPORES DURING RAIN EVENTS. Parker, S.P., Gleason, M.L., and Nutter, F.W., Jr. Iowa State University, Plant Pathology, 351 Bessey, Ames, Iowa 50011.

Six-wk-old potted tomato plants were placed at 30-cm intervals up to 180 cm from an inoculum source in the field for periods of 24 hours on five rain dates and five non-rain dates. The mean number of lesions per leaf on plants exposed on rain dates ranged from 70 to 1680, whereas 0.0-0.46 lesions per leaf developed on plants exposed on non-rain dates. The number of lesions per leaf was linearly related to total rainfall which coincided with 24-hour exposure periods (R^2=0.86, prob>F=0.0001). Following spray-inoculation of the center plant in each row, percent defoliation within rows of tomato plants increased at the apparent infection rate of 0.282±0.015 units per day. The study confirms that rain is the primary mechanism for spore dissemination and an important factor contributing to the development of Septoria leaf spot epidemics in tomatoes.

A529

A DEVICE FOR QUANTITATIVE DEPOSITION OF PROPAGULES OF *PYRENOPHORA TRITICI-REPENTIS* ONTO LEAVES OF WHEAT (*TRITICUM AESTIVUM L.*). C. K. Evans, R. M. Hunger, and W. C. Siegerist, Plant Pathology Department, Oklahoma State University, Stillwater, OK 74078-9947.

A device is described with which propagules of *Pyrenophora tritici-repentis* (PTR) are quantitatively deposited onto a defined area of a wheat leaf. This is accomplished by attaching an atomizing sprayer (DeVilbiss Pulmo-Aide Model 5601D) to the top of a clear acrylic plastic cylinder so that the sprayer is 20 cm from a stage of acrylic plastic. This stage supports the wheat leaf which is covered with a plastic cover sheet with a rectangular area removed to expose a specific area of the leaf. With this device, five concentrations of a conidial suspension nearly free of other PTR propagules were inoculated onto wheat leaves and onto specific areas of glass microscope slides for comparison. All tests were repeated once. Conidia counts were made with a stereo microscope and wheat leaf areas were determined with a video imaging system. Analyses indicated the device uniformly deposits PTR propagules, but deposition onto glass slides overestimated the number of propagules deposited onto wheat leaves. This device can be used to estimate infection efficiencies of PTR isolates.

A530

COMPARISON OF METHODS FOR ESTIMATING THE TIMING AND DURATION OF DEW PERIODS. M. L. Gleason, S. E. Taylor, T. M. Loughin, and K. J. Koehler, Departments of Plant Pathology, Agronomy, and Statistics, Iowa State University, Ames, IA 50011.

Two alternative models for estimating the timing and duration of dew periods were tested: 1) relative humidity >90% = dew, and 2) a statistical model utilizing relative humidity, temperature, and wind speed. Hourly data, including wetness as measured by flat-plate electronic sensors, from six Iowa weather stations in 1991 were used to develop the second model. Model 2 used a statistical classification program called CART to eliminate hours in which no dew was likely to occur, followed by stepwise linear discriminant analysis. Both models were validated with 1992 data sets from 15 weather stations in Iowa, Illinois, Nebraska, and Kansas. Of a total of 17,487 hours in which dew was possible, presence or absence of dew was predicted correctly in 78.6% of cases for Model 1 and 83.5% for Model 2. Duration of dew periods was predicted within 2 hr on 67.2% of days for Model 1 and 76.0% of days for Model 2. The results indicate that Model 2 increases the accuracy of dew prediction in comparison to a model that relies solely on relative humidity.

A531

SPATIAL DYNAMICS OF DISEASE SYMPTOM EXPRESSION DURING PHYTOPHTHORA EPIDEMICS ON BELL PEPPER. Jean B. Ristaino, Robert P. Larkin, and C.Lee Campbell. Department of Plant Pathology, North Carolina State University, Raleigh, NC 27695-7616.

Epidemics caused by *Phytophthora capsici* on bell pepper were monitored in two commercial fields in grids of 400 contiguous quadrats, 1 m in length, with 2-3 plants

per quadrat. Incidence of quadrats with plants with wilt, crown lesions, or dead plants increased from 0.5-15.8, 3.0-16.8 and 1.5-15.3% in field 1 and 0.3-16.5, 12.8-23.5 and 10.8-31% in field 2. Incidence of quadrats with plants with stem lesions remained below 2% in both fields until day of the year 211, and never exceeded 16%. Core cluster size for plants with wilt symptoms, crown lesions or dead plants increased from 4-40, 5-17, 2-38, and 5-45, 26-36, and 25-28 distance class units in fields 1 and 2, respectively. In field 1, reflected clusters coalesced with the core cluster of plants with wilt, crown lesions or dead plants. In field 2, reflected cluster size increased from 4-27 and 2-33 distance class units for plants with crown lesions or dead plants but these clusters did not coalesce with the core cluster. In both fields, clusters of plants with stem lesions remained distinct and separated by 1-3 rows. Greater spread of disease occurred within rows than across rows in both fields.

A532

CHARACTERIZATION OF WATERMELON VIRUS EPIDEMICS IN FLORIDA. Mora-A, Gustavo, Webb, S.E., Purcifull, D.E., Zettler, F.W. and Kok-Yokomi, M.L. Plant Pathology Department, University of Florida, Gainesville, FL 32611 and CFREC-Leesburg, Leesburg, FL 34748

Spatial patterns and temporal dispersion of epidemics induced by natural virus infection of watermelon plants were characterized in four experimental plots, 0.2 ha each, at Leesburg, Florida. *Uroleucon* sp. was the most common aphid vector observed visiting on plants (40 of 60 aphids) and in green tile traps (167 of 763 aphids) from 14 April to 20 June, 1993. Aphid flight activity was highest during the first and second week of May. Mosaic symptoms were detected in 1 of 1200 plants on 30 April. ELISA detected watermelon mosaic virus 2 in 97% of 300 plants in one plot on 7 June. Papaya ringspot virus type W and zucchini yellow mosaic virus were not detected. Epidemic rates were estimated by linearization of the logistic and Gompertz models and correlated with spatial patterns. Two-dimensional class analysis indicated strong and moderate aggregation at the initial and the logistic face of the epidemics, respectively.

A533

RELATIONSHIP OF TIME OF INFECTION BY POTATO VIRUS Y ON SEED TRANSMISSION. A. M. Mondjana, D. I. Rouse, and T. L. German, Department of Plant Pathology, University of Wisconsin, 1630 Linden Dr. Madison, WI 53706.

Potato Virus Y (PVY) causes a major seed transmitted disease of potato. Transmission during the season is mainly by aphids. This research was conducted to determine the effect of time of plant infection by PVY on disease transmission through progeny tubers. The impact of the disease on yield and tuber quality was also examined. Field plots were established in a randomized block design with four replicates. Treatments included: PVY infected seed tubers and mechanical inoculation of foliage at 50, 70, and 90 days after planting (DAP) for potato cultivar Russet Burbank and Russet Norkotah. The proportion of infected progeny tubers decreased with later inoculations. This relationship appeared to be linear. In 1992, a frost occurred in mid-June, just after the first mechanical inoculation, resulted in a significant reduction of infected progeny tubers. Yield and tuber quality were affected by the infected tuber treatment but not by other treatments.

A534 Withdrawn

A535

BIOLOGICAL AND CHEMICAL CONTROL OF TARGET SPOT OF FLOAT-GROWN TOBACCO SEEDLINGS. R. L. Ashby, B. H. Ownley, B. B. Reddick[1], and D. O. Onks[2]. [1]Entomology and Plant Pathology Dept., University of Tennessee, Knoxville 37996 and [2]Highland Rim Expt. Station, Springfield, TN 37172

Target spot (Rhizoctonia leaf spot) of tobacco, caused by the soilborne fungus *Thanatephorus cucumeris* (anamorph *Rhizoctonia solani*) has caused significant losses in tobacco transplants grown in float systems in Tennessee. There also has been an increase in the incidence of target spot on field plants, which is probably related to the increase in production of transplants by the float system. Currently there are no control measures recommended for target spot on float plants. In this study we tested the efficacy of four chemicals and four biological control agents for control of target spot on tobacco transplants in the float system. Three treatments, *Bacillus* sp. strain BA55, Fluazinam, and Dithane, applied weekly to tobacco leaves, provided substantial control of target spot compared to other treatments and the untreated check.

A536

FURTHER STUDIES OF ACREMONIUM WILT OF GRAIN SORGHUM. H.A. El-Shafey, E.M. El-Assiuty, A.Z. El-Abedeen and T. F. Ibrahim. Plant Pathology Institute, Agricultural Res. Center, Giza, Egypt.

Acremonium wilt of grain sorghum, caused by *Acremonium strictum*, can be observed in most Egyptian sorghum fields. Under field conditions, infection rates as high as 50% can be observed. In pots, the % of infected plants increased with inoculum density up to 100 g inoculum/25 cm-diameter pot and then decreased. The % of infected plants was also correlated with age of the plants when they were transplanted into pots containing infected soil. Infection reached 100% with 21-day old seedlings and then decreased. Highest disease incidence was obtained when fungal inoculum was applied to the pot soil 30 days after sowing. Other inoculation techniques have also been examined. *A. strictum* isolates, recovered from either maize or sorghum, varied in pathogenicity, but were more virulent on sorghum than on maize. *B. subtilis* is antagonistic to *A. strictum* growing on nutrient agar plates. Evaluation of resistance of grain sorghum breeding materials to *A. strictum* is in progress.

A537

CORRELATION OF PHENOTYPES OF *DISCULA DESTRUCTIVA* WITH dsRNA. J. M. Yao, S. D. McElreath, and F. H. Tainter. Department of Forest Resources, Clemson University, Clemson, SC 29634-1003.

Isolates of Discula destructiva grown on potato-dextrose agar (PDA) or oak medium (McElreath and Tainter, 1993, Current Microbiology 26:117-121) sometimes have sectors with different pigmentation, morphology, growth rate, and/or conidiation. Sectors of several isolates with phenotypic differences were subcultured on cellophane membranes on PDA. Mycelia were harvested and extracted for dsRNA. Double-stranded RNA was present in subcultures of some sectors and absent in others from the same isolate. Sometimes differences in the number of dsRNA segments occurred in extractions of different isogenic sectors.

A538

DOUBLE-STRANDED RNA IN THE DOGWOOD ANTHRACNOSE FUNGUS. S. D. McElreath, J. M. Yao, and F. H. Tainter. Department of Forest Resources, Clemson University, Clemson, SC 29634-1003.

Previously we reported the detection of dsRNA in 11/12 isolates of Discula associated with dogwood anthracnose (McElreath and Tainter, 1991, Phytopathology 81:1157). Seventy-three additional isolates have now been examined. Eighty-one of eighty-two isolates of Discula destructiva contained dsRNA. Double-stranded RNA was not demonstrated, after repeated attempts, in 1 isolate of D. destructiva nor in 3 isolates of Discula, Type 2. Three major size classes were revealed by agarose gel electrophoresis. These were: large (ca. 8-12 kbp), medium (ca. 3-4 kbp), and small (ca. 1.5-2.5 kbp). Additional segments were present in some isolates. Twenty-three isolates had one or more large segments. The most common banding profile was 0 large, 2 medium, and 2 small bands.

A539

HYBRIDIZATION OF DOUBLE-STRANDED RNA FROM ISOLATES OF *DISCULA DESTRUCTIVA*. J. M. Yao, S. D. McElreath, and F. H. Tainter. Department of Forest Resources, Clemson University, Clemson SC 29634-1003.

Dot-blot hybridization of dsRNA from eighty isolates of Discula destructiva, the dogwood anthracnose fungus, was used to determine their genetic relatedness. The isolates were obtained from different geographic locations from New York to Alabama and their dsRNA segments varied in size and number. There were no significant differences in the percentages of isolates which hybridized with each of

6 probes constructed from dsRNA isolates from different states and with different electrophoretic banding profiles. Each probe hybridized with about 60% of the isolates. Double-stranded RNAs from D. destructiva appear to have more genetic relatedness than dsRNAs from Cryphonectria parasitica, the chestnut blight fungus, suggesting a more recent common origin.

A540

A BIOASSAY FOR STUDYING TOXIC METABOLITES OF *DISCULA DESTRUCTIVA*. D. E. Wedge, S. D. McElreath, and F. H. Tainter. Department of Forest Resources, Clemson University, Clemson SC 29634-1003.

An in vitro bioassay was developed to investigate the toxicity of metabolites of Discula destructiva, the dogwood anthracnose fungus. Isolates of D. destructiva and Discula, type 2, were grown under identical culture conditions. Radish (Raphanus sativus) seeds were germinated in vitro on Murashige-Skoog media supplemented with serial dilutions of each culture filtrate. Root and shoot growth were dramatically inhibited by high dosages of culture filtrate for all isolates tested. Different degrees of inhibition were observed among the isolates. Inhibition of primary root and shoot elongation occurred at high dosages and stimulation of roots occurred at low dosages for some isolates. Concomitantly, inhibition of secondary lateral roots continued throughout the dosage range tested. This study supports the hypothesis that D. destructiva produces a plant growth regulatory phytotoxin(s) that may affect root and shoot growth of dogwood (Cornus spp.). A bioassay of this type may be useful for testing the virulence of isolates of D. destructiva.

A541

CULTIVAR RESISTANCE TO BLACK STEM ON Amaranthus spp. IN MEXICO. Ma. del Consuelo Sánchez E. Chapingo, University Mexico 56230.

So far, sources genetic resistance of among different races of Amaranthus spp. to stem black, caused by Macrophoma sp., are not known. Therefore, Azteca, Mercado, Mixteca, Nepal, Mexicana and Sudamericana races of Amaranthus were evaluated. Healthy plants of Amaranthus were artificially inoculated, primary inoculum being obtained from diseased plants collected in the field and increased in Potato-Dextrose-Agar (PDA) at room temperature until picnidial formation. The experiment was carried out utilizing a randomized block design, which included 5 treatments and 14 replicates. The results indicated differences among races. The Sudamericana race may be considered highly susceptible, since all plants died 6 days after inoculation; The Mercado and Mixteca races, on the other hand, may be graded as susceptible; The Azteca race showed tolerance, The Mexicana and Nepal races are definitely tolerant since the damage they suffered was not severe and none of the inoculaded plants died.

A542

COMPARISON OF *DIDYMELLA BRYONIAE* AND RELATED *PHOMA* SPECIES FROM CUCURBITS. A. P. Keinath, M. W. Farnham, and T. A. Zitter, Clemson Univ., Coastal REC and USDA, ARS, U.S. Vegetable Lab., Charleston, SC 29414 and Cornell Univ., Ithaca, NY 14853.

Didymella bryoniae (anamorph *Phoma cucurbitacearum* [= *Ascochyta cucumis*]), which causes gummy stem blight of cucurbits, occurs throughout the eastern USA. Other *Phoma* spp., such as *P. exigua*, also have been reported to cause symptoms of gummy stem blight. Twenty-seven isolates of *D. bryoniae* and *Phoma* spp. were obtained from diseased watermelon, cantaloupe, and other cucurbits grown in commercial fields in SC and NY. *D. bryoniae* could be distinguished from *Phoma* spp. when grown on quarter-strength potato-dextrose agar at 24 C with 12 hr photoperiod. Percentage monoseptate conidia ranged from 0 to 17% for *D. bryoniae* isolates, whereas no *Phoma* isolate produced any septate conidia. All 19 *D. bryoniae* isolates were pathogenic to watermelon 'Charleston Gray' and cantaloupe 'Classic' when inoculated with 2 mL of 10^5 conidia/mL. Six isolates of *Phoma* were nonpathogenic and 2 isolates from watermelon were significantly ($P \leq 0.05$) less virulent than *D. bryoniae* on these hosts. *D. bryoniae* isolates from SC and NY share common characteristics. Genetic analysis of isolates with RAPD markers is in progress.

A543

RELATIONSHIP BETWEEN DEVELOPMENT OF APOTHECIA AND SCLEROTINIA STEM ROT OF CANOLA IN GEORGIA. D. V. Phillips and P. L. Raymer, University of Georgia, Griffin, Georgia 30223.

Canola (*Brassica napus* L.) planted during October or November is consistently damaged in the rosette and/or flowering stage by Sclerotinia stem rot. Rosette stage infection has been observed from early December until bolting (late February-early March), with disease incidence as high as 32%. Infection during the flowering and early pod-filling stage (late February-early May) is even more common. Disease incidences approaching 100% have been observed, with incidences of 30-50% frequently observed the first time canola is planted in a field. Since March 1990, apothecia in canola fields were counted biweekly (weekly during the flowering period) each canola season. Apothecia were observed between March 1 and April 15 each year, with the peak numbers observed during late March or early April. This corresponds very closely with the flowering period for all well-adapted cultivars. Peak apothecial counts were 21.2, 21.6, 19.8, and 1.4 per m^2 for 1990, 1991, 1992, and 1993, respectively. During four crop seasons, only one apothecium was observed before bolting, during extensive rosette stage infection.

A544

ROOT ROTS AND DAMPING-OFF OF GINSENG SEEDLINGS IN ONTARIO. R.D. Reeleder and R.A. Brammall, Agriculture Canada Research Station, Delhi ON, N4B 2W9, and Horticultural Research Institute of Ontario, Simcoe ON, Canada N3Y 4N5.

Root rot and damping-off diseases are major problems in the establishment of ginseng (Panax quinquefolius) in Ontario; however, little is known with respect to the identity of seedling root pathogens. Pathogenicity trials of fungi recovered from diseased roots were carried out in order to determine whether observed symptoms could be reproduced. Isolates of Pythium spp, Cylindrocarpon spp, and Rhizoctonia solani were used. Inoculation of seedlings with Pythium isolates resulted in rapid maceration of roots or damping-off. Cylindrocarpon destructans produced an orange-brown rot of seedling roots. Inoculation with R. solani resulted in rapid invasion of stem tissue followed by collapse of the plant. Although these fungi were able to reproduce symptoms observed, other microorganisms such as Fusarium spp. are commonly recovered form diseased roots and may be implicated in root rots of seedlings.

A545

VARIETY, FOLIAR NUTRIENTS AND RATE OF SEEDING EFFECTS ON THE DISEASE SEVERITY AND THE YIELD OF BEAN (*Phaseolus vulgaris* L.). Flores-Revilla, C. and Nieto-Angel, D. Fitopatología. CP. Montecillo, México. 56230.

The complex of plant pathogens that attack bean crops at Puebla, México, reduces the yield 35 to 48% and there are not efficient and economical control methods to increas it. This is why this research evaluated the effects of some control practices in order to diminish the disease severity and to increase the crop yield. The experiment was stablished during the rainy season at Huejotzingo, Puebla in 1992. A randimized block design with a subdivided land plots arrary and four replications was used. Three varieties (Flor de Mayo Bajío, Negro Puebla y Mantequilla Calpan), Three seeding densities (86,000, 172,000 and 344,000 plants/ha) and microelements aplications (with or without) were tested. As a result, eighteen treatments were obtained. The rust (*Uromyces phaseoli*), anthracnose (*Colletotrichum lindemuthianum*) and common bligth (*Xanthomonas campestris* pv.*phaseoli*) severity and the crop yield were measured in three plant strata. The disease severity was analized by the area under the diseases progress curve method. Flor de Mayo Bajío was resistant to rust. Mantequilla Calpan and Negro Puebla withstood anthracnose and common bligth. Rust developed better in the upper and middle strata while anthracnose did it in the middle and lower strata, and common bligth it in the lower stratum. Disease degree was directly proportional to the rate of seeding. Larger yieldings were obtained on the Flor de Mayo Bajío and Mantequilla Calpan varieties at both seeding densities 172,000 and 344,000 plants/ha.

A546

CROP YIELD LOSSES BECAUSE OF FOLIAR DISEASES IN STRIP INTERCROPPING. C. A. Martinson and R. O. Hartwig*, Department of Plant Pathology, and *USDA/ARS Midwest Area, National Soil Tilth Laboratory, Iowa State University, Ames, IA 50011.

Pathogens that survive in crop debris have caused foliar disease problems in soybean, oat, and corn intercropped in repeated narrow (3.8 m wide) strips and with crops rotated in the aforementioned sequence. In 1992, a 15 m length section of each strip was sprayed weekly with chlorothalonil until about 3 weeks before plant maturity; disease incidence, disease severity, and crop yield from the sprayed plots were compared with an adjacent unsprayed 15 m section in every strip. Twelve replications were used. Dry weather in May and June prevented significant disease development in oat; frequent rains thereafter allowed for some Septoria blight in soybean resulting in a 3.4% yield increase in fungicide treated sections of a strip. Corn yields were increased 4.0 and 6.0% with chlorothalonil sprays and partial control of gray leaf spot, common rust, and Northern leaf blight. Yield gains with strip intercropping were not negated by leaf diseases.

A547

INFECTION OF POTATO BY *RHIZOCTONIA SOLANI* AG-2-2 PATHOGENIC TO SUGAR BEET. R.A. Kuznia, D.M. Rustad, and C.E. Windels. Northwest Expt. Stat., Univ. Minnesota, Crookston 56716.

Potato cultivars Norland and Kennebec were grown in steamed soil in the greenhouse for 5 wk (21 C). Soil was then inoculated with four isolates of *R. solani* AG-2-2 (pathogenic to sugar beet) by placing one colonized corn kernel at a 4-cm depth at each of four sites 3-cm from the stem base (27 C). At 8 wk after inoculation (WAI), root weights of Norland were significantly reduced by AG-2-2 isolates in one trial but not the other; root weights of Kennebec were unaffected by AG-2-2 isolates. *R. solani* AG-2-2 was isolated from 6.5% of discolored root segments of Norland and 5.8% of Kennebec (8 WAI). At 4 WAI, sclerotia of *R. solani* AG-2-2 formed on seed pieces of Norland (mean=77%; range=67-100%, depending upon isolate); sclerotia were not found on seed pieces of Kennebec until 8 WAI (mean=62.5%; range=50-75%). *R. solani* AG-2-2 was isolated from 28 and 56% of potato stems of Norland at 4-and 8-WAI, respectively, and from 25% of Kennebec at both times. Thus, crop rotation of potatoes and sugar beet may maintain population levels of *R. solani* AG-2-2.

A548

EFFECT OF WATER POTENTIAL ON GERMINATION,GROWTH, AND SCLEROTIAL PRODUCTION OF *Macrophomina phaseolina*. G. Olaya and G. S. Abawi. Dept. of Plant Pathology. Cornell University, Geneva, NY 14456.

Macrophomina phaseolina (Mp) is a major pathogen of beans in areas with drought and high temperature conditions.The influence of osmotic water potential (Ψs) on growth parameters of Mp was determined at 30 C on Potato Dextrose Agar (PDA) and Potato Dextrose Broth (PDB) adjusted to different water potentials with either KCl, NaCl, or sucrose. Radial growth of Mp on PDA was maximal at Ψs values between -12.2 and -18.8 bars, but growth was reduced at lower Ψs values. Dry weight of Mp colonies grown on PDB was maximal at Ψs values between -20.3 and -33.4 bars. Production of sclerotia of Mp on PDB was not affected by Ψs of -6.7 to -39.2 bars, but was completely inhibited at Ψs of -82.7 bars. After 48 hours of incubation, the germination of sclerotia of Mp on PDA was not significantly affected at Ψs of -3.2 to -47.6 bars, however germination was drastically reduced with further reduction in Ψs. The influence of Ψs adjusted by KCl, NaCl, or sucrose on Mp followed a similar pattern, but growth of Mp was much greater in media adjusted with sucrose.

A549

PREVALENCE OF ANTHRACNOSE DISEASES IN GEORGIA IN 1992. W. Uddin, Extension Plant Pathology, University of Georgia, Athens, GA 30602.

A computer database was developed for the Extension Plant Pathology Plant Disease Clinic at the University of Georgia to monitor the prevalence of diseases on various hosts in Georgia. A total of 2697 samples from a variety of sources, including landscapes, forests and commercial agriculture, was processed in the Plant Disease Clinic in 1992. As a group, anthracnose diseases were the most common and were diagnosed on 30 different hosts from 32 counties. *Colletotrichum* spp. were isolated more frequently (57%) than *Gloeosporium* spp. (43%) from diseased specimens. Although most of these samples were ornamentals and shade trees, increased numbers of field and fruit crop samples, especially strawberries, were diagnosed with anthracnose in 1992 compared to the previous year. Investigations of the prevalence of all phases of anthracnose in strawberries in Georgia, including crown, bud and fruit rot, are currently underway, and will be reported at a later time.

A550

SURVEY OF INOCULUM DENSITY AND DISTRIBUTION OF SOYBEAN SEEDLING BLIGHT PATHOGENS IN IOWA. S.S.A. Rizvi, X.B. Yang, G.L. Tylka, and D.C. McGee. Department of Plant Pathology, Iowa State University, Ames, IA 50011.

The inoculum density of *Pythium* spp.,the causal organism of soybean seedling blight was studied in Iowa soils in late April and early May. Soil samples were collected from a depth of 4-6 cm at 74 sites across Iowa. Dilution plate method was used to isolate *Pythium ultimum* and related species. Propagules of *Pythium* spp. ranged from 14-180 g^{-1}, and 1-97 g^{-1} soil for *Phytophthora* spp. We detected up to 5600 eggs of SCN per 100 cm^3. The pathogenicity of *Pythium* spp.was determined by inoculating soybean cultivar "IA 2007", grown in sterilized soil injected with 0.5-16% (w/w, inoculum on oats) levels of *Pythium* spp. Both pre- and post- emergent infections were observed in soils injected with 0.5-12% (w/w) levels of *Pythium* while only pre- emergent infection occurred in 14% and 16% levels of *Pythium* spp. This information could be of value in developing a system to predict the occurrence of damping off before planting in Iowa and the potential benefit of seed treatment.

A551

TRANSFER OF RESISTANCE TO *XANTHOMONAS CAMPESTRIS* PV. *CAMPESTRIS* INTO *BRASSICA OLERACEA* BY PROTOPLAST FUSION. Lise N. Hansen & Elizabeth D. Earle. Department of Plant Breeding and Biometry, Cornell University, Ithaca, NY 14853-1902.

Black rot caused by the bacterium *Xanthomonas campestris* pv. *campestris* is one of the most serious diseases of *Brassica oleracea* vegetable crops. Sources of resistance to the disease within *B. oleracea* are insufficient and available control means are limited. Certain lines of *B. napus* contain good resistance controlled by a dominant gene; however, crossing the two species sexually is very difficult. Alternatively, protoplast fusion may overcome the interspecific crossing barrier. Somatic hybrids have been produced by protoplast fusion between a rapid cycling *B. oleracea* and a highly resistant *B. napus* line. Inoculations with the pathogen have identified four resistant somatic hybrids. For transfer of the resistance into *B. oleracea*, the hybrid plants have been crossed directly to R.C. *B. oleracea*, and to a bridge line, 'line 15'. Progeny has been obtained from the crosses to 'line 15'.

A552

AN ELISA PROCEDURE DEVELOPED FOR DETECTION OF LOW POPULATIONS OF *XANTHOMONAS CAMPESTRIS* PV. *VESICATORIA* (Xcv) IN TOMATO LEAVES. G. C. Somodi, J. B. Jones and J. W. Scott. University of Florida, GCREC, 5007 60th Street East, Bradenton, FL 34203

An ELISA method was developed to detect low populations of Xcv in resistant tomato genotypes derived from Hawaii 7998. Xcv cells suspended in saline containing ground leaf tissue were diluted 1:1 (v:v) with a lysis buffer (in g/L: KH_2PO_4, 2; Na_2HPO_4, 11.5; EDTA disodium, 0.14; thimerosal, 0.02; lysozyme, 0.2), incubated 16 hr at room temperature, and applied to microtiter plates that had been coated with a polyclonal antibody. A mouse monoclonal antibody and alkaline phosphatase conjugated goat-antimouse were subsequently applied. Populations as low as 10^4 cfu were detected, with sensitivity increased 10-fold by the lysis buffer and an additional 10-fold by an ELISA Amplification System (Gibco BRL) over conventional ELISA. When leaves of resistant and susceptible genotypes were infiltrated with Xcv and sampled over time there was a 0.93 correlation between this ELISA procedure and populations determined by direct plating.

A553 Withdrawn

A554

PATHOGEN X LIGHT INTERACTION IN RESISTANT ALFALFA INFECTED WITH *VERTICILLIUM ALBO-ATRUM*. B. W. Pennypacker, D. P. Knievel, M. L. Risius, and K. T. Leath. Agronomy Dept., Penn State University and USDA-ARS, U.S. Pasture Lab., Univ. Park, PA. 16802

The effect of photosynthetic photon flux density (PPFD) on resistance to *V. albo-atrum* (*Vaa*) was tested in two comparative, factorial, greenhouse experiments. Significant Vaa x PPFD x week interactions were found in disease rating and height. Significant *Vaa* x PPFD interactions were detected in leaf dry weight, dark respiration, and quantity of Rubisco. *Vaa* caused greater growth suppression, and more symptoms under 40% PPFD. Dark respiration and amount of Rubisco were reduced at 40% PPFD,

whereas dark respiration was greater in infected plants at 70 and 100% PPFD. *In vitro* Rubisco activity was increased significantly in all infected plants. Net photosynthesis (Pn) was not affected by the pathogen, but was significantly lower at 40 and 70% PPFD in all plants. Lack of a *Vaa* x PPFD interaction in Pn, coupled with increased growth suppression and symptom expression in infected plants under 40% PPFD is evidence that quantity of photosynthate, not rate of Pn controls the expression of resistance to *Vaa* in alfalfa.

A555

TRANSGENIC APPLE PLANTS CONTAINING LYTIC PROTEINS HAVE INCREASED RESISTANCE TO *ERWINIA AMYLOVORA*. J. Norelli[1], H. Aldwinckle[1], L. Destéfano-Beltrán[2], and J. Jaynes[2]. [1]Depart. of Plant Pathology, Cornell Univ., Geneva, NY 14456. [2]Depart. of Biochemistry, Louisiana State Univ., Baton Rouge, LA 70803.

Cecropin B and attacin E are lytic proteins native to the hemolymph of *Hyalophora cecropia*, the giant silk moth. SB-37 and Shiva-1, substitution analogs of cecropin B, and attacin E possess a broad spectrum of activity against both gram negative and gram positive bacteria. Apple (*Malus domestica*) transgenics containing genes encoding these lytic proteins were obtained by *Agrobacterium tumefaciens* mediated transformation. Integration of lytic protein genes into the apple genome was confirmed by Southern analysis. Northern analysis of a M.26 transgenic containing the attacin E gene (T1), indicated the presence of attacin E mRNA in plants inoculated with *Erwinia amylovora*. When *in vitro* grown plants of T1, M.26, and Liberty (resistant control) were inoculated with *E. amylovora* the $\log_{10}$ of the inoculum concentration necessary to kill 50% of the plants was 5.4, 4.4, and 5.6, respectively. In greenhouse trials T1 was significantly more resistant to fire blight than M.26.

A556

INHERITANCE OF LATENT PERIOD LENGTH IN THE MAIZE; *EXSEROHILUM TURCICUM* PATHOSYSTEM. M. L. Carson, USDA, ARS, N.C. State University, Raleigh, NC 27695.

Latent period length (days to necrotic lesion formation after inoculation with *E. turcicum*) was measured on individual plants of parental inbred lines, F_1, F_2, and backcross generations resulting from crosses of two partially resistant inbred lines (69-1 and Mo17) with the highly susceptible inbred A632. Experiments were conducted using both seedlings in the greenhouse and on vegetative plants in the field. Additive gene action accounted for all of the significant variation among the generation means. Estimates of heritability ranged from 49% (greenhouse) to 83% (field). Numbers of effective factors ranged from 13 to 15 in 69-1 to 4.7 in Mo17. Latent period length appears to be a major component of partial resistance to *E. turcicum* that can be selected for on greenhouse seedlings or on vegetative plants prior to anthesis in the field, thus potentially increasing the efficiency of selection.

A557

EVALUATION OF SEVEN TOMATO GENOTYPES FOR RESISTANCE TO *FUSARIUM OXYSPORUM* F.SP. *RADICIS-LYCOPERSICI*. R.J. McGovern[1], L.E. Datnoff[2], and C.S. Vavrina[3], University of Florida, Plant Pathology Department, Immokalee, FL[1] 33934, Belle Glade, FL 33430[2], Horticultural Sciences Department, Immokalee, FL 34221[3].

Seedlings and mature plants of six south Florida commercial tomato cultivars, Agriset 761, Merced, Olympic, PAP 34283, Solarset, Sunny, and a new cultivar, NVH 4471, were screened in the laboratory and field for resistance to *Fusarium oxysporum* f.sp. *radicis-lycopersici* (FORL), the causal agent of Fusarium crown and root rot of tomato. Surface disinfested (0.5% NaOCl) seeds of each genotype were grown on carnation leaf agar inoculated with an FORL isolate. The presence or absence of typical hypocotyl discoloration was recorded 11 days after incubation of seedlings at 20C with a 12 hour photoperiod. Seven week old transplants of each genotype were planted in a commercial tomato field in southwest Florida naturally infested with FORL. Randomly selected plants of each genotype were dissected longitudinally and rated for crown rot symptoms using a 0-3 scale, 3 weeks following final harvest. *In vitro* incidence of crown rot was significantly lowest in NVH 4471, and both field incidence and severity were significantly reduced by this genotype. Merced and Agriset were intermediate in susceptibility to FORL. Merced produced the highest yield, 20.8 MT/ha, while Agriset and NVH 4471 were intermediate, producing respectively, 17.8 MT/ha and 17.4 MT/ha.

A558

QTLs ASSOCIATED WITH RESISTANCE TO *Xanthomonas campestris pv campestris* AT THE PRIMARY LEAF AND ADULT PLANT STAGES. L. E. A. Camargo, P. H. Williams, T. C. Osborn. Dept. of Agronomy, Univ. of Wisconsin, Madison, WI, 53705.

Although good sources of resistance to black rot are available in *Brassica oleracea* little is known about the genetic control of resistance. We investigated the genetics of resistance in a segregating population derived from a cross between a resistant cabbage (Badger Inbred-16) and a susceptible broccoli (OSU CR-7) inbred line. An RFLP linkage map was developed using F2 individuals and F3 lines were evaluated for the amount of diseased leaf area (DLA) by inoculating primary leaves of 2 week old plants in the green house and young leaves of adult plants in the field. The map position and effects of loci controlling quantitative variation for resistance were determined and compared for both growth stages.

A559

TWO NEW DOMINANT GENES FOR RUST RESISTANCE IN PEARL MILLET. J. P. Wilson, USDA-ARS Forage and Turf Research Unit, University of Georgia, Coastal Plain Experiment Station, Tifton, GA 31793.

Rust, caused by Puccinia substriata var. indica, is a late-season disease of pearl millet which can significantly reduce yield and digestibility of the forage. Rust resistant plants were selected from two landraces from Burkina Faso, BF 122 and BF 201, and the resistances backcrossed into an improved inbred. Segregation of resistance in backcross F_2 and testcross progeny seedlings is generally consistent with the segregation of single, dominant genes for resistance from both of the landraces. Seedlings of inbreds Tift 85DB, ICML 11, and backcross derivatives of BF 122 and BF 201 were inoculated in the greenhouse with three single-uredinium isolates of P. s. var. indica. Differential reactions to infection by the isolates indicate that the resistance genes from BF 122 and BF 201 differ from each other and are different from Rr_1 in Tift 85DB and Rpp_1 in ICML 11.

A560

ANALYSIS OF DEFENSE TRANSCRIPT ACCUMULATION AND PHYTOALEXIN PRODUCTION IN SOYBEAN, TOBACCO, AND TOMATO IN RESPONSE TO STRAINS OF PSEUDOMONAS SYRINGAE. X. P. Sun, J. L. Jakobek, and P. B. Lindgren, Department of Plant Pathology, North Carolina State University, Raleigh, N.C. 27695-7616

Our previous analysis of bean-Pseudomonas syringae interactions suggested that plants have a general, nonspecific mechanism for the induction of defense transcripts and phytoalexins by pathogenic and non-pathogenic bacteria which is distinct from the more specific mechanism associated with the induction of the hypersensitive response. To test this hypothesis we have expanded our analysis to additional plant-bacterial interactions. Defense transcipt accumulation and phytoalexin production during interactions of soybean, tobacco and tomato and incompatible P. syringae strains, P. syringae Hrp$^-$ mutants, P. fluorescens, and E. coli were studied. Although there was slight variation during specific interactions, defense transcripts and phytoalexins accumulated during these interactions irrespective of the development of a hypersensitive response. We believe these results support the above hypothesis.

A561

Disease resistance screening in maize to Exserohilum turcicum and Bipolaris maydis using colonized sorghum grain and a mechanical dispenser. D.P. Jeffers, and J.A. Mihm. CIMMYT, Apdo. Postal 6-641, 06600 Mexico, D.F., Mexico.

A technique has been devised to improve the efficiency and the quality of inoculum application of Exserohilum turcicum and Bipolaris maydis for disease resistance breeding in maize. Cultures of the fungi are increased on sterile low tannin sorghum grain. The colonized grain is dried and then passed through a 5 mm mesh wire screen to separate the grains and make a more uniform particle size. A mechanical dispenser, designed for infesting with insects, was modified to deliver 2 cc of inoculum/plant at the V4-V5 growth stage. A person can inoculate 2,500 plants/hour and deliver the same volume of inoculum to each plant, thus improving uniformity in the epidemic generated.

A562

ENVIRONMENTAL STABILITY OF FORAGE YIELD AND QUALITY IN PEARL MILLET HYBRIDS HETEROGENEOUS FOR RUST RESISTANCE. J. P. Wilson, W. W. Hanna, and R. N. Gates. USDA-ARS Forage and Turf Research Unit, University of Georgia, Coastal Plain Experiment Station, Tifton, GA 31793.

The rust-susceptible pearl millet hybrid 'Tifleaf 1', the resistant hybrid 'Tifleaf 2', and two experimental 3-way hybrids with different proportions of resistant and susceptible plants were evaluated for rust resistance and forage yield and quality in 1990, 1991, and 1992. Different environmental conditions were simulated by varying planting date, planting density, and fungicide applications across the three years. Rust severity of forage was negatively correlated with late season green yield, dry matter yield, in vitro dry matter digestibility, and digestible dry matter yield. The stability across environments of the 3-way hybrids was intermediate to Tifleaf 1 and Tifleaf 2 for rust severity and the yield and quality measurements negatively correlated with rust severity. Identification and utilization of additional sources of resistance would be beneficial to improve the performance of 3-way hybrids.

A563

WHEAT STREAK MOSAIC VIRUS RESISTANCE IN FOXTAIL MILLET, Setaria italica (L.) BEAUV. A. Marcon, J. E. Watkins, S. G. Jensen, E. M. Ball, Department of Plant Pathology, University of Nebraska-Lincoln, USDA-ARS, Lincoln,NE 68583-0722; D.D. Baltensperger, University of Nebraska Panhandle Research Center, Scottsbluff, 69361

Wheat streak mosaic virus (WSMV), vectored by the wheat curl mite, Eriophyes tulipae, causes a serious disease of wheat, resulting in an estimated 6.3 million dollars in losses in Cheyenne County, NE in 1993. Breaking the disease cycle by eliminating oversummering hosts can decrease the prevalence of the disease. Setaria italica is a potential oversummering host for WSMV. We screened 240 foxtail millet lines and varieties, by mechanically inoculating with WSMV. Reactions were evaluated using symptom development and virus titer as determined using ELISA. Foxtail millet lines I.Se (453, 454, 474), NESE (4, 13, 18, 25, 42, 44, 49, 50, 53, 54, 121) and cultivars 'Karang', 'Arzan' and 'Erzen' were the most susceptible. Cultivar 'Red Siberian' and lines I. Se 462 and NESE (3A, 27, 39 and 116) showed the best resistance. We have identified genetic resistance to WSMV within foxtail millet germplasm.

A564

GENETIC ASSOCIATION IN *PHASEOLUS VULGARIS* OF A HOST RESPONSE CONDITIONING RESISTANCE AND/OR LETHAL NECROSIS TO TEN POTYVIRUSES. M.L. Fisher, J.F. Murphy, and M.M. Kyle, Dept of Plant Breeding, Cornell University, Ithaca NY 14853.

Genetic analysis has shown an association between host factor(s) that affect 10 bean potyviruses. Conditional resistance or a necrotic response similar to the breakdown of that resistance is closely linked, if not identical, to the I gene for resistance to bean common mosaic potyvirus (BCMV). While all 10 viruses cause typical mosaic disease symptoms on i/i near-isolines, responses on I/I plants fall into three classes. I/I plants are resistant to the first set of viruses at low temperatures (25°C) (virus cannot be recovered), however, above 30°C, inoculation leads to pinpoint lesions followed by systemic veinal necrosis. Graft-inoculation of I/I plants with a BCMV-infected plant leads to systemic necrosis at any temperature, suggesting that necrosis is not strictly a function of high temperature but relates to the presence of virus. The second group of viruses do not infect I/I plants at any temperature and systemic necrosis is never observed. Viruses in the third class always produce a systemic veinal necrotic response on I/I plants regardless of temperature. We are continuing to investigate how a single host factor or closely linked factors can condition either resistance or necrosis to a number of distinct, but closely related potyviruses.

A565

EXPRESSION OF RESISTANCE TO SCLEROTINIA TRIFOLIORUM IN PROGENY OF ALFALFA PLANTS SELECTED FOR RESISTANCE BY STEM INOCULATIONS. R. G. Pratt and D. E. Rowe, USDA, ARS, Forage Research Unit, Mississippi State, MS 39762

Four plants of alfalfa cultivar Delta, of 494 screened for resistance to S. trifoliorum by stem inoculations, were selected as resistant phenotypes and polycrossed. The four half-sib families of first-generation progeny from these plants were evaluated for resistance in comparison to Delta. Three families were significantly more resistant than Delta when evaluated by stem and whole-plant inoculation techniques. The fourth family gave intermediate responses by both methods of inoculation. A composite population of the three most resistant families developed less severe disease than Delta with natural infection in the field during two growing seasons. These results indicate that selection for resistance to S. trifoliorum by the stem inoculation technique may give significant heritable, whole-plant resistance to the pathogen that is expressed under both artificial and natural conditions.

A567

EVALUATION OF MARYBLYT FOR DETERMINING ANTIBIOTIC SPRAY SCHEDULES FOR FIRE BLIGHT CONTROL ON APPLES IN ALABAMA. E. J. Sikora and E. M. Bauske, Department of Plant Pathology, Auburn University, AL 36849-5624.

A conventional spray schedule for fire blight control on apples in Athens (north), Chilton (central), and LaFayette (east), Alabama was compared to a schedule recommended by the computer model MARYBLYT. Total number of antibiotic sprays used at each site was compared to the number of sprays recommended by the model. Field evaluation of the conventional and MARYBLYT spray schedules to determine effect on disease development was conducted at the Chilton site only. MARYBLYT recommended 8 sprays at Athens and 7 at LaFayette and Chilton. Cooperators following the conventional schedule applied 4 sprays at Athens, 5 at LaFayette, and 6 at Chilton. Disease incidence was low at Chilton and Athens but high at LaFayette. At Chilton, conventional and MARYBLYT systems proved equal in disease control. MARYBLYT predicted correctly the onset of blossom and shoot blight symptoms.

A568

EVALUATION OF LOW-INPUT FUNGICIDE SPRAY PROGRAMS FOR CONTROL OF *ALTERNARIA SOLANI* ON FIELD TOMATOES IN ALABAMA. E. J. Sikora, E. M. Bauske, G. W. Zehnder, K. L. Bowen, and W. S. Gazaway, Department of Plant Pathology, Auburn University, AL 36849-5624.

Two low-input fungicide spray programs were evaluated on fresh market 'Olympic' tomatoes in Cullman, AL in 1992. Treatments were: weekly applications of mancozeb, one at label rates and one at reduced rates; applications of mancozeb at label rates as recommended by TOM-CAST; and an unsprayed control. Early blight symptoms were rated weekly. No yield differences were observed among the three spray programs though all increased yields (26-43%) compared to the control. TOM-CAST recommended four fewer sprays. The lower fungicide-rate treatment used approximately 32% less product than the standard treatment. Disease severity was highest in the unsprayed control and lowest in the two weekly-fungicide-spray programs.

A569

EFFECTS OF TILLAGE, ROW WIDTH, AND CULTIVARS ON FOLIAR DISEASES OF DOUBLE-CROP SOYBEAN.
J. A. Wrather, S. C. Anand, and S. R. Kendig, University of Missouri - Delta Center, P. O. Box 160, Portageville, MO 63873.

Experiments were conducted over two years, 1985 and 1986, to determine the effect of tillage systems, row widths, and soybean cultivars on foliar diseases in double-crop soybean. The soybean cultivars Pershing, susceptible to bacterial blight, and Avery, resistant to bacterial blight, were each planted in 38-cm and 75-cm row widths in conventional-tilled and non-tilled soil following winter wheat. There was an interaction between tillage treatment and cultivar for the severity of bacterial blight. Tillage did not influence bacterial blight severity on Avery whereas the disease severity was higher on tilled compared to no-tilled Pershing soybean. There was an interaction between tillage, cultivar, and year for the severity of Septoria brown spot. Generally, Septoria brown spot severity was higher in 1986 than 1987, lower on Avery than Pershing both years, and higher in tilled than no-tilled conditions. Row width did not affect the severity of these diseases.

A570

SOUR BUNCH ROT COMPLEX INTEGRATED MANAGEMENT ON WINERY GRAPE IN MEXICO. **R. Jaime-Garcia. Campo Experimental Costa de Hermosillo, CIRNO-INIFAP, Apdo. postal 1031, 83000 Hermosillo, Son., Mexico.**

Sour bunch rot (SBR) complex is a major problem in winery grape in The Coast of Hermosillo, Sonora, area. Vines of winery grape (Cv. Carignane) were subjected to leaf removal (LR) and a check (without leaf removal) in a split plot design with integrated treatments of fungicides, insecticides and gibberelic acid directed to grape clusters. SBR complex was significantly reduced in the LR treatments. Total infection (measured as the product of incidence times severity) was measured as 27.08% in the check and 12.24% in the LR treatments. A good control of powdery mildew combinated with an application of benomyl at blooming time reduced the SBR problem in about 53%. The solely application of fungicides to control of powdery mildew or fungi penetrating at blooming time (Botryiodiplodia theobromae) reduced the SBR problem in 30% and 25% respectively. Application of insecticides or gibberelic acid did not reduce the SBR complex in this experiment. The overall grape yield was not significantly different among treatments and the control regardless of leaf removal. Grape yield of LR was significantly higher than in the check.

A571

TACTICAL AGRICULTURAL TEAMS: INNOVATION IN TEACHING INTEGRATED PEST MANAGEMENT. J. Keith Waldron, Department of Entomology, Cornell University, Ithaca, NY 14853, Philip Sutton, Cornell Cooperative Extension, Genesee County, Batavia, NY 14020, and James VanKirk, Cornell Cooperative Extension, Cayuga County, Auburn, NY 13021.

Tactical Agricultural (TAg) Teams is a field oriented educational program designed to enhance participant Integrated Pest Management (IPM) and Integrated Crop Management (ICM) skills. TAg teams are comprised of 3 to 7 farmers, an agribusiness person, and an extension representative who facilitates the training sessions. Intensive training is offered in pest and crop management and other production topics to help producers optimize the economic and environmental efficiency of crop management decisions. Production alfalfa and corn fields serve as "classrooms" for addressing key pest and crop management concerns at critical crop growth stages or seasonal times. Pre- and post testing of TAg participants indicates this format allows for the very successful transfer of a variety of pest and crop management information. Survey information indicates TAg participants had greater than 90% compliance with IPM recommendations. Since 1990, TAg's intensive IPM and ICM training has been transferred to program participants who collectively manage over 50,000 acres of field crops in 24 counties.

A572

USE OF SDS-PAGE TO ANALYZE PROTEINS OF METHAM-TREATED MICROSCLEROTIA OF VERTICILLIUM DAHLIAE. Cheryl Ann Engelkes and Deborah R. Fravel, USDA, ARS, Biocontrol of Plant Diseases Laboratory, Beltsville, MD 20705.

A procedure was developed to analyze proteins from microsclerotia (MS) of Verticillium dahliae, grown for 1-5 wk in Czapek-Dox broth, collected on 100 µm mesh, and placed in soil treated with 0, 0.045 or 0.45 µl/g soil (≈ 0, 93.5 or 935 L/ha) of metham (Vapam®) for 2 days. Retrieved MS were ground for 50-250 sec with a mini-beadbeater cell disrupter. Water-soluble proteins (10-290 µg protein[Bradford]/g MS[fr wt]) were concentrated by ultrafiltration (10,000 MWCO). Protein profiles were created using the Phastsystem SDS-PAGE and detected with a combination of Coomassie blue and silver stains. The number of protein bands (43-94 kDa) of V. dahliae MS decreased with increasing rates of metham. SDS-PAGE protein bands which occur from MS exposed to sublethal rates of metham can be used as markers to characterize weakening of MS.

A573

CHARACTERIZATION OF AN ANTIGEN THAT BINDS TO A PYTHIUM ULTIMUM-SPECIFIC MONOCLONAL ANTIBODY. F. Avila and G. Yuen, Department of Plant Pathology, University of Nebraska-Lincoln, Lincoln, NE 68583-0722

A monoclonal antibody (MAb) specific to P. ultimum (Pu) was previously used to identify Pu in pure culture by ELISA. Other assays utilizing this MAb are being developed to study interactions of Pu with microorganisms in roots and soil. Sensitivity of the assays in such natural environments may be reduced due to masking of antigen characteristics. For this reason, studies were conducted to characterize the antigen, localize it within the cell, and identify factors that influence antigen-Ab binding. When mycelium was treated with proteinase, antigen binding was lost. Deglycosylation of the mycelium by laminarinase or by periodate oxidation decreased antigen-MAb binding. These results indicate the antigen is a glycoprotein and the epitope may be located in the glycosidic moiety. Western blot analysis indicated that the antigen molecule has an apparent molecular weight of 46 Kd. The antigen is thermostable, and binding with the MAb increases after boiling the antigen for 5-30 min. Immunofluorescence indicates that the antigen occurs on the cell wall with highest level in hyphal tips.

A574

Utilization of Environmental Horticulture Data via a Consumer 1-800 Number. David L. Clement, M. K. Malinoski, A. S. Lavigna, and J. S. Westrope. Cooperative Extension Service, Maryland Institute for Agriculture and Natural Resources, 12005 Homewood Road, Ellicott City, MD 21042.

The Home and Garden Information Center provides information on Environmental Horticulture to residents in Maryland via a toll free phone number. Taped information is available 24 hours a day, 7 days a week and Horticulture Consultants are available 5 days a week. Prerecorded information includes a "Tip of the Week" as well as answers to the most frequently asked questions. The phone data is handled by a file server running Novell NetWare and software developed on dBase IV version 1.5. Ornamental and turf calls account for approximately 50% of the calls. Phone data can be sorted according to topic and call frequency by county, month, and year. This data has been used to track disease and insect outbreaks through the state, to prioritize programming at the Center, and to supplement other state agency surveys. Since its inception three years ago, the Center has handled over 134,000 phone calls. Approximately 73,000 callers were helped with their questions by the Horticulture Consultants while the remainder obtained self help information from the prerecorded tapes. About 10,000 fact sheets have been mailed, 1,500 soil test requests were filled, and 1,600 plant samples have been diagnosed. The Center has recorded over 250 tapes and published 50 fact sheets on Environmental Horticulture issues.

A575

A NEW RAPID METHOD FOR PLOTTING LIVE OAK TREES IN OAK WILT INFECTION CENTERS. A. D. Wilson, USDA Forest Service, Southern Hardwoods Laboratory, P.O. Box 227, Stoneville, Mississippi, 38776.

An algorithm and methodology were developed for calculating and plotting tree positions within oak wilt research grid-plots. The method utilized a laser survey instrument to collect horizontal distance and azimuth data between reference (origin) trees and target trees. Corrective equations were devised for transforming azimuth to polar coordinate bearings (directions), permitting tree position calculations for any grid-plot orientation relative to magnetic north. Polar coordinate data were then converted to rectangular coordinates for plotting target tree positions from reference tree positions. A software program with coordinate plotting capability was used to produce maps of research plots and to determine coordinates of each tree relative to any desired point of origin within the plot grid. Unknown intertree distances between individual trees were determined from coordinate data once all trees were plotted in the grid. Thus, the procedure avoided the need to take polar coordinate data for all possible permutations of tree combinations. The method is useful in early diagnosis, epidemiology, disease suppression, and other oak wilt studies requiring accurate information of intertree distances and tree positions relative to the expanding edge of infection centers. It is also applicable to epidemiological studies of other forest diseases when coordinate research plots are used.

A578

USE OF A SELF-STUDY PLANT DISEASE HERBARIUM IN TEACHING PLANT PATHOLOGY. A.B.A.M. Baudoin, Dept. of Plant Pathology, Physiology and Weed Science, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061.

A teaching herbarium of self-study modules was developed for use in laboratory courses. Modules were designed to meet three objectives: (1) to allow students with different crop interests to study disease problems of crops of their choice during the same laboratory session; (2) to present a realistic, field-like situation, in which one studies a disease by first answering questions based on the kinds of information available in the field (color prints of field appearance, written descriptions of the disease situation, and preserved display samples), then progresses to information available only after laboratory study (samples for microscopic examination, preserved cultures of pathogens, microscope preparations, results of serological tests); and (3) to allow students to practice use of the literature available for aiding disease diagnosis and prescribing control methods.

A579

TRANSFORMATION OF BIPOLARIS SOROKINIANA WITH THE GUS-GENE AND APPLICATIONS FOR FUNGAL POPULATION STUDIES.
E. Liljeroth, H-B. Jansson and W. Schäfer. Department of Plant Breeding Research, The Swedish University of Agricultural Sciences, S-268 31 Svalöv, Sweden; Department of Microbial Ecology, Lund University, S-223 62 Lund, Sweden; Institut für Genbiologische Forschung Berlin GmbH, Ihnestr. 63, D 1000 Berlin 33, Germany

Bipolaris sorokiniana, a fungal pathogen of cereals, was transformed with the GUS (β-glucuronidase) gene under a constitutive promotor. Transformants that showed a stable and constant level of GUS expression after several conidiation cycles, and after several months of growth on non-selective media, could be selected. However, some transformants lost their GUS-activity after conidiation. The stable transformants did not differ from the wildtype in growth rate or pathogenicity. A significant, positive correlation was found between GUS-activity and ergosterol content in barley roots infected with a transformed strain indicating that GUS can be used as a marker to study fungal population development in plant tissue.

A580

FLUORESCENCE-BASED PCR ASSAY FOR DETECTION OF GRAPEVINE FANLEAF VIRUS. DA Knorr, AJ Blasband, A Rowhani, and DA Golino, Applied Biosystems, Foster City, CA, 94404, and Plant Pathology Department, University of California, Davis, CA 95616.

An assay using fluorescently-labelled nucleotides or oligonucleotide primers in combination with the polymerase chain reaction (PCR) has

been developed for the detection of grapevine fanleaf virus (GFLV). Primers were selected based on published sequences for the capsid protein gene of GFLV and either labelled with a fluorescent tag or left untreated. Reverse-transcribed PCR products specific for GFLV were analyzed by laser-exited fluorescence on an ABI model 373A DNA Sequencer running GeneScanner™ software. These tools allow automatic quantitation and band sizing with respect to internal standards, as well as correction of differences between samples in different lanes and on different gels. Virus was detected from as little as 400 ng of grape tissue. This assay is also being adapted for automation using the ABI Catalyst Molecular Biology LabStation™.

A581

Characterization of Intraspecies Diversity on Colletotrichum gloeosporioides with Isozyme Analysis. P. Kaufmann and G. J. Weidemann. Department of Plant Pathology, University of Arkansas, Fayetteville, AR 72701.

Colletotrichum gloeosporioides is a heterogeneous fungal species consisting of numerous host specific populations. Polyacrylamide gel electrophoresis isozyme analysis was examined as a method to characterize genetic diversity in several host specific populations of Colletotrichum gloeosporioides. Enzymes including glucose dehydrogenase, glucose-6-phosphate dehydrogenase, nicotinamide dinucleotide phosphate dehydrogenase, phosphoglucoisomerase, and superoxide dismutase provided polymorphisms which distinguished isolates on the basis of host preference. Preliminary results suggest that isozyme analysis may be a useful tool for characterizing intraspecific population diversity within Colletotrichum gloeosporioides.

A583

PCR AMPLIFICATION OF DNA FROM BACTERIAL PATHOGENS OF SUGARCANE. S. A. Lopes and K. E. Damann, Dept. of Plant Pathology & Crop Physiology, Louisiana State University Agricultural Ctr., Baton Rouge, LA 70803.

Clavibacter xyli subsp. xyli and Xanthomonas albilineans cause ratoon stunting and leaf scald diseases respectively. To develop a method for early diagnosis we are trying to amplify unique segments of bacterial DNA which are specific to each pathogen. Bacterial DNA was obtained by disruption of cell walls with glass beads or microwaves, followed by phenol/chloroform treatment. Succesful amplification has been obtained using primers for the internal spacer region between 16s and 23s rRNA genetic loci (Jensen et al, 1993), and with degenerate primers for an internal segment of the recA gene (Duwat et al, 1992), and with several RAPD primers. The efforts to apply these results to vascular extracts and their implications for diagnosis will be presented.

A584

ACCLIMATION OF SOME ARBUSCULAR MYCORRHIZAL FUNGI TO HIGH SOIL ALUMINUM. H. T. Bartolome-Esteban and N. C. Schenck, Plant Pathology Department, University of Florida, Gainesville, FL 32611.

Isolates of arbuscular mycorrhizal fungi that do not normally germinate in a 100% Al-saturated soil because of low tolerance to soil acidity were gradually exposed to increasing soil Al saturation. The fungi were pot-cultured with bahia grass initially in 12.5%, then 25%, and finally 50% Al-saturated soils. Of the twelve isolates studied, only *Glomus etunicatum* (Isolate LETC 329), *Acaulospora longula* (ALGL 316), *Entrophospora colombiana* (ECLB 356), and *Glomus mosseae* (LMSS 313) germinated in a 100% Al-saturated soil after gradual exposure to Al. Isolate ECLB 356 had the highest spore germination and the most extensive hyphal growth. The frequency of developing tolerance to Al and the maximum germination obtained with acclimation were low; however, the study demonstrated that development of tolerance to Al is possible by acclimation. This phenomenon may be important in the utilization of mycorrhizal fungi in highly acidic and Al-saturated tropical soils.

A585

CROP OILS ENHANCE CONIDIA GERMINATION OF THE MYCOHERBICIDE COLLETOTRICHUM TRUNCATUM. G. H. Egley and C. D. Boyette. USDA-ARS, Stoneville, MS

During attempts to enhance efficacy of C. truncatum for biocontrol of the weedy pest, Sesbania exaltata, we found that 85 and 50% of the conidia germinated in safflower and unrefined corn oil respectively at four hours while the conidia were suspended in water-oil emulsions (1:1, v/v). Germination was less than 5% in water alone or in water-oil emulsions of soybean, olive, peanut, refined corn oil or mineral oil. In other studies, the water-unrefined corn oil emulsion enabled 35 to 70% (0 to 12% in water only) of the conidia to germinate on glass slides or on detached S. exaltata leaves while incubated 24 hr in a low humidity (35% RH) atmosphere. The requirement of additional moisture for infection was also greatly reduced when the conidia were sprayed in the water-corn oil emulsion onto the weed. The unrefined corn oil enhanced C. truncatum germination. The water-oil emulsion also retained water that alone was sufficient for conidia germination on plant leaves.

A586

CHARACTERIZATION OF A NEW SPECIES OF PYTHIUM PRODUCING MULTIPLE OOSPORES FROM BENTGRASS IN NORTH CAROLINA.
Z. G. Abad, H. D. Shew, L. T. Lucas, and L. F. Grand. North Carolina State University, Raleigh, NC 27695-7616.

Pythium sp. nov. was isolated from roots and crowns of bentgrass (Agrostis palustris) from 2 golf courses in NC. The species is distinctive from other Pythium spp. with the exception of P. multisporum in the production of multiple oospores (1-6 per oogonium). The species is also distinguished by the occurrence of some oogonia (approx. 20 %) with an uncommon pedicel with 2-4 swollen elements originating at varying distances below the oogonia, and the occurrence of 2 or 3 oogonia from the same branch of the oogonial hypha (approx. 2%). This is the second report of a species of Pythium that produces multiple oospores. The discovery of this species could provide new clues for understanding the nature of oosporogenesis in species with multiple oospores.

A587

UROCYSTIS TRILLII ON *TRILLIUM VIRIDESCENS* IN ARKANSAS. R.D. Cartwright, B.E. Walker, and G.E. Templeton, Dept. of Plant Pathology, University of Arkansas, Fayetteville, AR 72701.

Sori were observed on leaves and stems of *T. viridescens* at one location in northern Arkansas during April - June in 1992 and 1993. Sori appeared as small chlorotic spots becoming black and erumpent at maturity (4-10 mm). Microscopic examination showed the fungus to be *Urocystis trillii*, a rarely reported smut of *Trillium* spp. in the northwestern U.S. and Japan. Infected tissue had abundant, intracellular, hyaline, septate hyphae 2-4 um in diameter that eventually formed large (20-52 (41) um) , brown, spherical to irregularly shaped, spore balls containing 4-18 thick-walled brown fertile cells, and an outer layer of hyaline, sterile cells. This is the first report of *U. trillii* on *T. viridescens* and the first reported occurrence in the eastern United States.

A588

INFLUENCE OF GOSSYPOL ON GROWTH AND SCLEROTIAL PRODUCTION OF ASPERGILLUS FLAVUS. R.K. Garber and P.J. Cotty, USDA, ARS, Southern Reg. Res. Center, New Orleans, LA 70179

Aflatoxins are toxic fungal metabolites that limit use of cottonseed infected by *A. flavus*. Another toxic compound, gossypol, occurs in most cottonseed and is of plant origin. The current investigation sought to determine potential interactions between gossypol and *A. flavus*. Radial growth of all *A. flavus* isolates was inhibited by gossypol (20-400 µg/ml) in MES buffered Czapeks-Dox agar pH 5 to 7. Greatest inhibition occurred at pH 6.5 and 7.0. Gossypol stimulated increased sclerotial production with maximum stimulation occurring between pH 6 and 7. Several isolates failed to produce sclerotia in the absence of gossypol acetate within this pH range. At pH 7.0, gossypol stimulated sclerotial production even at 20 µg/ml. Gossypol may influence *A. flavus*/cotton interactions by slowing fungal invasion, reducing aflatoxin production, and stimulating sclerotial formation . Addition of gossypol to media may provide for more reliable production of sclerotia for ecological, physiological and taxonomic studies.

A589

INCIDENCE AND STABILITY OF DOUBLE-STRANDED RNA WITHIN THE ASPERGILLUS FLAVUS GROUP. K.S. Elias and P.J. Cotty, USDA, ARS, SRRC, P.O. Box 19687, New Orleans, Louisiana 70179.

Phenotypic traits such as hypovirulence, killer strains, and toxin production are directly associated with infection of fungi by double-stranded (ds) RNA. These observations lead to questions on distribution of dsRNA within *A. flavus* populations and the relation of dsRNA to aflatoxin production and phenotypic variability. Nearly 100 isolates in the *A. flavus* group, both culture collection isolates and recent field accessions, were analyzed for dsRNA via standard cellulose CF-11 methods. While roughly 10% of the fungal isolates contained dsRNA, no two isolates contained identical dsRNAs based on electrophoretic migration in agarose gels. The dsRNA content of isolates also differed in stability and quantity. Attempts to cure 6 isolates of dsRNA by single conidium transfer, nitrogen metabolism spontaneous mutant selection, and cycloheximide treatment met with variable results since partial, total and no curing was observed. The culture media used affected the presence and amount of dsRNA produced. No correlation between dsRNA and aflatoxin-producing ability was observed.

A590

VEGETATIVE COMPATIBILITY ANALYSIS OF ATOXIGENIC ASPERGILLUS FLAVUS ISOLATES. P.J. Cotty, USDA, ARS, Southern Regional Research Center, P.O. Box 19687, New Orleans, LA 70179.

Nineteen percent of *A. flavus* isolates collected from 32 fields in five states failed to produce aflatoxins in liquid fermentations (limit of detection 5 PPB). Atoxigenicities were often stable through two to four evaluations. However, some isolates produced aflatoxins when either mutated into a niaD$^-$ phenotype or transferred by single conidium. The 190 isolates initially identified as atoxigenic were placed in 78 vegetative compatibility groups on the basis of complementation between nitrate non-utilizing mutants generated on chlorate supplemented medium. Thirty-two percent (62 individuals) were assigned to three VCGs. However, 49 isolates were assigned to VCGs represented by single isolates. The three most common VCGs were isolated from 10 to 13 fields located in 2 to 4 states. Tester mutants of these three VCGs failed to complement mutants of 80 toxigenic isolates collected from three distinct areas. The results support suggestions that certain *A. flavus* VCGs have relatively stable atoxigenic phenotypes.

A591

FRACTAL ANALYSIS OF SUBSURFACE GROWTH OF GLIOCLADIUM VIRENS. JJ. Classen, A.D. Whittaker, C.M. Kenerley. Departments of Agricultural Engineering and Plant Pathology & Microbiology. Texas A&M University, College Station, TX 77843.

The fractal dimension of mycelial growth from an alginate prill of a genetically-modified isolate of *Gliocladium virens* in the soil subsurface was investigated. The isolate is capable of producing the bacterial enzyme organophosphate hydrolase. A system was developed to allow microscopic images of colony growth over a seven day period to be digitized by a Quantimet 570 for image processing. A combination of image processing algorithms and manual corrections give binary images at each sampling time of fungal colonies growing in soil and ground lignite. These images were analyzed for the fractal dimension of the colony by a box counting algorithm. This algorithm overlays the image with square boxes of decreasing size; at each box size, the number of boxes covering a part of the colony are counted. The size of each box is related to the number of counts at that box size by the fractal dimension. These data will be useful in assessing mycelial extension as a component of pesticide degradation by a genetically modified fungus.

A592

VARIATION IN DNA AND PROTEIN PROFILES AMONG TRIPLY CLONED ISOLATES OF THREE *SPIROPLASMA CITRI* LINES DIFFERING IN TRANSMISSION OR SUBCULTURING HISTORY. P. D. Zuck, M.E. Shaw, and J. Fletcher. Department of Plant Pathology, Oklahoma State University, Stillwater, OK 74078.

Differences in protein profiles, RFLPs, extrachromosomal DNAs, viral integration and insect transmissibility occur among three lines derived from *Spiroplasma citri* strain BR3 (BR3-T, -G, and -P) which differ in transmission or subculture history. To test for variation within the lines, six to eight triply cloned sublines of each line were isolated. Proteins were analysed by polyacrylamide gel electrophoresis and Western blotting using anti-spiralin serum. *Eco*RI digested total DNAs were analysed on ethidium bromide stained agarose gels and by Southern blot hybridization using a cloned SVBR3 virus probe. The most notable difference was the PAGE mobility of spiralin among the BR3-G sublines, which also has been noted in other laboratories for different *S. citri* strains. Other differences among the clonal isolates appeared minor, so future comparisons of lines will be carried out using the total line population.

A593

COMPARISON OF PCR AMPLIFICATION OF DNA AND ELISA FOR THE DETECTION OF *XYLELLA FASTIDIOSA* IN PLANT EXTRACTS. G.V. Minsavage[1], D.L. Hopkins[2], R.M.V.B.C. Leite[3], and R.E. Stall[1], [1]Department of Plant Pathology and [2]CFREC-Leesburg, University of Florida, Gainesville, FL 32611, and [3]Instituto Agronomico do Parana Londrina, PR, 86001, Brazil.

Because of low titer, *Xylella fastidiosa* is often difficult to detect. A 7.4 kb *Eco* RI fragment of genomic DNA of *X. fastidiosa* strain PCE-RR was labelled, used as a probe, and found to be conserved in 18 strains tested. A 1.0 kb internal *Eco RV* fragment of the probe was sequenced and used to design primers that amplify portions of genomic DNA specific to *X. fastidiosa*. Extracts were obtained both by maceration of grape petioles and by vacuum extraction of citrus stems in phosphate buffer containing sodium ascorbate and acid-washed PVPP. Both extracts containing various concentrations of *X. fastidiosa* were assayed by ELISA and by PCR amplification of the specific DNA fragment. With ELISA, 3×10^4 bacteria per ml could be detected. PCR amplification was 100-fold more sensitive, detecting 3×10^2 bacteria per ml.

A594

LIGHT AND TRANSMISSION ELECTRON MICROSCOPY OF CITRUS LEAVES AFFECTED BY CITRUS VARIEGATED CHLOROSIS AND PECOSITA. R. H. Brlansky and D. S. Howd. University of Florida, CREC, Lake Alfred, FL.

Leaf samples were collected from citrus variegated chlorosis and pecosita affected sweet orange trees from Sao Paulo State, Brazil and Missiones, Argentina respectively. Symptoms included chlorotic patterns similar to zinc deficiencies with small brown lesions. Sections of leaf pieces were prepared for and examined by light (LM) and transmission electron microscopy (TEM) and were compared to sections of leaf tissue from healthy greenhouse citrus. Using LM, the chlorotic areas of affected leaves were similar to healthy tissue; but with TEM, numerous osmiophilic globules, loss of starch granules, and breakdown of chloroplasts was observed. Brown lesions containing opaque material with the breakdown of cell walls in the spongy parenchyma cells. were seen with LM. In TEM sections, there was a thickening and collapse of cell walls, osmiophilic globules, opaque material, destruction of normal cell constituents and necrosis. Xylem-inhabiting bacteria were found in nearby xylem of some small leaf veins.

A595

THE BEET LEAFHOPPER TRANSMITTED VIRESCENCE AGENT IS ASSOCIATED WITH DISEASED POTATOES IN UTAH. C. D. Smart, S. V. Thomson*, K. Flint* and B. C. Kirkpatrick. Dept. of Plant Pathology, University of California, Davis 95616 and *Department of Biology, Utah State University, Logan, UT 84322.

Potato plants exhibiting symptoms resembling psyllid yellows including chlorosis, stunting, aerial tuber formation and purple apical leaves, were collected near Provo, Utah. DNA was extracted from infected plants and their tubers, and Southern blot analyses were performed using cloned aster yellows chromosomal and plasmid DNA probes as well as beet leafhopper transmitted virescence agent (BLTVA) plasmid probes. Hybridization occurred between diseased potato DNA and the BLTVA probe, but not with AY-MLO probes. Nucleotide sequence of 16S ribosomal RNA and 16/23S spacer regions showed that the MLO associated with the diseased potatoes was indistinguishable from the BLTVA-MLO.

A596

GUT AND SALIVARY GLAND BARRIERS TO TRANSMISSION OF THREE *SPIROPLASMA CITRI* LINES ACQUIRED BY THE LEAFHOPPER *CIRCULIFER TENELLUS*. A. C. Wayadande & J. Fletcher. Dept. of Plant Pathology, Oklahoma State University, Stillwater, OK 74078.

Barriers to transmission of three lines of Spiroplasma citri strain BR3 which differ in transmission or subculturing history were determined within the leafhopper vector, Circulifer tenellus. Spiroplasmas were recovered by cultivation from groups of leafhoppers which had previously acquired BR3-T (maintained by leafhopper transmission), BR3-G (maintained by graft transmission > 8 y), or BR3-P (passed over 130 times in liquid medium) from membrane sachets. Since spiroplasmas were recovered six weeks after feeding, these data suggest that the gut wall does not act as a barrier to transmission. However, when leafhoppers which had been injected with the spiroplasmas fed on membrane sachets, only BR3-T was recovered from the

cultured feeding solutions, indicating that the salivary gland presented a barrier to BR3-G and BR3-P transmission. Additionally, S. citri BR3-T was recovered from feeding solutions when BR3-T-injected non-vector Dalbulus maidis was tested.

A597

DNA FINGERPRINTING ANALYSIS OF VIRAL DNA INTEGRATION INTO THE GENOMES OF *SPIROPLASMA CITRI* LINES DIFFERING IN INSECT TRANSMISSIBILITY. Y.H. Sha, J. Fletcher, and U. Melcher. Departments of Plant Pathology and Biochemistry & Molecular Biology, Oklahoma State University, Stillwater 74078

Genomic DNA fingerprints of three lines of *Spiroplasma citri* strain BR3 differing in transmission history showed minor differences. BR3-G (a graft transmitted, insect non-transmissible line) lacked three *Eco*RI fragments, while BR3-P (a cultured, insect non-transmissible line) lacked one, when compared to the insect transmissible line, BR3-T. The genomes of these *S. citri* lines were probed with a DNA probe of virus SVBR3, which naturally infects *S. citri* BR3. Viral DNA hybridized to the genomes of all three lines, and results indicated that viral DNA integrated into the *S. citri* genomes at 6-9 sites. Viral DNA hybridized to bands of 3.1 and 25.4 kb of BR3-T, and to a band of 7.1 kb of BR3-G and BR3-P. Thus, hybridization to 3.1 and 25.4 kb bands correlated with insect transmissibility and hybridization to a 7.1 kb fragment correlated with non-transmissibility. Viral DNA integration in the spiroplasma genome may cause disruption or rearrangement of a gene or genes required for insect transmission of spiroplasmas.

A598

PHYLOGENETIC ANALYSIS OF 16S rRNA AND RIBOSOMAL PROTEIN GENES SEQUENCES INDICATES MYCOPLASMALIKE ORGANISMS ARE MONOPHYLETIC. D.E. Gundersen[1,3], I.M. Lee[1], S. Rehner[2], R.E. Davis[1] and D.T. Kingsbury[3]. [1]USDA-ARS, [2]MPPL, [3]SBML, Beltsville, MD 20705, and [3]Dept. Micr. and Imm., George Washington U., Wash. D.C. 20037.

16S rDNA from each of 24 mycoplasmalike organisms (MLOs) representing 10 MLO 16S rRNA groups and 16 subgroups was PCR-amplified and partially (about 50%) sequenced. Phylogenetic relationships within MLOs and among the MLOs and other prokaryotes, including Mollicutes from animal sources, were assessed using parsimony (PAUP program). Within MLOs, 10 terminal groups were resolved, which correspond to the 10 major 16S rRNA groups previously established by Lee et. al. (1993 Phytopathology) on the basis of RFLP analysis. Analysis among MLOs and other prokaryotes indicated that the MLOs are monophyletic. Their closest relative examined was Acholeplasma laidlawii, confirming results of others based on analysis with limited MLO strains. The phylogenetic relationships among MLOs were further supported by analyses of PCR-amplified MLO ribosomal protein genes (primers developed by Lim and Sears, 1992. J. Bacteriol.). These results validate the classification of MLOs based on characteristic RFLP patterns of 16S rDNA sequences.

A599

MYCOPLASMALIKE ORGANISM (MLO) 16S rRNA GROUP-SPECIFIC PRIMER PAIRS FOR PCR-BASED CLASSIFICATION OF MLOs ASSOCIATED WITH PLANTS AND INSECTS. I-M. Lee, D.E. Gundersen, R.W. Hammond, and R.E. Davis. Molecular Plant Pathology Laboratory, Agricultural Research Service, USDA, Beltsville, MD 20705.

Mycoplasmalike organism (MLO) 16S rRNA group-specific primer pairs R16(I)F1R1 and R16(V)F1R1 for PCR were designed on the basis of partial MLO 16S rRNA sequences amplified with the universal primer pair R16F2R2. The primer pair R16(I)F1R1 specifically initiated amplification of 16S rDNA sequences among MLO strains in the MLO group 16SrI, while the primer pair 16R(V)F1R1 initiated amplification in the group 16SrV. Neither primer pair initiated amplification of 16S rDNA sequences from MLO strains in other groups or from other prokaryotes including animal Mollicutes and plant pathogenic bateria. An MLO group-specific primer pair is particularly useful for epidemiological study of MLO-induced diseases, allowing sensitive detection of specific MLO strains from plant and insect sources. Nested PCR assays using the pair R16F2R2 and a group-specific primer pair further increased sensitivity in MLO detection and provided a means for specific detection of a given MLO strain in the case of mixed infection.

A608

NEMATODE MIGRATION TO ROOTS OF ENDOPHYTE-INFECTED AND ENDOPHYTE-FREE TALL FESCUE. Gwinn, K. D., E. C. Bernard, D. J. Trently, and P. L. Jennings. Entomology and Plant Pathology, The University of Tennessee, Knoxville, TN 37901-1071.

Meloidogyne marylandi (Mm) juveniles (J2) do not parasitize endophyte (*Acremonium coenophialum*)-infected (E+) tall fescue, but reproduce well in endophyte-free (E-) plants. *Pratylenchus scribneri* (Ps) colonizes E+ and E- plants equally but does not reproduce in E+ plants. The objective of this research was to determine if nematode migration is influenced by endophyte status of the host. E+ and E- tillers were transplanted to greenhouse pots divided with cardboard inserts. After 3 wk, cardboard inserts were removed and replaced with screen-enclosed soil containing Ps (4000 nematodes/pot) or Mm eggs (7000/pot). After 10 d, nematodes were counted. Ps migrated to E+ and E- rhizospheres and invaded roots in equal numbers. More Mm J2 migrated to E+ rhizosphere than to E- rhizospheres, but J2 numbers were greater in E- roots ($P \leq 0.05$).

A611

POST-INFECTION DEVELOPMENT OF MELOIDOGYNE INCOGNITA ON THREE COTTON LINES WITH DIFFERENT LEVELS OF RESISTANCE. Bing Tang,[1] G. W. Lawrence,[2] R. G. Creech,[1] J. N. Jenkins,[3] and J. C. McCarty, Jr.[3] [1]Departments of Agronomy, [2]Plant Pathology and Weed Science, and USDA-ARS, Crop Science Res. Lab., Mississippi State University, Mississippi State, MS 39762.

Susceptible (M-8), tolerant (T-78) and resistant (M-315) cotton, Gossypium hirsutum L., germplasms were compared for effects on stages of development of Meloidogyne incognita race 3. Post-infection stages were classified into seven developmental groups to indicate penetration, life stage development, population density and reproduction. Resistance in M-315 was characterized by a significant reduction in nematode development 8 days after inoculation, fewer juveniles developing into adults, and reduced (compared to M-8) number and size of root galls. For T-78, M. incognita development was reduced 26 days after inoculation, fewer late stage juveniles developed into adult females or egg laying females, and gall size was reduced compared to M-8.

A614

EFFECTS OF NATURALLY OCCURRING AROMATIC COMPOUNDS ON PARASITIC NEMATODES IN COTTON. E. M. Bauske, V. Estaún, R. Rodríguez-Kábana, and J. W. Kloepper. Auburn University, AL, 36849.

The effects of benzaldehyde, citral, menthol, furfural, and α-terpineol on nematodes were determined in a greenhouse experiment using naturally infested soil. Compounds were applied at 0, 0.1, 0.25, and 0.5 ml/kg soil and ten days after treatment, 'Deltapine 50' cotton seed was planted. Nematodes in the soil and roots, percentage root colonization by arbuscular mycorrhial fungi (AM), root galling, and plant height were assessed. None of the compounds adversely affected plant height. Terpineol at 0.1 and 0.25 ml/kg and the lowest levels of citral and menthol had no effect on AM colonization. All compounds studied reduced *Meloidogyne incognita* larvae in roots and soil and reduced the number of galls/g root. *Hoplolaimus galeatus* populations in roots decreased with all levels of benzaldehyde, citral, and menthol, were not affected by terpineol, and were increased by all levels of furfural. Low and high levels of benzaldehyde and all levels of furfural and terpineol increased populations of *Paratrichodorus minor* in soil. No *Tylenchorhynchus* spp. were found in soil treated with either citral or menthol and populations increased in soil treated with furfural.

A620

CHEMICAL MANAGEMENT OF THE LANCE NEMATODE (HOPLOLAIMUS MAGNISTYLUS) ON COTTON. G. W. Lawrence[1] and K. S. McLean.[2] [1]Department of Plant Pathology and Weed Science, Mississippi State University, Mississippi State, MS, 39762 and [2]Department of Agriculture, Northeast Louisiana State University, Monroe, LA 71209.

The efficacy of nematicides to reduce the lance nematode (Hoplolaimus magnistylus Robbins, 1982) populations and effects on growth and yield of cotton (Gossypium hersutum) cv. DPL-20 were examined. The test was conducted in a field naturally infested with H. magnistylus with an average initial nematode population density of 172 nematodes/250 cm^3 soil. Nematicide treatments consisted of 1,3-dichloropropene (1,3-D), aldicarb, and 1,3-D + aldicarb. Nematode population development was followed monthly for 185 days. At harvest, seed cotton yields were significantly increased in the plots treated with 1,3-D + aldicarb (28 liters/ha + 0.56 kg ai/ha) compared with the untreated control. Seed cotton yield was increased 353 kg/ha. Yields were numerically greater in the treated plots when compared with the control; however, all increases were not significant. Hoplolaimus magnistylus population densities increased to a high of 989 nematodes/250 cm^3 soil at 185 days after planting. All nematicide treatments significantly reduced H. magnistylus at harvest.

A621

SURVEY FOR TOBACCO NECROSIS STRAINS OF POTATO VIRUS Y IN POTATO PRODUCTION AREAS OF FLORIDA. L.G. Brown and D.P. Weingartner. Florida Department of Agriculture and Consumer Services and University of Florida, Gainesville, Florida, 32614.

Around 1989 PVY^N was detected in the potato seed production in certain areas of Canada and remains a problem despite extensive eradication efforts by the Canadian government and growers. The Florida Department of Agriculture & Consumer Services (FDACS) was concerned that PVY^N had been introduced on seed potatoes from Canada. The Florida survey design detected PVY^N in the Homestead area in 1992, and in Manatee and St. Johns counties in 1993. Serological detection was made with Maby^N 295.5. Confirmation of the serological tests was by inoculation to Burley 21 tobacco. The survey encompassed over 80% of the 47,000 acres of commercial potato production in Florida. After the initial detection the re-sampling of positive farms demonstrated that viral incidence in most fields was < 0.01% with the highest incidence being < 0.03%. Re-sampling verified the precision of the survey method which was designed to detect the virus at a 0.1% incidence with a 95% confidence. Tissue samples were concurrently tested for potato virus Y^O, potato leafroll virus, potato virus M, and potato virus X.

A622

IN VITRO SELECTION OF SOYBEAN CULTIVARS FOR STIMULATION OF *STRIGA HERMONTHICA* SEED GERMINATION. M. O. Alabi, D. K. Berner, and B. A. Okusanya. The International Institute of Tropical Agriculture, PMB 5320, Oyo Road, Ibadan, Nigeria.

An *in vitro* technique was developed to screen soybeans (non-hosts) for efficiency in germinating *Striga hermonthica* seeds. Surface disinfested *S. hermonthica* seeds were placed on 8-mm glass fiber filter paper discs (25-30 seeds/disc) placed on moistened filter paper in petri dishes. Seeds were conditioned by incubating at 30°C in darkness for 10 d. Roots of 7-d-old soybean seedlings were cut into 1 cm pieces, weighed and placed into a 2-cm-diam. aluminum ring centered on moistened filter papers in a petri dish. Glass fiber discs with the conditioned *S. hermonthica* seeds were placed around the central ring in 4 radii of 3 concentric rings with the first one touching the germination source. After incubating for 48 hours at 30°C, percent *S. hermonthica* seed germination was determined. Distances from the germination source and root weight were used as covariates in analyses of cultivar differences. Results showed significant differences among 58 soybean cultivars in *S. hermonthica* seeds germinated. Interestingly, germination significantly decreased with increasing root weight and increased with distance from root cuttings.

A623

FIELD INFESTATION METHODS FOR *STRIGA HERMONTHICA*. D. K. Berner and E. I. Aigbokhan, International Institute of Tropical Agriculture, PMB 5320, Oyo Road, Ibadan, Nigeria.

Erratic infection of hosts by *Striga hermonthica* in field trials makes selection of resistant materials and evaluation of control tactics difficult. Artificial infestation methods with *S. hermonthica* seeds were tested for ease of usage and efficacy in reducing field variability in infection. Methods tested were: 1. sand as a carrier material with a) 14 d and 7 d *S. hermonthica* seed conditioning in the field, b) 14 d and 7 d conditioning in the laboratory c) no conditioning and 2. water as a carrier material with a) 14 d and 7 d laboratory conditioning and c) no conditioning. Two maize cultivars were used. Numbers of emerged *S. hermonthica* plants, maize yield, and coefficients of variation were used as criteria of assessment. Results showed that methods 1a gave lower infection with higher variability than the other methods. Among the other methods there were no significant differences for the criteria assessed. Any of these other methods could be used to produce uniformly infected fields, the choice dependent on ease of usage and availability of materials and labor.

A624

RESPONSE OF *STRIGA HERMONTHICA* SEEDS TO DIFFERENT GERMINATION STIMULANTS AND STIMULANT CONCENTRATIONS. E. S. Ariga and D. K. Berner. International Institute of Tropical Agriculture, PMB 5320, Oyo Road, Ibadan, Nigeria.

Surface disinfested *Striga hermonthica* seeds were placed on 8-mm glass fiber discs (25-50 seeds/disc) placed on moistened filter paper in petri dishes. The seeds were conditioned by incubating in darkness at 28 C for 11 d. Three populations of *S. hermonthica* seeds were used. Germination stimulants were isolated from 5 g of dried, ground cotton roots, stems and leaves and dried, ground cowpea stems. Cotton samples were soaked in 200 ml of distilled water for 1 hr and then filtered. Cowpea samples were soaked in 50, 100, 200, 400, 800, 1000, 1500, and 2000 ml of water to form different concentrations of stimulant. Concentrations of 1, 5, 10, 20, 40, and 100 ppm of synthetic germination stimulant (GR24) were also tested for parasite germination. To each disc of conditioned seeds, 0.13 ml of stimulant was applied. Germination was determined after incubation at 28 C for 48 hr. Results showed that cotton roots were the most effective stimulant among the plant materials tested. Increasing concentrations of both cowpea stem extracts and GR24 resulted in reductions in *S. hermonthica* seed germination.

A627

CONDITIONS FOR PROPAGATING AND STORING PEANUT MOTTLE POTYVIRUS IN TISSUE CULTURE. P. Bagade, G. R. Buss, and S. A. Tolin. Departments of Crop and Soil Environmental Sciences, and Plant Pathology, Physiology and Weed Science, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061.

In a study of the inheritance of resistance in soybean to peanut mottle potyvirus (PMV), we have had difficulties in consistently recovering virus from peanut or soybean leaves desiccated over $CaCl_2$ and stored for longer than a few months. Attempts were made to use a dual soybean callus+virus culture method that we have used successfully for soybean mosaic virus. Leaves of soybean or peanut infected with PMV were used to establish callus cultures on a modified MS medium. Transfers were made to fresh media at two-month intervals by selecting firm, dark-green sections of each callus. Infectivity assays of early passage calli were positive but indicated a low virus titer. After four subcultures no infectivity was obtained and no viral antigen was detected by a tissue immunoblot assay. It is postulated that, because PMV is unevenly distributed in mottled leaves of soybean and peanut, the non-infected cells in a callus overgrow the infected cells resulting in loss of virus in the callus. Studies are in progress to determine the effect on PMV in tissue culture of the symptomology and developmental stage of explant source leaves, the portion of callus used for subculturing, and the growth conditions using Nicotiana benthamiana as well as different soybean cultivars.

A628

DETECTION OF PLANT VIRUSES USING NON-RADIOACTIVE DOT BLOT HYBRIDIZATION. R. Creamer and K. Harper, Department of Plant Pathology, University of California, Riverside, CA 92521.

A dot blot hybridization system for colorimetric detection of plant viruses was developed using the Genius® kit system. Gemiviruses, including squash leaf curl virus, beet curly top virus, and tomato geminiviruses -MX1 and MX2, zucchini yellow mosaic potyvirus, lettuce infectious yellows virus, and beet yellows closterovirus were detected using this system. For detection of geminiviruses, plant material was extracted using either a sodium-sulfite or Tris buffer followed by two phenol:chloroform treatments. Sodium hydroxide denaturation was performed on extracts before blotting. Material from plants to be tested for the presence of RNA viruses could be similarly extracted using the Tris buffer. These extracts or supernatant could be blotted after formaldehyde/SSC extraction and heat denaturation. However, in order to prevent very strong interference or blocking of the detection signal, membranes containing blots of crude plant homogenate required a treatment of Pronase and Proteinase K prior to hybridization. This colorimetric system was less sensitive than radioactive dot blot hybridization, but was successfully done in laboratories with minimal equipment.

A629

THREE VARIANTS DERIVED FROM A SINGLE CULTURE OF IMPATIENS NECROTIC SPOT VIRUS. I. ELECTRON MICROSCOPY. R.H. Lawson, M.M. Dienelt and H.T. Hsu. USDA, ARS, FNCL, Beltsville, MD 20705-2350

A defective isolate of Impatiens necrotic spot virus from gloxinia, INSV-Igg lacks virions but develops dense, chain-like masses of nucleocapsid protein (N protein). These structures react in thin section with antiserum to INSV N-protein in immunogold labeling tests. When INSV-Igg was passaged through four series of eight mechanical transfers each in Nicotiana benthamiana at high temperature, (HT) (27/24 day/night) and at low temperature, (LT) (21/18 day/night), three stable cultures were distinguished. At LT, the culture remained identical to the original. At HT, virions were always formed and two HT cultures were recovered. INSV-HT-1 failed to react serologically with INSV antiserum and lacked chain-like N protein. INSV-HT-2 formed chain-like N protein and was serologically reactive. A fourth INSV culture INSV-B from Impatiens, was maintained in N. benthamiana at LT and HT and displayed serological and cytological characteristics similar to those of INSV-HT-2. When the four viral cultures were grown at LT and HT in Impatiens Accent Salmon, their characteristic cytopathological and serological properties remained unchanged. Isolates could be further differentiated by their systemic effects in Impatiens.

A630

THREE VARIANTS DERIVED FROM A SINGLE CULTURE OF IMPATIENS NECROTIC SPOT VIRUS. II. SYMPTOMATOLOGY AND SEROLOGY. H.T. Hsu, R.H. Lawson & M.M. Dienelt. USDA,ARS,FNCL, Beltsville, MD 20705.

An isolate of Impatiens necrotic spot virus, INSV-HT-1 was compared on Impatiens Accent Salmon with the original INSV-Igg culture, a second high temperature culture, INSV-HT-2, and an isolate from Impatiens INSV-B. HT-1, HT-2 and INSV-Igg induced primary local lesions in Impatiens leaves 3-4 days after inoculation. Local lesions first appeared as faint chlorotic spots within necrotic rings. Necrotic lesions expanded rapidly within 24-48 hours. Inoculated leaves collapsed and abscised from the stem at the base of petiole. HT-1 produced systemic stem necrosis 7-9 days post inoculation. The presence of the virus was confirmed by infectivity assay in Nicotiana benthamiana and by electron microscopy. HT-2 produced systemic necrotic lesions on some leaves with occasional stem necrosis. No systemic invasion was observed in Impatiens inoculated with INSV-Igg two weeks after inoculation. INSV-B induced diffuse chlorosis and small necrotic spots on Impatiens leaves 5-6 days post inoculation. Systemic line pattern and areas of tissue discoloration without stem necrosis were present in INSV-B infections. Igg, HT-2 and INSV-B, but not HT-1, react serologically with antiserum prepared to INSV N protein.

A631

DETECTION OF APPLE CHLOROTIC LEAFSPOT CLOSTEROVIRUS AND APPLE STEM GROOVING CAPILLOVIRUS USING RT-PCR. G. R. Kinard and S. W. Scott. Department of Plant Pathology and Physiology, Clemson University, Clemson, SC 29634-0377.

Apple chlorotic leafspot virus (ACLSV) and apple stem grooving virus (ASGV) both infect rosaceous fruit trees. Although latent in many cultivars, both are associated with severe graft incompatibilities in some scion/rootstock combinations. Both viruses occur in low concentrations in woody hosts. Detection methods include graft bioassays onto woody indicators and ELISA, which have limitations. We have selected two pairs of primers from published sequences that specifically amplify a 548 nt fragment of the ACLSV genome and a 419 nt segment of the ASGV genome when used in the reverse transcriptase polymerase chain reaction (RT-PCR). Ten pg of purified virus (≈ 500 fg of viral RNA) can be detected by agarose gel electrophoresis of the PCR product. The primers selected amplified fragments from both European and American isolates of ACLSV and an American isolate of ASGV. RT-PCR readily detects both viruses in total nucleic acid extracts (TNA) of virus-infected Chenopodium quinoa Willd., but is less effective for TNA from rosaceous fruit species. Research is in progress to optimize this system for the rapid, reliable detection of ACLSV and ASGV in apple leaf tissue.

A632

NON RADIOACTIVE PROBES GENERATED FROM DSRNA. R. A. Valverde, R. A. Arancibia, and F. Can. Dept. of Plant Pathology and Crop Physiology, Louisiana Agricultural Experiment Station, Louisiana State University Agricultural Center, Baton Rouge 70803.

DsRNAs from pepper cryptic virus, physalis mottle virus, a fungal virus and rice were extracted with phenol and purified with cellulose. After DNase treatment, dsRNA was boiled and cooled on ice. Resultant ssRNAs were cross-linked with horseradish peroxidase (HRP) following the direct labelling method of Amersham. DsRNA samples were run on agarose or acrylamide gels and electroblotted on nylon membranes after denaturation. Molecular hybridization was conducted overnight at 42 C. Luminol was used as chemiluminescent substrate for the detection of HRP labelled dsRNAs. Blots were then exposed to X-ray film. Successful labelling and hybridizations were obtained with all dsRNA types as sources of ssRNA. This method was used to determine sequence similarities among dsRNAs.

A633

A VIRUS-LIKE AGENT ASSOCIATED WITH THISTLE MOSAIC DISEASE. K.- K. Ahn, K. S. Kim, R. C. Gergerich and E. J. Anderson, Department of Plant Pathology, University of Arkansas, Fayetteville, AR 72701, USA.

Virus-like particles (VLPs) of unique morphology and viroplasm-like inclusions were found in field thistle (*Cirsium discolor*) showing mosaic symptoms. No VLPs or inclusions were observed in symptomless thistles. The VLPs were large, double-membrane bound, ovoid particles ranging from 100 to 150 nm in diameter. These particles are similar to the virus-like agents associated with wheat spot mosaic, fig mosaic, yellow ringspot of redbud, and rose rosette diseases. The VLPs were observed in the cytoplasm of all leaf cell types and many appeared to be closely associated with viroplasm-like inclusions. No dsRNAs were detected in symptomatic tissues. The particles and associated inclusions are morphologically similar to those reported for immature poxviruses in animal and insect cells. Poxviruses are large, enveloped dsDNA viruses that have not been reported in plants. The purification and molecular characterization of VLPs are in progress.

A634

ANALYSIS OF BARLEY YELLOW DWARF VIRUS STRUCTURE USING MONOCLONAL ANTIBODIES TO THE 22kDa AND 50kDa PROTEINS. S.-L. Cheng, L. L. Domier, and C. J. D'Arcy. Department of Plant Pathology, University of Illinois, 1102 S. Goodwin Ave., Urbana, IL 61801.

Two murine monoclonal antibodies (MAb) against BYDV-PAV-IL have been produced: MAb PAV-IL-22k is specific to the 22kDa core coat protein and MAb PAV-IL-50k is specific to the 50kDa protein. The 50kDa protein is a read-through protein fused to the carboxyl terminus of the 22kDa ORF, and is produced by read-through of a stop codon. MAb PAV-IL-22k reacts with BYDV-PAV-IL and BYDV-MAV-NY, which supports previous serological data. MAb PAV-IL-50k reacts with BYDV-PAV-IL and BYDV-RPV-IL, which are distantly serologically related, but share the aphid vector, *Rhopalosiphum padi*. Purified BYDV-PAV-IL trapped on sepharose linked to MAb PAV-IL-22k was labeled with fluorescein-conjugated MAb PAV-IL-50k. These results are evidence that the 50kDa protein is a structural protein on the external surface of the BYDV-PAV virion.

A635

CHIMERIC GEMINIVIRAL CONSTRUCTIONS BETWEEN BEAN GOLDEN MOSAIC VIRUS AND BEAN DWARF MOSAIC VIRUS INDICATE THAT DNA-B CONTRIBUTES TO SYMPTOM EXPRESSION AND POSSIBLY TO HOST RANGE. M. B. Bett and D. P. Maxwell. Dept. of Plant Pathology, University of Wisconsin, Madison, WI 53706.

A Guatemalan isolate of bean golden mosaic geminivirus (BGMV-GA) produces golden mosaic symptoms on *Phaseolus vulgaris* cvs. Topcrop and Pinto 114, dwarf symptoms on *P. vulgaris* cv. Red Kidney and no symptoms on *Nicotiana benthamiana*. A Colombian isolate of bean dwarf mosaic virus (BDMV-CO) produces dwarf symptoms on Topcrop and *N. benthamiana* and no symptoms on Pinto 114 and Red Kidney. Nucleotide sequence comparison of the common region and phylogenetic analyses have placed BGMV-GA and BDMV-CO in different phylogenetic clusters. Pseudorecombinants constructed with DNA-A of one virus and DNA-B of the other were not infectious. However, when beans were inoculated with DNA-A of BGMV-GA and a chimeric DNA-B, in which the common region from BGMV-GA replaced the common region in the DNA-B of BDMV-CO, dwarf symptoms occurred on Topcrop, variable symptoms occurred on *N. benthamiana*, and no symptoms occurred on Pinto 114 or Red Kidney. The results suggest that DNA-B contributes to symptom expression and possibly to host range.

A636

IN SITU **LOCALIZATION OF BARLEY YELLOW DWARF VIRUS IN OATS.** Petra H. Nass[1)], Birute P. Jakstys[2)], and Cleora J. D'Arcy[1)]. Department of Plant Pathology[1)] and Center for Electron Microscopy[2)], University of Illinois, South Goodwin Ave, Urbana, IL 61801.

Barley yellow dwarf luteovirus (BYDV) is restricted to the phloem tissue of its host. An immunogold localization assay was developed to identify viral coat protein on a subcellular level. Seven-day-old Coast Black oat seedlings were inoculated with BYDV-PAV-IL by 20-30 viruliferous *Rhopalosiphum padi* L. during a 48-72hr inoculation access. At 3, 5, 7 and 10 days after the beginning of the inoculation access, 1 mm^2 samples were taken from the midrib of the primary leaf. Samples were fixed in 0.5% glutaraldehyde/3.5% paraformaldehyde and 1% OsO_4 in 50mM sodium cacodylate buffer, ph 7.0. After a dehydration series of ethanol and propylene oxide, samples were embedded in Spurr's resin. Viral particles were labeled with either polyclonal or monoclonal antibodies. Polyclonal antibodies were cross adsorbed with sap from non-infected oats. Primary antibodies were detected with gold conjugated protein A. In infected plants viral coat protein was observed mainly in the cytoplasm, whereas no label was found in non-infected plants.

A637

GENOME STRUCTURE OF RNA-2 OF A TOBACCO RATTLE VIRUS STRAIN FROM THE PACIFIC NORTHWEST. M.R. Sudarshana and P.H. Berger, Dept. of Plant, Soil, and Entomological Sciences, University of Idaho, Moscow, ID 83844.

Genomic RNA-2 from tobacco rattle virus (TRV) strains is highly variable in the number of open reading frames (ORFs), coat protein (CP) homology, and length of the 3'-end homologous to RNA-1. To understand the genome structure of a Pacific Northwest strain of TRV, RNA-2 (ca. 3.3 kb) of TRV-ORY was reverse transcribed and a cDNA library constructed. Two overlapping cDNA clones that span about 2200 bases were sequenced using dideoxy chain termination reactions. The 5'-end was identified based on homology with RNA-2 of TRV-TCM. Two ORFs were found organized as in RNA-2 of TRV-TCM and the first ORF coded for a putative CP which was most closely related to pepper ringspot tobravirus (PRV). The second ORF was found to be unrelated to any of the ORFs of other tobraviruses. The identity of the second ORF is being investigated. It appears that TRV-ORY is an anomalous strain, much like TRV-TCM, and possibly arose out of recombination of a TRV strain with PRV.

A638

IDENTIFICATION OF PLANT PROTEINS WHICH BIND NON-BEETLE TRANSMISSIBLE VIRUSES. T. MAHMOOD, R. C. Gergerich, and E. J. Anderson. Department of Plant Pathology, University of Arkansas, Fayetteville, AR 72701.

Non-beetle transmissible viruses are not translocated when introduced into the vascular system of plants. Adsorption or attachment to some plant component(s) in the vascular system may immobilize these viruses and prevent translocation and infection (Ann. Rev. Phytopath. 25:111-123). Two non-beetle transmissible viruses, tobacco ringspot virus (TRSV) and the cowpea strain of tobacco mosaic virus (CP-TMV), were used to identify plant components that might bind these viruses. An extra protein was detected when purified TRSV or CP-TMV was incubated with a crude extract of plant proteins, pelleted by ultracentrifugation, and then analyzed by SDS-PAGE. This protein was absent in purified virus or when beetle-transmissible southern bean mosaic and bean pod mottle viruses were similarly incubated with plant proteins. With TRSV, the additional protein was 17 KDa whereas that for CP-TMV was about 16 KDa. These putative binding proteins for CP-TMV and TRSV in Black Valentine bean extracts were also detected by virus overlay of protein blots (Biochem. Biophys. Acta 856:19-26).

A639

CHARACTERIZATION OF A SEED-TRANSMITTED CUCUMBER MOSAIC VIRUS (CMV) ISOLATE FROM SPINACH (*Spinacia oleracea* L.). Y. Yang[1], J. C. Correll[1], T. E. Morelock[2] and E. J. Anderson[1]. [1]Department of Plant Pathology and [2]Department of Horticulture and Forestry, University of Arkansas, Fayetteville, AR 72701.

Cucumber mosaic virus (CMV) has been reported to be seed-transmitted in a number of plant species but, to our knowledge, not in spinach (*Spinacia oleracea* L.). Seed lots from two commercially grown varieties of spinach, suspected of CMV infection, were tested by seed grow-out and enzyme-linked immunosorbent assays (ELISA). Results indicated that CMV was seed-transmitted in spinach and the infection rate of seedlings was as high as 15%. The coat protein gene and flanking sequences of this spinach isolate of CMV were amplified from infected leaf nucleic acid preparations by polymerase chain reaction (PCR) and the resulting cDNA was cloned and sequenced. Coat protein cDNA sequence analysis revealed 96% sequence identity with CMV-Q, a member of subgroup S (II, ToRS), and only 74% sequence homology with CMV-Y, a member of subgroup WT (I, DTL), indicating that this CMV isolate from spinach is a member of subgroup S(II, ToRS).

A640

CHARACTERIZATION OF COAT PROTEIN GENES OF CITRUS TRISTEZA VIRUS STRAINS FROM COLOMBIA. M. Guzman Barney*, K.L. Manjunath, V.J. Febres, S.S. Pappu, H.R. Pappu, R.F. Lee**, and C.L. Niblett. *Biotechnology Institute, National University, Bogota, Colombia; Plant Pathology Department, University of Florida, Gainesville, FL 32611 and **Citrus Research and Education Center, Lake Alfred, FL 33850.

Citrus tristeza virus (CTV) is widespread and severe in Colombia. Lethal quick decline (QD) strains generally preclude the use of sour orange (SO) rootstock, and stem pitting (SP) strains severely affect citrus on QD-tolerant rootstocks. However, near Mompox, a hot, humid area in northern Colombia, QD and SP strains are absent, SO is the predominant rootstock, and citrus is infected only with mild CTV strains. Coat protein (CP) genes of mild and severe Colombian strains were amplified and sequenced. The CP genes of several severe strains contained a unique EcoR1 restriction site; mild strains did not. The deduced amino acid sequences and the serological reactivities of the mild strain CPs from Colombia were consistent with those of other known mild strains. (Supported in part by COLCIENCIAS, Bogota, Colombia).

A641

SEQUENCE VARIATION IN THE AMINO TERMINUS OF THE CAPSID PROTEIN OF TWO ISOLATES OF DASHEEN MOSAIC POTYVIRUS. S.S. Pappu, H.R. Pappu, R.J. Lastra* and C.L. Niblett. Plant Pathology Department, University of Florida, Gainesville, FL 32611, USA and *CATIE, Turrialba, Costa Rica.

Dasheen mosaic virus (DMV) is a potyvirus that infects members of the *Araceae* family. Western blots of DMV isolates from Florida (LA, TEN and HS) and Costa Rica (CR1) revealed differences in molecular weight of capsid proteins (CPs) of these isolates. To determine the molecular basis for this variability, the capsid protein gene (CPG) of the Florida isolate TEN whose CP is smaller than that of LA was cloned and sequenced. Comparison of the TEN CP sequence to the already known sequence of LA CP revealed sequence differences in the amino terminus of the CP. The CP of isolate TEN is 314 residues long and is 15 amino acid residues shorter than that of LA. This difference was due to a 12 base insertion and a 57 base deletion in the 5' region of CPG of TEN as compared to LA. Otherwise, both isolates were highly homologous in the CP (95.8%) and the 3' non-coding region (88.97%).

A642

EFFECT OF THE SALIVARY GLAND BASAL LAMINA ON TRANSMISSION EFFICIENCY OF BARLEY YELLOW DWARF VIRUS (BYDV) BY APHIDS. M.L. Peiffer, F.E. Gildow, and S.M. Gray. Departments of Plant Pathology. Penn State University, University Park, PA 16802 and USDA-ARS, Cornell University, Ithaca, NY 14853.

Ability of virions of the RPV and PAV isolates of BYDV to penetrate aphid accessory salivary gland basal lamina (ASG-BL) was associated with transmission efficiency. Individual aphids were injected with 1 ng RPV, allowed to feed 24 h on oats, then examined by electron microscopy. 64% of *Rhopalosiphum padi (Rp)* transmitted RPV. *Sitobion avenae (Sa), Schizaphis graminum (Sg), Metopolophium dirhodum (Md)* and *R. maidis (Rm)* did not transmit RPV. The density of virions in the ASG-BL in *Rp, Sa, Sg, Md* and *Rm* was 20, 57, 2.5, 13 and 0 virions/10 µm, respectively. Virus was observed intracellularly only in *Rp*. When individual aphids were injected with 4 ng PAV, the percentage of *Rp, Sa, Sg, Md* and *Rm* transmitting to oats was 54, 15, 4, 8 and 0%, respectively. Densities of PAV in the ASG-BL of the same species were 37, 4.5, <1, 10 and 0 virions/10 µm. This suggests that in *Rm*, the ASG-BL is a barrier to virus ingress into the gland. Differences in ability of virions to attach to and penetrate BL of aphids suggests differences in BL composition influence vector specificity.

A643

THE RELATIONSHIP BETWEEN FEEDING AND VIRUS RETENTION TIME IN BEETLE TRANSMISSION OF PLANT VIRUSES. R. Y. Wang, R. C. Gergerich, and K. S. Kim. Department of Plant Pathology, University of Arkansas, Fayetteville, AR 72701.

Retention time is an important factor in determining temporal patterns of plant virus dissemination and overwintering in viruliferous vectors. Three leaf-feeding beetles, Mexican bean beetle (Epilachna varivestis), bean leaf beetle (Cerotoma trifurcata), and spotted cucumber beetle (Diabrotica undecimpunctata howardi), were used to study retention of southern bean mosaic virus (SBMV) in leaf-feeding beetles. After beetles acquired SBMV from virus-infected Black Valentine bean leaves, they were viruliferous for less than four days unless they did not feed, in which case they were viruliferous for longer periods. To test the hypothesis that virus retention time is determined solely by the amount of feeding after virus acquisition, Mexican bean beetles and spotted cucumber beetles were held at 5-7 C for 10, 20, and 30 days

without feeding after virus acquisition from SBMV-infected leaves. The same virus transmission rate and pattern were observed for beetles after this cold treatment as for those control beetles that were not subjected to the cold treatment, suggesting that feeding is a factor that limits plant virus retention by leaf-feeding beetles.

A645

ENHANCED COEXISTENCE BETWEEN NEAR-ISOGENIC MANNITYL OPINE CATABOLIZING AND NON-CATABOLIZING *PSEUDOMONAS SYRINGAE* STRAINS ON MANNITYL OPINE-PRODUCING TOBACCO PLANTS. M. Wilson[1], I. Hwang[2], M.A. Savka[2], S.K. Farrand[2], and S.E. Lindow[1]. University of California, Berkeley, 94720.[1] University of Illinois, Urbana-Champaign.[2]

The plasmid pYDH208, encoding catabolism of mannopine and agropine, was introduced into *Pseudomonas syringae* Cit7. Replacement series experiments were conducted with the near-isogenic strain pair *P. syringae* Cit7(pYDH208) and Cit7::*xyl*E on tobacco plants (*Nicotiana tabacum* cv. Xanthi) transformed to produce mannityl opines and on parental wild type plants. When inoculated individually onto wild type plants the populations of both strains were similar and when combined the strains competed equally for limiting resources. When inoculated individually onto transgenic plants the population of Cit7(pYDH208) was 4-fold higher than that of Cit7::*xyl*E. When combined, while the relative competitiveness of the strains was unaltered, the ability of Cit7(pYDH208) to utilize mannityl opines enhanced the level of coexistence with the near-isogenic strain Cit7::*xyl*E.

A646

GENOTYPIC VARIATION IN COLOMBIAN ISOLATES OF PSEUDOMONAS SOLANACEARUM RACE 2 DETERMINED BY RFLP ANALYSIS. [1]Granada, G., [2]Howell, M., and *[2]Cook, D. [1]Instituto Colombiano Agropecuario, Colombia. [2]Department of Plant Pathology and Microbiology, Texas A&M University.

Recent severe epidemics of Moko disease caused by *Pseudomonas solanacearum* on improved plantain varieties have raised questions about increased genetic variability of the pathogen in Colombia. 28 isolates of *P. solanacearum* were analyzed for restriction fragment length polymorphism (RFLP) at eight different RFLP loci. Nineteen of these isolates were identical to a multi-locus genotype (MLG), MLG 25, which was determined previously to be widely distributed on plantain throughout Colombia and Peru. The remaining nine isolates, however, belong to three previously unrecorded multi-locus genotypes: MLGs 47, 48, and 51. MLG 51, from northern Colombia, has no new alleles, but is comprised of a mixture of alleles associated with either MLG 25 or the banana/plantain MLG 28 occurring in Venezuela and Honduras. MLG 47 from northern Colombia and MLG 48 from southwestern Colombia contain new alleles, and thus may indicate expansion of the genetic variation present in Colombian plantain isolates of *P. solanacearum*. MLG 47 contains a new allele of the major *P. solanacearum hrp* locus, but otherwise is identical to MLG 25. MLG 48 contains two new alleles, and two known alleles not found previously in isolates of Race 2.

A647

POTENTIAL OF ENDOPHYTIC BACTERIA FOR BIOLOGICAL CONTROL OF FUSARIUM WILT OF COTTON. C. Q. Chen, G. Musson, E. Bauske, and J.W. Kloepper. Dept. of Plant Pathology, Biological Control Institute, Auburn University, Auburn, AL 36849-5409.

Endophytic bacterial strains collected from healthy cotton stems and roots were evaluated for biological control potential in a model system with cotton Fusarium wilt. Bacteria were stab-inoculated into 7-day old cotton plants near the base of the stem. Controls were inoculated with sterile water. Ten days later, plants were stab-inoculated with 10^6 spores/ml of a pathogenic isolate of *Fusarium oxysporum* f.sp. *vasinfectum*. In primary screens, bacterized plants were visually compared to non-bacterized plants using a 0 to 4 disease rating scale. Forty-one of 243 strains were chosen for reduced symptom expression. In the secondary screen completely randomized design experiments were used. Fifteen of 41 strains caused significant disease reductions compared to the control. In the advanced screen, these 15 strains were further tested using randomized complete block design experiments. In repeated testing several strains provided significant protection. Strains included nine isolates of *Pseudomonas* sp. and two isolates of *Phyllobacterium rubiacearum*. The results indicate that some endophytic bacteria have potential as biological control agents.

A648

DNAK AND THE HEAT STRESS RESPONSE OF Pseudomonas syringae pv glycinea race 4. L. M. Wolfson, S. A. Snyder, and J. E. Partridge. Department of Plant Pathology, University of Nebraska, Lincoln, 68583-0722.

Pseudomonas syringae pv glycinea race 4 (Psg4) responds to supraoptimal temperatures with the production of DNAK. Sustained temperatures above 32C and temperature shocks above 38C lead to death. Because of its molecular chaperone function, as expected DNAK synthesis is constitutive at all temperatures; however, temperatures above 31C induce elevated levels to be produced. Cells are "protected" from the lethal effects of a 40C heat shock when pretreated at 31C. Increased quantities of DNAK may be demonstrated at all temperatures above 31C; however this increase diminishes at tempertures above 35C. Growth curve response indicates increasing length of doubling times above 32C with little or no growth above 38C. This research indicates that Psg4 responds to heat shock by producing DNAK but that it does not acclimate to sustained or cyclic supraoptimal temperatures.

A649

DIFFERENTIATION OF *STREPTOMYCES* STRAINS BY ANALYSIS OF CELLULAR FATTY ACIDS. T.C.R. Ndowora; L.L. Kinkel; R.K. Jones; and N.A. Anderson. Dept. of Plant Pathology, U of MN, St. Paul 55108.

Streptomyces scabies causes potato scab. Antibiotic-producing *Streptomyces* species from scab suppressive soil inhibit growth of the pathogen *in vitro*. Distinction of pathogenic and suppressive isolates is critical to studying the interactions between these groups *in vivo*. Cellular fatty acid (FA) compositions were analyzed for a random collection of 13 pathogenic and 7 suppressive strains isolated from potato tubers. Fatty acids were extracted from 72h cultures of each isolate and converted to FA methyl esters (FAMEs). The FAMEs were analyzed using gas chromatography. Suppressive and pathogenic *Streptomyces* strains were distinguishable on the basis of their cellular FA compositions. The predominant FAs found were 16:0 ISO (26.9%) for the pathogenic and 15:0 ANTEISO (25.7%) for the suppressive isolates. Subgroupings obtained within the pathogenic and suppressive groups were similar but not completely identical to those resulting from traditional taxonomic, heterokaryosis, and pairing tests.

A650

DETECTION AND DIFFERENTIATION OF *XANTHOMONAS CAMPESTRIS* PATHOVARS BY POLYMERASE CHAIN REACTION. M. A. Sulzinski, G. W. Moorman, and C. P. Romaine. Department of Biology, University of Scranton, Scranton, PA 18510, and Department of Plant Pathology, The Pennsylvania State University, University Park, PA 16802

Two sets of highly conserved enterobacterial consensus sequences were used as targets for polymerase chain reaction (PCR) amplification: (a) Enterobacterial Repetitive Intergenic Consensus [ERIC] and (b) Repetitive Entragenic Palindromic [REP]. Nucleic acid was extracted from a series of *X. campestris* pathovar isolates, including *pelargonii*, *citri*, *citrumelo*, and *begoniae*, as well as from other phytopathogenic bacteria. After PCR amplification using ERIC or REP primers, amplicon products were separated by agarose gel electrophoresis. With either primer pair, fingerprints were essentially identical within bacterial pathovars, but were unique for each species or pathovar tested. Application of this technique for pathogen-free certification of floral stock is being investigated.

A651

EXTRACELLULAR COMPLEMENTATION OF PIGMENT AND EPS PRODUCTION IN *XANTHOMONAS*. A. R. Poplawsky, W. W. C. Chun. Plant Pathology Division, PSES, Universtiy of Idaho, Moscow ID 83843

Most xanthomonads produce yellow pigments (xanthomonadins) and extracellular polysaccharide (EPS). Although no biological role is known for the xanthomonadins, EPS is implicated in pathogenesis. Insertion mutations in either *pig*B or *pig*C of *X. campestris* pv. *campestris* (*Xcc*) reduced or eliminated pigment and EPS production. Both traits were restored either by functional copies of *pig*B and *pig*C, or by growth in culture adjacent to strains of five pathovars of *Xc* or mutant strains containing functional copies of *pig*B and *pig*C. This effect was reversible, transferable in media and eliminated by dialysis. Although *pig*B and *pig*C mutants were fully pathogenic, epiphytic population levels of a *pig*B mutant averaged less than 10% of the parent strain levels. Thus, a small, non-transforming, diffusible extracellular factor may be important for epiphytic development in *Xanthomonas*.

A652

TYROSINE PHOSPHORYLATION OF FLAGELLIN IN *PSEUDOMONAS SOLANACEARUM*. James Bina, Merelee Atkinson, and Caitilyn Allen. Department of Plant Pathology, University of Wisconsin-Madison, Madison, WI 53706.

We recently described a cytoplasmic membrane-associated tyrosine kinase activity in *Pseudomonas solanacearum*, incitant of bacterial wilt disease. When membrane fractions are incubated with γ-^{32}P-ATP a 90 kd protein is strongly radiolabelled and a 50 kd protein is weakly radiolabelled. Kinetic evidence suggests that the tyrosine kinase activity, unusual among prokaryotes, is intrinsic to the 90 kd protein. Western blots probed with anti-phosphotyrosine monoclonal antibody indicate that both proteins contain phosphorylated tyrosine residues. The 50 kd phosphoprotein has many of the properties of flagellin, the protein monomer that polymerizes to form the flagellar filament. Regulatory mutants affected in production of polygalacturonase (*pehR* mutants) exhibit reduced phosphorylation of the 90 kd protein. Since both polygalacturonase production and regulation of motility are associated with virulence in this bacterium, we are investigating the possible role of this tyrosine kinase in plant pathogenesis.

A653

PLASMID AND GENETIC DIVERSITY OF PSEUDOMONAS SYRINGAE POPULATIONS WITH DIFFERING EXPOSURES TO COPPER AND STREPTOMYCIN BACTERICIDES. G.W. Sundin[1], D.H. Demezas[2], D.E. Monks[1], and C.L. Bender[1], Department of Plant Pathology[1] and Microbiology[2], Oklahoma State University, Stillwater, OK 74078.

During a two-year field survey, we studied populations of epiphytic Pseudomonas syringae from Pyrus calleryana trees at three nursery sites. The bactericidal spray regime differed at each site with trees receiving sprays of copper and streptomycin (I), copper alone (II), or no sprays (III). A copper-resistant, streptomycin-sensitive (Cu^r Sm^s) phenotype was most prevalent in strains from sites I and II (48.8 and 84.7%), while the majority of strains from site III were sensitive to copper and streptomycin (56.3%). The Cu^r Sm^r phenotype was observed in 36.9, 9.3, and 43.3% of strains from sites I, II, and III, respectively. Plasmids were isolated from 358 strains, and six Cu^r, one Sm^r, and six Cu^r Sm^r plasmids were identified based on size differences and homology to specific DNA probes. The genetic diversity of 100 randomly-selected strains from sites I and III was examined using arbitrarily-primed polymerase chain reaction. The data indicate plasmid transfer and plasmid rearrangements have played a role in the evolution of copper and streptomycin resistance in these populations.

A654

CHARACTERIZATION OF AGROBACTERIA ISOLATED FROM AERIAL TUMORS ON *FICUS BENJAMINA* L. H. Bouzar, W. S. Chilton, N. C. Hodge, Y. Dessaux, and J. B. Jones, Gulf Coast Research & Education Ctr., University of Florida, 5007 60th St. East, Bradenton FL 34203

In the spring of 1991, galls were observed on pruned branches of one-year-old weeping fig trees grown in a Florida nursery. Both tumorigenic and nontumorigenic agrobacteria were recovered from galls. According to traditional diagnostic tests, carbon source utilization, and fatty acid content, nontumorigenic strains were identified as *A. tumefaciens* (i.e., biovar 1), whereas tumorigenic strains could not be assigned to any of the previously reported *Agrobacterium* spp. Tumors incited by these strains contained nopaline and two new opines, chrysopine and santhopine (Dransart *et al.*, Proc. 8th Intl. Conf. Plant Path. Bact., Versailles, 1993). Tumorigenic strains catabolized nopaline, chrysopine and santhopine, but not mannopine, octopine, succinamopine, leucinopine, cucumopine, or mikimopine. Nontumorigenic *A. tumefaciens* strains were unable to degrade any of those opines.

A655

EXOPOLYSACCHARIDES PRODUCED BY PATHOGENIC AND SAPROPHYTIC FLUORESCENT PSEUDOMONADS ASSOCIATED WITH MUSHROOM PRODUCTION. William F. Fett, J. M. Wells and C. Wijey. USDA, ARS, Eastern Regional Research Center, Philadelphia, PA 19118.

Over 230 strains of fluorescent pseudomonads were isolated from nine Pennsylvania grower lots of mushroom (Agricus bisporus) and from discolored lesions on mushroom caps. Of these, 30% exhibited mucoid growth upon initial isolation on Difco Pseudomonas agar F (PAF). Exopolysaccharides (EPS) were tested for neutral sugars and uronic acids by colorimetric assays. Derivatives of the sugars were analyzed by gas-liquid chromatography (GLC). Results indicated that most saprophytic strains of P. fluorescens produced a galactoglucan previously named marginalan. The EPS of strains identified as the mushroom pathogen P. gingerii gave a unique profile upon GLC. Strains of P. fluorescens (= P. "reactans"), weakly pathogenic on mushroom, produced one of three EPS's: marginalan, an EPS high in uronic acid or an EPS containing galactose and ribose. Strains of the mushroom pathogen P. tolaasii were all nonmucoid on PAF.

A656

CHARACTERIZATION OF *Pseudomonas syringae* INVOLVED IN KERNEL BLIGHT OF BARLEY. C. Martinez-Miller, S.L. Siemsen, and D.C. Sands. Dept. of Plant Pathology, Montana State University, Bozeman, MT 59717.

Kernel blight has been a serious disease of malting barley, reducing seed and malting quality. Kernel blight has been described as black-brown lesions on the lemma with well-defined edges when caused by *P. syringae pv. syringae*, or at the embryo end when caused by *Cochliobolus sativus*, *Alternaria spp.* or other fungi. In Montana, *P. syringae* seems to cause both symptom types. Two groups of *P. syringae* are present. One group does not produce syringomycin. The second produces syringomycin, hybridizes to an internal fragment of the cloned *syrB* gene and does not react with an antiserum prepared against the first group. Isolations from field samples suggest an association between syringomycin producers and symptoms at the embryo end or between the non-syringomycin producers and symptoms on the lemma. In the greenhouse, it appears that strains from both groups can cause both types of symptoms.

A657

DIFFERENTIAL EPIDEMIOLOGICAL FITNESS OBSERVED FOR TWO STRAINS OF *XANTHOMONAS CAMPESTRIS* PV. *CAMPESTRIS*. T. Shigaki and A. M. Alvarez. Department of Plant Pathology, University of Hawaii at Manoa, Honolulu, HI 96822

Latent spread of *Xanthomonas campestris* pv. *campestris* was confirmed in cabbage seedbeds by using pathovar-specific monoclonal antibodies (MAbs). Bacteria collected from guttation droplets of symptomless plants were grown on esculin-trehalose medium in microtiter plates and identified by ELISA by strain-specific MAbs. One strain (G2-12), which causes blight-like symptoms and reacts with MAb A11, spread more rapidly than another strain (A249), which causes typical black rot symptoms and reacts with MAb X21. Four weeks after inoculation, the mean incidence was 19.6% and 4.8% for G2-12 and A249, respectively. Of seedlings infected with G2-12 and A249, 9.1% and 22.2%, respectively, showed visual symptoms. This differential spread may be related to strain differences in epidemiological fitness, or efficiency of infection through hydathodes. Rapid latent spread in the seedbed may be a significant source of initial inoculum for field epidemics.

A658

CHARACTERISTICS OF A MONOCLONAL ANTIBODY TO *CLAVIBACTER MICHIGANENSIS* SUBSP. *MICHIGANENSIS*. A. Alvarez[1], M. Derie[2], A. Benedict[1], and R. Gabrielson[2]. [1]University of Hawaii, Honolulu, HI 96822, and [2]Washington State University, Puyallup, WA 98371.

A monoclonal antibody (clone 103-142, subclass IgG2a) designated Cm-1 was generated with specificity for *Clavibacter michiganensis* subsp. *michiganensis* (*Cmm*). It showed bright immunofluoresence with *Cmm* cells and reacted with a heat stable extracellular polysaccharide extracted from culture filtrates of *Cmm*. Cm-1 reacted in ELISA with all but one of eighty-eight virulent strains of *Cmm* and weakly with five of five *C.m.* subsp. *sepedonicum* strains. It did not react with 13 avirulent strains otherwise resembling *Cmm* or 89 strains from bacterial genera of plant and animal origin. When 61 cultures selected from tomato seed assays on the basis of their colony morphology on modified SCM agar medium were screened by ELISA, every isolate reacted. All cultures appeared typical of *Cmm* on nutrient broth yeast extract agar medium except two with orange pigmentation. The antibody may be of value in detection of *Cmm* in seeds.

A659

IMPROVED FORMULATIONS OF ATOXIGENIC *ASPERGILLUS FLAVUS* FOR APPLICATIONS TO AGRICULTURAL FIELDS.

D. J. Daigle and P. J. Cotty, SRRC, ARS, USDA, P.O. Box 19687, New Orleans, LA 70179-0687.

Production of spores by alginate encapsulated mycelia of an atoxigenic strain of *A. flavus* was investigated. In laboratory tests mycelia, encapsulated in alginate pellets containing corn cob grits and wheat gluten, produced more conidia on a weight basis than either colonized wheat seed or sclerotia. Kaolin, a traditional filler, reduced spore yield when substituted for corn cob grits. Several adjuvants increased pellet spore yield and wheat gluten was selected as the most useful. Addition of pesticides to pellets was possible without eliminating pellet ability to release spores. This may permit production of pellets resistant to various fungi, bacteria, and insects that may reduce pellet efficacy in the field. Spore yield of alginate pellets did not diminish after two years storage at 8°C.

A660

ENDOPHYTIC NATURE OF ENTEROBACTER CLOACAE IN ROOTS OF CORN SEEDLINGS. C. W. Bacon, and D. M. Hinton. USDA, ARS, Russell Research Center, Athens, Ga 30613.

Surface-sterilized kernels of an unknown Italian corn cultivar produced seedlings with roots endophytically infected by Enterobacter cloacae. The identity of the bacterium was established with biochemical and fatty acid analysis. This is the first report which establishes that E. cloacae is biologically associated with plants. The nature of the association was examined with light, scanning, and transmission electron microscopy. The bacterium was randomly distributed intercellularly in several locations of the cortex, and the outer margin of the pericycle, usually adjacent to phloem cells. There were no instances of damage to host cells or evidence of decline of the corn seedling during a three-week observation period. The endophytic nature of this bacterium has important potential for biocontrol of corn pathogens, particularly Fusarium moniliforme.

A661

BIOLOGICAL CONTROL OF THE BACTERIAL RING ROT PATHOGEN BY ENDOPHYTIC BACTERIA ISOLATED FROM POTATO. A. M. Van Buren, C. Andre, and C. A. Ishimaru, Department of Plant Pathology and Weed Science, Colorado State University, Fort Collins, CO, 80523.

Endophytic bacteria isolated from surface-sterilized stems of potato plants were evaluated as biological control agents effective against Clavibacter michiganense subsp. sepedonicum. Of 198 strains evaluated, 21-32%, depending on medium, produced antimicrobial agent(s) active against C. m. subsp. sepedonicum in vitro. Several strains (26%) prevented symptom development of bacterial ring rot in potato miniplants post-inoculated with C. m. subsp. sepedonicum. Four strains prevented bacterial ring rot symptoms in repeated greenhouse trials, and were selected for further study in replicated field trials in 1993. Population studies of one these strains, CICA90, a fluorescent pseudomonad, revealed that the bacterium could be recovered from root washes and from surface-sterilized roots and stem sections of inoculated plantlets. Endophytic bacteria may provide another strategy for management of bacterial ring rot.

A662

USE OF AN INVERT EMULSION CARRIER TO INCREASE INFECTION OF LEAFY SPURGE (EUPHORBIA ESULA L.) BY ALTERNARIA ANGUSTIOVOIDEA. S. M. Yang and D. R. Johnson, USDA, ARS, Frederick, MD 21702.

An invert emulsion carrier (IEC) was developed to improve infection of leafy spurge by A. angustiovoidea in the absence of dew. The oil phase of the IEC contained 20 ml mineral oil (Plastodent, Inc., Bronx, NY 10461), 2 ml Myverol 18-19 (M. Edwards, Eastman Chemical Products, Inc., Kingsport, TN), 80 ml Orchex 796 (Gil Chambers, Exxon Research and Engineering Co., Baytown, TX 77522), and 6 g paraffin wax. The water phase of the IEC contained 0.5 g sucrose, 0.1 ml Tween 20, and 100 ml tap water. Eighty-five percent of the conidia from the pathogen germinated in the IEC at 21-25 C and 35-50% relative humidity within 16-17 hrs, compared to 0% germination of conidia in aqueous dextrose solution controls. Suspensions of conidia in IEC were sprayed onto 3- to 5-week-old leafy spurge plants in the greenhouse or in field plots in the absence of dew. The Alternaria killed the plants two weeks after inoculation. Leafy spurge inoculated with an aqueous sucrose suspension of conidia in the same tests remained healthy. Results indicate that the IEC increases germination of conidia of A. angustiovoidea and infection and death of leafy spurge in absence of dew.

A663

BIOLOGICAL CONTROL OF THREE ROOT PATHOGENS OF WHEAT BY BACILLUS SPECIES. Dal-Soo Kim, R. James Cook, and David M. Weller. Washington State University and USDA-ARS, Pullman, WA 99164-6430

Bacillus spp. were selectively isolated from roots of either seedlings or stubble of wheat from fields with a history (10-20 yr) of continuous wheat, and presumably where the rhizosphere microbiota was highly adapted to roots of wheat. Two thousand isolates were tested for in vitro antibiosis against Rhizoctonia solani AG8, Gaeumannomyces graminis f. sp. tritici, and Pythium irregulare. Of those, 300 were selected for preliminary tests against rhizoctonia root rot on wheat grown from seed coated with the respective isolates. Eighteen strains selected from this test were then screened for activity against take-all and pythium root rot. Of three isolates active against take-all, one (BcL324) also significantly increased emergence of seedlings in pot tests with soil naturally infested with Pythium spp. All three isolates were tested as rifamficin-resistant mutants in a field plot naturally infested with Pythium spp. and artificially infested with R. solani AG8. Only strain BcL324 resulted in both significantly higher emergence (due mainly to Pythium control) and significantly fewer rhizoctonia lesions on seminal roots.

A664

PRODUCTION OF BIOCONTROL AGENT-FORTIFIED COMPOST-AMENDED POTTING MIXES FOR PREDICTABLE DISEASE SUPPRESSION. [1]M.E. Grebus, [2]K.A. Feldman, [1]C.A. Musselman, and [1]H.A.J. Hoitink. [1]Dept of Plant Pathology, OARDC/The Ohio State Univ., Wooster, OH 44691; [2]Earthgro, P.O.Box 143, Lebanon, CT 06249.

Compost prepared in windrows from a mixture of spruce and hemlock bark was inoculated after peak heating (60 C) with the biocontrol agents *Trichoderma hamatum 382* and *Flavobacterium balustinum 299*. It was then formulated into a potting mix containing compost, sphagnum peat and perlite. Mix prepared with biocontrol agent-fortified compost consistently suppressed Pythium root rot of cucumber as well as Rhizoctonia damping-off and Fusarium wilt of radish. The control mix not inoculated with the biocontrol agents consistently suppressed Pythium root rot. However, it did not consistently suppress Rhizoctonia and remained conducive to Fusarium wilt, even after 4 mo storage. In conclusion, only the biocontrol agent-fortified compost-amended mix consistently provided biological control of all three diseases.

A665

USE OF A PETAL DISK ASSAY TO IDENTIFY BACTERIA FOR BIOCONTROL OF *BOTRYTIS* ON PETUNIA. A. B. Gould, D. Y. Kobayashi, and M. S. Bergen, Department of Plant Pathology, Rutgers University, PO Box 231, New Brunswick, NJ 08903.

Several hundred bacteria indigenous to petunia flowers were screened for biocontrol of Botrytis blight using a petal disk assay. Flowers washed in sterile water were shaken in buffer and sonicated to dislodge adherent bacteria. Bacteria were isolated from the buffer solution and stored in sterile water. Selected bacteria were then grown in nutrient broth for 2 days, centrifuged, and resuspended in 0.001% Tween-20 to 10^8 cells/ml. Petal disks from 4-month-old plants, cut with a 14 mm diameter cork borer from surface sterilized flowers, were dipped in the bacterial suspensions and placed in the bottom of large (15 mm) multi-well plates. After 24 h, each disk was inoculated with 0.1 ml of a suspension containing 100 *B. cinerea* spores and evaluated for fungal infection within 7 days. A number of bacteria with potential for biocontrol of *Botrytis* have been identified.

A666

BIOLOGICAL CONTROL OF PHYTOPHTHORA ROOT ROT OF PEPPER IN SOUTHERN NEW MEXICO. S.E. Indiogine, C.M. Liddell, C.L. Biles. New Mexico State University, Department of Entomology, Plant Pathology and Weed Science, Box 3BE, Las Cruces, New Mexico 88003.

Growth chamber experiments were conducted to assay the efficacy of several fungal and bacterial biological control organisms against the pathogen *Phytophthora capsici*. The fungi were: *Pythium oligandrum* NM2060, *Trichoderma harzianum* NM2056, and *Fusarium oxysporum* C-14. The bacteria were: *Pseudomonas putida* N1R, *Erwinia herbicola* SR2 and SR3, and *Streptomyces griseoviridis* Mycostop™. The most effective bacterial organism was *E. herbicola* SR3; somewhat less effective were Mycostop and SR2. *P. putida* N1R was not effective. Preliminary experiments with fungi would indicate *P. oligandrum* NM2060 was the most effective biological control agent. In order to approximate the behavior of the fungal biocontrol agents on the root surface, the fungi were grown on a cellophane film placed on agar medium. The cellophane is made of cellulose and thus simulates the root surface. Phase-contrast microscopy indicated coiling of biocontrol fungi around pathogen hyphae and sporangia, following attraction to these structures.

A667

BIOLOGICAL CONTROL OF FUSARIUM CROWN AND ROOT ROT OF TOMATO. L. E. Datnoff, University of Florida, EREC, Belle Glade; S. Nemec, USDA-ARS, Orlando, and K. Pohronezny, EREC, Belle Glade.

Because of the potential loss of fumigants, development of fungicide insensitivity, and lack of commercially available resistant genotypes, experiments were conducted to evaluate commercial formulations of Glomus intraradix (GI) and Trichoderma harzianum (TH), alone and in combination, for the control of Fusarium crown and root rot of tomato (FCRR), caused by Fusarium

oxysporum f. sp. radicis-lycopersici. Tomato seeds cv. Sunny were planted into soil non-infested and infested with the biocontrol agents. After 6-7 weeks, plants were transplanted into commercial tomato fields with a previous FCRR history. Large fruits (≥ 6.27 cm) were harvested, counted and weighed at maturity. Disease incidence also was recorded. In 1991 and 1993, disease incidence in the controls decreased from 48%-57% to 25%-32%, 14%-47%, and 18%-20% for TH, GI, and TH + GI, respectively. Numbers of large fruit in the biocontrol treatments increased over the controls 13%-26%, 4%-17%, 15%; whereas, weights increased 6%-23%, 3%-21%, 15%-16% for TH, GI, and TH + GI, respectively. In summary, biocontrol agents can be effective for reducing FCRR and increasing tomato yields.

A668

SPATIAL AND TEMPORAL DYNAMICS OF A SUPPRESSIVE *STREPTOMYCES* STRAIN IN THE RHIZOSPHERE. A.D. Ryan, L.L. Kinkel, N.A. Anderson. Dept. of Plant Pathology, U. of Minn., St Paul, 55108.

Streptomyces strain 93 is suppressive to potato scab caused by *Streptomyces scabies*. Knowledge of its colonization dynamics on potato roots is important in determining spread and persistence of the agent in the rhizosphere. The distribution of strain 93 in the root system of potato plants was determined at a range of inoculum levels. Seed pieces were inoculated with the suppressive strain by dipping them in an inoculum slurry or by incorporating the strain into the soil on a vermiculite base at planting. Roots were sampled at 6, 8 and 10 weeks after planting. At each date 2 roots were taken from each of 4 plants and divided into 10 cm segments starting from the seed piece. Each root segment was assayed for populations of strain 93, total *Streptomyces* and total bacteria. Populations of strain 93 first increased and then decreased following inoculation. Populations of strain 93 were generally found to be evenly distributed among root segments at each sampling time as were populations of total *Streptomyces* and total bacteria.

A669

PREVENTING DECAY IN DOUGLAS-FIR AND SOUTHERN PINE WITH SCYTALIDIUM LIGNICOLA. T. Highley and L. Ferge, Forest Products Laboratory, Madison, WI, 53705.

We determined 1) the ability of the bioprotectant Scytalidium lignicola to colonize and survive in wood exposed in the field, 2) decay resistance of blocks removed from treated S. lignicola wood and 3) effect of S. lignicola on strength of wood. After two years exposure in the field, S. lignicola was still isolated throughout pine log sections. Decay fungi were not isolated from treated sections but were isolated from untreated controls. Unsterilized blocks removed from treated pine sections after one year's exposure in the field were resistant to decay. However, decay prevention was lost when blocks were sterilized. Southern Pine and Douglas-fir timbers (15.2 cm X 15.2 cm X 30.5 cm) treated with S. lignicola were completely colonized by one month. Like the pine sections, unsterilized wood removed from the timbers was decay resistant but resistance was lost with sterilization. S. lignicola had little affect on strength of wood.

A670

CONTROLLING EXISTING SAPSTAIN FUNGAL GROWTH OF CERATOCYSTIS COERULESCENS BY REACTING WITH HYDROXY RADICALS OR METABOLITES OBTAINED FROM BJERKANDERA ADUSTA AND TALAROMYCES FLAVUS. S. Croan, Forest Products Laboratory, Madison, WI, 53705.

The purpose of this study was to determine if hydroxy radicals from the Fenton reaction and metabolites from Bjerkandera adusta and Talaromyces flavus (1) decolorize stains in wood and (2) eradicate existing sapstain fungus, Ceratocystis coerulescens We studied the interaction of the sapstain fungus C. coerulescens against the test fungi B. adusta and T. flavus in modified dual cultures on agar medium. The metabolites obtained from test fungi and the hydroxyl radical generated by the Fenton reaction were examined on pine veneer disks stained by C. coerulescens. Our results indicate that the test fungi were necrotrophic parasitic and pathogenic to the sapstain fungus C. coerulescens. The sapstained pine veneer disks were decolorized and the existing hyphae of C. coerulescens were killed by reaction with hydroxy radicals or the combination of metabolites from the antagonists.

A671

INHIBITION OF ALTERNARIA PANAX SPORE GERMINATION AND BIOCONTROL OF ALTERNARIA LEAF BLIGHT ON AMERICAN GINSENG BY PSEUDOMONAS CEPACIA STRAIN AMMD. A.E. Joy and J.L. Parke, Department of Plant Pathology, Russell Labs, 1630 Linden Drive, Madison WI 53706.

Alternaria panax causes Alternaria leaf and stem blight, a serious disease of cultivated American ginseng. The most effective disease control method consists of frequent applications of mancozeb, a fungicide which is not labelled for use on ginseng. Biological alternatives for disease control are being sought. In vitro spore germination assays demonstrated that the germination of *A. panax* conidia was prevented or reduced after incubation with cultures of *Pseudomonas cepacia* strain AMMD at 10^8 CFU's/ml. In plant bioassays, 4-week-old ginseng seedlings were sprayed with a suspension of *A. panax* conidia either alone or together with cultures of the bacterium at 10^9 CFU's/ml. Ten days later Alternaria lesion development was significantly less among plants co-inoculated with the pathogen and the bacterium as compared to those inoculated with the pathogen alone. Field trials are underway to test the biocontrol potential of this bacterium in a commercial ginseng garden.

A674

ASSESSMENT OF SOIL POPULATIONS OF PHOMA TERRESTRIS, PYTHIUM IRREGULARE AND FUSARIUM SP. ASSOCIATED WITH RED ROOT ROT OF CORN IN DELAWARE. W. Mao, R. B. Carroll and D. P. Whittington, Dept. of Plant and Soil Sciences, Univ. of Delaware, Newark, DE 19717.

A split-split plot was utilized in Newark, DE in 1992 to assess soil populations of Phoma terrestris (P.t.), Pythium irregulare (P.i.) and Fusarium sp. and their association with red root rot (RRR) of corn. Soil was fumigated with Busan 1020. Treatments consisted of inoculation with all combinations of the fungi and a control. Inoculum was applied with seed at planting. Hybrids were DK 572 and DK 582 which are susceptible and resistant, respectively, to RRR. Soil was collected every 15 days from May 25 until harvest and selective media was utilized to determine colony forming units (CFU's). Significant differences ($P \geq 0.01$) were found between treatments, hybrids and sampling time for all three fungi. P.t. and P.i. developed most rapidly and the highest CFU's were obtained for the combined treatments with these fungi. These treatments also gave significantly higher ($P \geq 0.01$) severity ratings for RRR, indicating synergism and a causal role in RRR development.

A675

CERTIFIED WHEAT SEED PRODUCTION AND KARNAL BUNT IN THE YAQUI VALLEY, SONORA, MEXICO, FROM 1982-83 TO 1992-93. Pablo Sanchez-Villanueva, National Seed Inspection and Certification Service, Cd. Obregon, Sonora; Guillermo Fuentes-Davila, CIMMYT, Apdo. Postal 6-641, 06600 Mexico, D. F.

Karnal bunt (KB) incidence, favored by rainfall and high relative humidity during the flowering stage of the wheat plant, has been erratic in fields registered for certified seed production as records indicate since 1982-1983. When these conditions are minimal, the level of samples without infected seeds has been high, as in 1983-84, 1986-87, 1987-88, 1989-90 and 1992-93 which had 85.2, 93.4, 77.5, 80.7 and 53.7% of the samples KB-free, respectively. High disease incidence occurred in 1982-83, 1984-85, 1985-86, 1988-89, 1990-91 and 1991-92. The percentages of samples with no infected seeds were 20.6, 27.6, 32.4, 30.6 and 21.3, respectively. According to the internal quarantine No. 16, only KB-free seed should be certified. This disposition, however, was followed during 1986-88, 1989-90 and 1992-93. In 1988-89, 5 infected seeds/kg were allowed, and in 1990-91 and 1991-92 ten. These measures were taken because in years with high KB incidence the volumes obtained for certified seed were very low, and were unable to satisfy the demand for seed in the valley. However, after several years of adjustments made by the seed industry, seed lots with infection will no longer be accepted for certification.

A676

AN INOCULATED NURSERY TO SCREEN SOYBEAN VARIETIES FOR DISEASE REACTIONS IN SOUTHERN LOUISIANA. J.S. Russin, T.R. Betz, G.B. Padgett, and J.P Snow. Department of Plant Pathology and Crop Physiology, Louisiana State University Agricultural Center, Baton Rouge, LA 70803.

Rhizoctonia foliar blight (Rhizoctonia solani AG1(RS)) and stem canker (Diaporthe phaseolorum var. caulivora (DPC)) are serious problems on soybean in Louisiana. Because new soybean varieties are released constantly, a disease nursery was established to provide information on varietal responses to these important diseases. Plots were two rows wide (76 cm) and 12 m long. Varieties were planted in four blocks and were grouped by maturity to facilitate planting and harvesting. A portion (6 m) of each row was inoculated with either RS or DPC. Remaining portions of rows were noninoculated controls. Mycelia of RS (anastomosis groups IA and IB) were grown on acidified potato dextrose broth, filtered, and comminuted in a blender, and equal amounts of IA and IB mycelial slurries were combined prior to inoculation. Using a pump sprayer, inoculum (2-3x10^5 mycelial fragments/ml) was applied at 305 ml/30 row-m to plants in mid-late vegetative stages. Mycelia of DPC were grown on PDA plates that supported autoclaved, senesced soybean stems. After 2-3 months, plate contents were comminuted and filtered as described. Inoculum (1-2x10^6 ascospores/ml) was applied at a rate of 415 ml/30 row-m. Plants were inoculated twice during V3-5. Overhead irrigation was applied as needed. Disease ratings were made throughout reproductive stages. This approach has provided reliable estimates of disease incidence and severity for several years.

A678

EFFECTS OF RHIZOCTONIA SOLANI AG-3 ON TUBER SET OF KENNEBEC POTATOES. S. S. Leach and G. Black. USDA/ARS N. E. Plant, Soil and Water Laboratory, University of Maine, Orono, ME 04469-5753.

Two greenhouse studies were conducted in 1991 and 1993 with Kennebec mini-tubers free of R. solani AG-3 and planted in non-infested or R. solani AG-3 infested soil. The results of the first study showed that 25% fewer stems, stolons and tubers were formed in infested soil. No stolons or sprouts were pruned or malformed tubers produced in non-infested soil. Eighty five percent more stolon swellings, a precurser of tuber formation, were formed in non-infested soil. In the second study the results were similar for Kennebec and nine other cultivars. These findings show that R. solani is more detrimental to yield and tuber quality than previously reported.

A679

SYSTEMIC MOVEMENT OF *XANTHOMONAS FRAGARIAE* IN INOCULATED STRAWBERRY PLANTS. R. D. Milholland, D. F. Ritchie, M. E. Daykin, and W. A. Gutierrez. Dept. of Plant Pathology, N.C. State University, Raleigh, NC. 27695-7616.

Bacterial densities of *Xanthomonas fragariae*, the causal agent of angular leaf spot of strawberry, were assayed in 5mm sections of leaves, petioles, crowns, and roots of cv. Chandler 2,4,6,8 and 12 wk after inoculation of the leaves using a cotton swab. Plants were grown in 110 cm diameter clay pots and watered such that the foliage did not become wet. Plants were maintained under a 12 hr photoperiod at 15 C in a growth chamber for 6 wk, then transferred to 22 C for final 6 wk. The cfu of *X. fragariae* increased to 10^7 in leaves and 10^4 in petioles within 2 wk after leaf inoculation. By 6 wk, cfu were 10^8 and 10^5 in leaf and petiole sections, respectively. *X. fragariae* was detected in 1 of 4 root samples, but not in the crown 6 wk after inoculation. Symptoms developed rapidly after 2 wk at 22 C with cfu unchanged in leaves, decreased in petioles, not detected in the crown, and detected in 2 of 4 root samples at 10^2 8 wk after inoculation. Systemic movement of the bacteria also was examined histologically.

A680

COMPARISON OF PRE- AND POSTPOLLINATION APPLICATIONS OF TRIPHENYLTIN HYDROXIDE AND PROPICONAZOLE FOR CONTROL OF PECAN SCAB AND ANTHRACNOSE. K. L. Reynolds[1] and P. F. Bertrand[2], Dept. of Plant Pathology, Univ. of Georgia, [1]Athens, GA 30602, and [2]CES Rural Development Center, Tifton, GA 31793.

Pre- and postpollination applications of triphenyltin hydroxide (TPTH) and propiconazole for control of pecan scab (*Cladosporium caryigenum*) and anthracnose (*Glomerella cingulata*) were compared on four pecan cultivars. Both fungicides, whether applied either before or after pollination, were equally effective in controlling scab on the cultivars Schley, Stuart, and Desirable. On Wichita, a highly scab-susceptible cultivar, prepollination applications of propiconazole were more effective than TPTH in controlling leaf scab, but propiconazole applied postpollination was less effective than TPTH in controlling scab later in the season, particularly on the nuts. Wichita nuts were significantly smaller than the other cultivars, and nut size was significantly affected by fungicide treatment, corresponding to differences in scab severity. However, % kernel was unaffected by fungicide treatment in all cultivars. Fungicide treatment had no effect on nut quality of the less scab-susceptible cultivars. Anthracnose severity and frequency of pathogen isolation from nuts were not significantly affected by fungicide treatment.

A681

Fungicidal control of blackberry rosette (*Cercosporella rubi*). Barbara J. Smith[1], J. A. Fox[2], J. F. Killebrew[2], and C. P. Hegwood, Jr[2]. (1) USDA-ARS, Small Fruit Research Station, P.O. Box 287, Poplarville, MS 39470 and (2) Miss. Ag. For. Exp. Stn., Mississippi State, MS 39762.

Most blackberry cultivars in the southeastern U. S. are susceptible to rosette disease. Various fungicides were evaluated for rosette control on Cheyenne or Shawnee blackberries at 4 locations in south MS for 4 years. Disease severity was determined in early spring by counting the rosettes on each cane or by a visual rating (0=no disease to 8=100% infected). Benomyl and bordeaux mixture were more effective than DCNA, myclobutanil, ferbam and propiconazole; iprodione and metalaxyl were ineffective. Fungicide effectiveness was dependent upon timing of applications and was best when applications continued as long as rosettes were blooming. Benomyl applied twice before, once during and twice after harvest reduced rosettes from 40 on untreated plants to 3.3 on treated plants in one study and decreased the disease rating from 4.5 on untreated plants to 0.8 on treated plants in another study.

A682

SUPPRESSION OF STRAWBERRY BLACK ROOT ROT WITH MINERAL NUTRITION. W. H. Elmer and J. A. LaMondia. The Connecticut Agricultural Experiment Station, Box 1106, New Haven, CT 06504.

Our objective was to identify disease-suppressive fertilization regimes for black root rot of strawberry, incited by Rhizoctonia fragariae, and lesion nematodes. 'Honeoye' strawberry' crowns were planted in soils (pH 6.2) naturally infested with both pathogens. Treatments were $Ca(NO_3)_2$ or $(NH_4)_2S0_4$ (112 kg N/ha) with KCl, $CaCl_2$, K_2SO_4 or $CaSO_4$ (56 kg/ha) combined with and without a granular slow-release micronutrient product. All treatments received 2.3 liter nitrapyrin/ha. Thus, 16 treatments were each applied at planting to 4 replicate plots (2.7 m X 3.0 m). Leaf area/plant was estimated in July and August. In August plants were sampled for % black root rot using the line-intersect method and for nematodes by root extraction. Leaf samples were analyzed for N, P, K, Ca, Mg, Mn, Zn, Cu, Fe and Cl. Only NH_4-N resulted in significantly less black root rot and more leaf area and more runners per plant when compared to other treatments; nematodes were not affected. NH_4-N increased leaf concentrations of N, Cl, and Mn, and reduced P, K, Fe, and Cu more than NO_3-N. Levels of N, Mg, Mn, and the K:Cl ratio were highly correlated with decreasing black root rot. Fertilization regimes may be useful in suppressing strawberry black root rot.

A684

HISTOLOGY OF PREHARVEST COLONIZATION OF BING CHERRY FRUITS BY FUNGI. Frank M. Dugan and Rodney G. Roberts, USDA, ARS, Tree Fruit Research Laboratory, 1104 N. Western Ave., Wenatchee WA 98801.

Unblemished cherry fruits (*Prunus avium* cv. 'Bing') were collected weekly from petal fall to harvest at three orchards. Fruit were surface-disinfested in 100 $\mu g \cdot ml^{-1}$ NaOCl, then either plated onto agar media (weeks 1-4) or incubated in moist chambers (weeks 5-10). Colonization of receptacular and stylar scars by wound pathogenic strains of *Alternaria, Cladosporium, Aureobasidium,* and other fungi was detected as early as one week after petal fall and increased until 90-100% of fruits were colonized at harvest. Fruits were collected 1-6 weeks prior to harvest, incubated until hyphae were visible at 25-60X, then the stylar scars were excised, fixed, embedded in plastic, sectioned and stained. Hyphae were observed inside walls and/or lumina of stylar scar cells and within associated vascular tissues. Hyphae which apparently originated from scar tissue penetrated stomata and grew under the cuticle into adjacent tissue. The presence of hyphae within cells, vascular bundles, substomatal cavities and under the cuticle indicates that fungi can survive standard industrial surface disinfestation procedures, colonize necrotic tissue associated with stylar and receptacular scars, and subsequently cause decay.

A685

An Inoculation Technique for Rating Blueberries for Resistance to Mummy-berry (Monilinia vaccinii-corymbosi). V. Brewster, A.W. Stretch, M.K. Ehlenfeldt. USDA-ARS, Blueberry & Cranberry Research Center, HC01 Box 33, Chatsworth, NJ 08019.

Potted plants of 53 varieties of highbush and half-high blueberries were arranged in a randomized complete block design.Psuedosclerotia with apothecia were collected from the field and transplanted to pots filled with a peat:sand mixture. The mummy berry pots were placed at regular intervals among the blueberry pots and at evenly spaced intervals around the perimeter of the experimental plot to provide an even, consistent source of inoculum. The plot was irrigated for one hour every evening during ascospore release to optimize the infection process. Numbers of blighted twigs were counted over an 18 day period and percent shoot blight computed. Blighted shoot means were separated using the Waller-Duncan test and means ranged from 78% (Bluehaven) to 1% (Bluejay). This study attempts to optimize inoculum availability so that plants can be rated under the same conditions from year to year. This procedure will be used to examine sources and mechanisms for ongoing breeding programs.

A686

PHOMOPSIS SPP.; A PATHOGEN CAUSING LEAF AND SHUCK NECROSIS AND TWIG DIE BACK ON PECAN.
C.C. Reilly and M.W. Hotchkiss, USDA-ARS, Southeastern Fruit & Tree Nut Research Laboratory, P.O. Box 87, Byron, Georgia 31008

A *Phomopsis* spp. was isolated from pecan fruit, leaves, and twigs having necrosis and dieback symptoms in major growing regions of Georgia. The fungus overwinters on peduncles of the previous year's crop. Conidia were present at bud break (1 April) and were dispersed by rain splash. The abundance of spores decreases after early June but spores were detected throughout the growing season. Specific disease symptoms on fruit and leaves did not develop until mid August through mid September although the fungus was isolated from symptomless tissue throughout the growing season. Inoculation of detached fruit of 16 pecan cultivars produced symptoms similar to those detected in the field. There was a differential rate of disease severity which appeared to correlate with field observed severity. Inoculated leaves required 4 days to develop symptoms and the pathogen was readily isolated from the healthy-necrotic interface of the tissue.

A687

DISEASES OF BLUEBERRY FRUIT AT HARVEST IN NORTH CAROLINA. W. O. Cline and R. D.Milholland, Dept. of Plant Pathology, North Carolina State University, Raleigh 27695.

Blueberries were harvested from 11 cultivars and four breeding selections from four locations in 1989 and 1990. Annual disease losses at harvest averaged 9.6% and were primarily due to five diseases: mummy berry (*Monilinia vaccinii-corymbosi*) 5.6%, phomopsis soft rot (*Phomopsis vaccinii* (2.9%, phyllosticta rot (*Phyllosticta vaccinii*) 0.4%, ripe rot (*Colletotrichum* sp.) 0.4%, and alternaria rot (*Alternaria tenuissima*) 0.2%. Phomopsis soft rot occurred both as a localized calyx-end rot and as a soft rot detectable only by feel. Phyllosticta rot is an early season disease, and 2/3 of the infected fruit were collected at the first harvest date in 1990. Significant differences in disease levels occurred among cultivars and locations. Low levels of ripe rot were attributed to the 7-day harvest interval used. A previously unreported physiological disorder in the cultivar Cape Fear resulted in soft, unmarketable fruit.

A688

THE OVERWINTERING OF *ALTERNARIA MALI*, THE CAUSAL AGENT OF ALTERNARIA BLOTCH OF APPLE. N. Filajdić, and T. B. Sutton. Dept. of Plant Pathology, North Carolina State University, Raleigh, NC 27695.

Leaves, buds and stems of apples were investigated as possible overwintering sites of *Alternaria mali* in three orchards of Red Delicious in North Carolina. Orchards were characterized by different environmental conditions and Alternaria blotch intensities. Samples were taken monthly from October 1991 through May 1992. No lesions or conidia were found on shoots but conidia were present in buds and overwintering leaves. Leaves were the most important overwintering site, with an average of 100.8 conidia per leaf; 7.2 conidia were detected per bud. Conidial germination from leaves ranged from 34.4% in October 1991 to 64.2% in May 1992; 24.5% of the conidia detected in buds germinated following incubation. The greater number of conidia per leaf was recorded at an orchard with a history of high disease intensity, and one with low disease intensity, compared to a third orchard with moderate intensity. These results suggest that the number of overwintering conidia do not relate to the intensity of Alternaria blotch in the following year.

A689

CHARACTERISTICS OF COLLETOTRICHUM FROM PEACH, APPLE, PECAN, AND OTHER HOSTS. B. Bernstein, E. I. Zehr, R. A. Dean, Department of Plant Pathology and Physiology Clemson University, Clemson, SC 29634-0377 and E. Shabi, Volcani Center, P.O.B. 6, Bet Dagan 50-250 Israel.

Isolates of Colletotrichum from peach, apple, pecan and other hosts were examined morphologically and tested in vitro for benomyl sensitivity and similarity in RFLP patterns. Morphologically, the isolates separated into pink and gray types. Both types were isolated from peach, apple and pecan. Regardless of the host, pink isolates were tolerant to benomyl, ends of conidia were pointed, and had similar RFLP patterns. Gray isolates were sensitive to benomyl, ends of conidia were rounded, and isolates had similar RFLP patterns that were distinct from pink strains. Symptoms following inoculation with gray and pink isolates could not be distinguished on detached fruit. The two types appear to be C. acutatum (pink strains) and C. gloeosporioides (gray strains), respectively.

A690

LEAF REMOVAL FOR CONTROL OF BOTRYTIS BUNCH ROT OF GRAPE IN THE GUADALUPE VALLEY, MEXICO. J.Guevara. INIFAP-CECOEN. APDO.POSTAL 2197.Ensenada, B.C.México.

Some grapes cultivars grown in Guadalupe Valley, México are very susceptible to Botrytis bunch rot, these are Zinfandel and Chenin Blanc, in Zinfandel the lost yield due to this disease over the past three years has been up to 30%. The purpose of this study was to test the leaf removal in the fruit zone for control of Botrytis bunch rot of grape (cul tivar Zinfandel), under the climatic conditions of Guadalupe Valley in México, since this technique has been shown to be of significant value for control of Botrytis on grape, in coastal growing areas and San Joaquin Valley in California. Results of this study showed that this cultural practice reduced the incidence of Botrytis bunch rot in 9%. Removal of leaves also lowered the severety of the disease in 42%. Yield losses in control was 40%, whereas in the leaf removal treatment was only 3%.

A691

ROOT GRAFTS ARE NOT NECESSARY FOR DEVELOPMENT OF CITRUS BLIGHT IN THE FIELD. S. Nemec, D. Myhre, and J. Gardner, USDA-ARS, USHRL, Orlando, FL 32803; Soil and Water Science Dept., Univ. of Florida, Gainesville, FL 32611; and Univ. of California, Davis 95616.

Citrus blight was reported to be experimentally root-graft transmitted (Plant Dis. 68:979-980), but in this and later root-grafting studies insufficient controls were used. We established a test in April 1984 at Lake Alfred, FL, in which apparently healthy and blight-diseased sweet orange trees on rough lemon were root-grafted to healthy 3-yr-old Valencia sweet orange grafted on rough lemon receptor trees. Receptor trees were also planted ungrafted between two healthy and two blighted trees. By December 1989, receptor trees in all treatments exhibited leaf Zn def, an early symptom of blight. Mean syringe water uptake ranged between 0.06 to 0.07 $ml \cdot sec^{-1}$ and leaf Zn content between 16.5 to 53.0 $\mu g \cdot g^{-1}$, with no significance between treatments. In September 1990, visual Zn def and bark Zn content (range: 72 to 131 $\mu g \cdot g^{-1}$)were nonsignificant among treatments. In November 1991, visual Zn def, wilt symptoms, and wood Zn (range: 19.5 to 33.6 $\mu g \cdot ml^{-1}$) did not differ among treatments. Wood and bark Zn and water uptake were within limits of those parameters in trees with blight. Concurrent blight development in ungrafted controls and grafted plants indicates that root grafts are not necessary for natural blight occurrence.

A693

MANAGEMENT OF GRAPE POWDERY MILDEW USING FATTY ACID-BASED FUNGICIDES. S.D. Savage, R.W. Shutter, R.L. Warner and K.J. Jones, Mycogen Corporation, 4980 Carroll Canyon Road. San Diego, CA 92121.

Certain formulations of naturally occuring fatty acids have potent curative activity against established colonies of *Uncinula necator*. These materials have been effectively and safely applied to grape leaves and clusters throughout the growing season and have not impacted on wine quality. The formulations have no preventive or residual activity and thus represent a

novel class distinct from either contact or systemic fungicides. In small-scale field trials in California and Chile in 1992, the efficacy of these fungicides was demonstrated on a stand-alone basis, as well as in programs including sulfur or DMI fungicides. In 1993, commercial-scale trials were conducted throughout California using a formulation coded MYX-1446 F. The interest in this product for grapes is enhanced by concerns about sulfur residues at harvest, the need to manage DMI tolerant strains, and the utility of curative action.

A694

FLUAZINAM, A BROAD-SPECTRUM FUNGICIDE FOR CONTROL OF ORNAMENTAL DISEASES. D. M. Benson, North Carolina State University, Raleigh 27695-7629.

Fluazinam (ISK Biotech, Mentor, OH) controlled *Botrytis cinerea* (31% blight vs 81% in control 'ck') and *Rhizoctonia solani* (0% stem rot vs 65% ck) on poinsettia, *Phytophthora cinnamomi* (25% root rot vs 46% ck) on azalea, *P. parasitica* (0% damping-off vs 65% ck) on vinca, *Pythium aphanidermatum* (0% damping-off vs 44% ck) on impatiens, and *Thielaviopsis basicola* (5% black root rot vs 34% ck) on pansy. Sprays of a 50WP formulation at 0.6-1.2 g a.i./L, 50WP drenches at 0.06-0.23 g a.i./m^2 , and 500F drenches at 0.19-0.57 g a.i./m^2 were effective (P=0.05) as protective treatments on seed, cuttings, or plants growing in peat:vermiculite, polyfoam rooting cubes, or pinebark:sand. For damping-off, one drench of fluazinam at seeding was effective. For Botrytis blight, multiple applications at 7 days were used. On azalea, a 60-day application interval was not as effective for *P. cinnamomi* as metalaxyl.

A695

INCIDENCE AND SEVERITY OF DOGWOOD ANTHRACNOSE: QUANTITATIVE MEASUREMENTS OF DISEASE AND THE EFFECTS OF ENVIRONMENT ON EPIDEMICS IN CONNECTICUT. V. L. Smith and F. J. Ferrandino, Connecticut Agricultural Experiment Station, New Haven, CT 06504

Severity and incidence of dogwood anthracnose, caused by *Discula destructiva* Redlin, were quantitatively determined at 1 site in 1990 and at 3 sites in both 1991 and 1992. Fifty leaves were sampled at each site at weekly intervals; disease incidence was determined for each sample and lesion density on an area basis was determined for each leaf. Daily rainfall and maximum and minimum temperatures were recorded. Defoliation due to anthracnose was assessed in 1992 by counting the number of leaves on specific branches at weekly intervals. Data from 3 years indicated that dogwood anthracnose was monocyclic in Connecticut, with most infections occurring soon after leaf emergence in the spring. Limited rainfall after leaf emergence in 1992 constrained disease development, while abundant rain in spring of 1990 enhanced disease development. No defoliation due to anthracnose infection occurred. Asymptote disease severity (% diseased tissue) levels ranged from 4.3 % in 1990 to 0.45 % in 1991; disease severity in 1992 was intermediate at 1.87 %. Lesion density and disease incidence over all years were described by the negative binomial distribution, suggesting that lesions on leaves were spatially aggregated.

A696

EFFECT OF PREINOCULATION AND POSTINOCULATION WATER STRESS ON STEM INVASION OF SCOTS PINE BY SPHAEROPSIS SAPINEA. J. L. Wattermann, M. L. Gleason, E. Bradford, J. K. Iles, and P. H. Flynn, Departments of Plant Pathology and Horticulture, Iowa State University, Ames, IA 50011.

In a greenhouse experiment, the main stems of 5-yr-old Scots pine (Pinus sylvestris) were inoculated with Sphaeropsis sapinea, the causal agent of Sphaeropsis canker, before or after watering was withheld. Six wk after inoculation, incidence of top dieback was 77% for trees inoculated before severe water stress (-4.5 to -5.0 MPa) was imposed, 22% for trees inoculated after severe water stress, 11% and 0% for the respective uninoculated controls, and 11% for a nonstressed, inoculated control. Water stress resulted in longer cankers and more extensive colonization by the pathogen than in the nonstressed, inoculated control. However, these responses were significantly greater than for the nonstressed, inoculated control only when water stress followed inoculation. The results confirm that water stress exacerbates expansion of S. sapinea cankers in Scots pine and demonstrate that the timing of water stress relative to pathogen ingress can strongly influence canker development.

A697

LIGHT INTENSITY AND DROUGHT STRESS AS PREDISPOSITION FACTORS FOR DOGWOOD ANTHRACNOSE. D. Erbaugh[1], M. Windham[1], A. Stodola[2], and R. Augé[2], [1]Dept. of Ent. and Plant Path. [2]Dept. of Orn. Hort. and Land. Des., Univ. of TN, Knoxville, TN 37901.

Light intensity and drought stress were studied as predisposition factors for dogwood anthracnose. Two-year-old potted dogwood trees (Cornus florida L.) were placed outdoors in light treatments of 100, 50, 10, or 2% ambient light. One year later, trees were removed from the light treatments and inoculated (artificially or naturally) with Discula destructiva Redlin sp. Nov. After inoculation, trees were returned to their former light treatments and some of the trees from each treatment were subjected to drought. Disease progression was recorded as percentage of leaves with lesions. Inoculation method had no effect on disease progression. Maximum disease progression values for trees in well-watered treatments were < 5% at 100% light, < 5% at 50% light, 30% at 10% light, and 15% at 2% light. Drought increased disease severity on all shaded trees where disease progression increased 700, 40, and 100% in the 50, 10, and 2% light treatments, respectively.

A698

ROOT ROT OF *DRACAENA* CAUSED BY *PYTHIUM* SPECIES. M. Aragaki, J.Y. Uchida, and C.Y. Kadooka. Department of Plant Pathology, University of Hawaii, Honolulu, HI, 96822.

Several species of Dracaena, such as D. fragrans, D. deremensis, and D. marginata, are major export commodities for the Hawaiian foliage industry. In recent years, poor plant growth has been widespread at several nurseries in Hawaii. In the field, declining stock plants are typified by slow growth, chlorosis, and the production of substandard new growth. In pots, diseased Dracaena are characterized by stunted yellow leaves, wilted green leaves, leaf drop, and slow growth. Plant death also occurs. Pythium splendens was frequently associated with the root rots of declining Dracaena. In addition, a second, slower-growing Pythium species (tentatively P. graminicola) was also isolated. Inoculations with both species confirmed their pathogenicity to D. fragrans cv. Massangeana. Pythium graminicola was more virulent than P. splendens, destroying the root systems of potted Dracaena plants in 4 to 6 weeks under greenhouse conditions.

A699

EXTRACELLULAR ENZYMES OF TWO FUNGI ASSOCIATED WITH DOGWOOD ANTHRACNOSE. R.N. Trigiano, N.E.A. Gerhaty, and M.T. Windham, Agric. Expt. Stat., University of Tennessee, Knoxville, TN 37901-1071.

Discula destructiva Redlin sp. Nov. and an undescribed species of Discula are often isolated from anthracnose lesions on Cornus florida L. Five isolates of D. destructiva and three isolates of Discula sp. were obtained from various geographic regions in the eastern United States and grown in liquid media. Extracellular proteins were precipitated and assayed for enzymes that would degrade CMC, hemicellulose, and pectin or oxidize polyphenols. Lipase, amylase, and polyphenol oxidase (PPO) activities were assessed using agar media. Both species of Discula produced cellulase and pectinase, but none could degrade native cellulose. All D. destructiva isolates produced hemicellulase; whereas, only two isolates of Discula sp. could degrade the substrate. All isolates produced lipase but none were able to hydrolyze starch. PPO activity was expressed by D. destructiva cultures on agar medium as dark zones within 24 h after inoculation; protein extracts from liquid cultures were also positive for PPO. Discula sp. did not produce PPO.

A700

HOST FACTORS AFFECTING BROWN PATCH DISEASE SEVERITY IN TALL FESCUE. L. J. Giesler and G. Y. Yuen., Department of Plant Pathology, University of Nebraska-Lincoln, Lincoln, NE 68583-0722.

Nine cultivars of tall fescue (Festuca arudinacea Schreb.) differed in severity of brown patch disease, caused by Rhizoctonia solani Kühn, sustained in a growth chamber. Disease severity among the cultivars in the field had a medium correlation with relative levels of susceptibility found in the growth chamber (r = 0.60, P = 0.10). There was a higher correlation between blade density and disease severity in the field (r = 0.78, P = 0.01). 'Kentucky-31' and 'Monarch' had the lowest levels of susceptibility in the growth chamber, but differed significantly in amounts of disease sustained in the field, with seasonal averages of 3.4 and 5.6, respectively, on a ten point scale. 'Kentucky-31' and 'Monarch' had the lowest and highest blade densities, respectively, among the nine cultivars. These results suggest that plant density, in addition to susceptibility, can influence the development of brown patch disease under field conditions. Microenvironmental conditions in tall fescue canopies are being monitored to determine differences among cultivars.

A701

IRON II AND IRON III CHELATORS PRODUCED BY THE BROWN-ROT FUNGUS GLOEOPHYLLUM TRABEUM. J. Jellison, A. Enoki, B. Goodell,

M. Ishihara, N. Hayashi, H. Tanaka, Univ. of Maine, Orono, ME 04469; Kinki University, Nara, Japan; Forestry and Forest Products Research Institute, Tsukuba, Japan.

Brown-rot fungi are associated with the ability to rapidly attack, depolymerize and metabolize wood cellulose. The potential role of low molecular weight fungal metabolites including biological iron-chelators in the degradation process is being examined. Two distinct iron-binding metabolites have been isolated from the brown-rot fungus Gloeophyllum trabeum. Their relative activity in one-electron oxidation assays is influenced by the presence of iron. An iron III chelator has been isolated which can be shown to modify the percent crystallinity of Populus spp. wood in vitro. Induction in liquid culture was shown to be controlled by transition metal concentration.

A702

A TRIPLOID ELM RESISTANT TO DUTCH ELM DISEASE

J. L. SHERALD, Center for Urban Ecology, National Park Service, Wash., D.C. 20242; F. S. SANTAMOUR, JR., U.S. National Arboretum, Wash. D.C. 20002; R. K. HAJELA, N. HAJELA, AND M. B. STICKLEN, Pesticide Research Center, Michigan State Univ., East Lansing, MI 48824.

A triploid elm hybrid was found among the American elms on the National Mall in Washington, D.C. Counts at meiosis and mitosis showed a chromosome complement of 2n=3x=42. Alignment at meiotic metaphase I was 14 bivalents, likely contributed by autosyndetic pairing of American elm chromosomes, and 14 univalents of an unknown species. DNA digested with Hind III and probed with a 9.5 kbp cloned ribosomal DNA fragment from pea showed 2 bands not related to American elm. Twig crotch inoculations of hybrid ramets with *Ophiostoma ulmi* produced wilt in 15% of the branches compared to 53% in wild-type American elms. None of 22 triploid elms inoculated developed systemic wilt compared to 8 of 18 American elms inoculated.

A703

ENVIRONMENTAL INFLUENCES ON ASPEN REGENERATION FAILURE.

W.R. Jacobi[2], E.F. Kelly[1], C.A. Troendle[3], P.A. Angwin[3], and C.A. Wettstein[4]. Depts. of [1]Agronomy; and [2]Plant Pathology and Weed Science, Colorado State University; [3]USDA, Forest Serv.; and [4]USDA, Soil Conservation Serv., Fort Collins, CO 80523.

Canker (Cytospora chrysosperma and Dothiora polyspora)-induced regeneration failure may occur in aspen (Populus tremuloides) stands 3-8 years after harvest. Interacting site and environmental conditions that may predispose aspen sprouts to these pathogens are being investigated in seven paired plots. Data on sprout growth, previous stand make up, slope and aspect; soil moisture content and retention, structure, bulk density, texture/particle size, and nutrient content; and weather conditions were collected. Previous stand parameters, slope and aspect, and surface soil density did not differ between affected and unaffected stands. Preliminary analysis suggests weather patterns producing more than normal precipitation mediated, through soil conditions, restricted root development thereby causing a predisposing stress.

A704

PEST INTERACTIONS AND CANOPY GAPS IN PONDEROSA PINE STANDS.

J.E. Lundquist, Rocky Mountain Forest and Range Experiment Station, 240 W. Prospect Rd, Fort Collins, CO 80526.

We examined methods of describing how disease and other disturbance agents interact to alter the spatial and temporal characteristics of canopy gaps. Studies compared previously harvested and unharvested Pinus ponderosa stands in the Black Hills of South Dakota. Canopy profiles were characterized using various spatial statistics. Pattern isopleths were used to locate gaps. Observations were made for gap cause, size, and spatial distribution; snag and coarse woody debris condition; and understory type and abundance. Results indicated that disturbance agents were usually coupled concurrently and sequentially, and this can be displayed as networks of pathways in process models. Furthermore, management activities and grazing measurably simplified the patterns and processes of disturbance networks, and this can be characterized as shifts in the process model. Results also indicated that spatial scale was a component important to determining the ecological effects of gap causing disturbances. A model was developed to predict how various combinations of disturbance agents (e.g., root diseases, bark beetles, tree harvesting, wind, lightning, wildfire) might alter the condition and abundance of snags and woody debris on a community scale.

A705

COLONIZATION OF LIVE OAK ROOT SYSTEMS BY CERATOCYSTIS FAGACEARUM.

D.N. Appel, K.L. Ivors, Texas A&M University, College Station, TX 77843, and A.D. Wilson, USDA Forest Service, Southern Hardwoods Laboratory, Stoneville, MS 38776.

Root systems of live oaks growing in four rural oak wilt foci were excavated with heavy equipment and sampled for Ceratocystis fagacearum in the laboratory. The pathogen was isolated in high proportions from the root systems of symptomatic trees, and less frequently from symptomless trees located adjacent to diseased trees. The fungus was also isolated from roots growing in gaps up to 18 m between diseased and apparently healthy trees. The fungus survived in root systems for at least 3 yrs following death of the crown. In no case was the fungus isolated from roots in trenches dug 31 m beyond the symptomatic trees to control the disease. A failed control trench, dug 0.86 m deep, was re-excavated 4 yrs after treatment and roots were analyzed to determine how the pathogen crossed the barrier. The fungus was isolated from roots on both sides of the trench, but no root grafting or root growth across the trench were found. Roots were found crossing the barrier below the depth of the trench. The implications of these results to control of oak wilt in live oak by trenching and fungicide injections are considered.

A706

WATER TRANSPORT AND BINDING IN FUSIFORM RUST GALLS IMAGED BY MAGNETIC RESONANCE MICROSCOPY (MRM)

I.S. MacFall[1], P.C. Spaine[2], R.D. Doudrick[3], and G.A. Johnson[4,1,4] Dept. of Radiol., Duke Univ, Durham, NC 27710, [3]USDA Forest Service, Athens,GA, 30602, [3] USDA Forest Service, Gulfport, MS 39505

Galled stems from 10-mo to 2-yr old seedlings of slash and loblolly pine previously inoculated with *Cronartium quercuum* f. sp. *fusiforme* were compared with healthy stems by MRM. Following transpirational uptake of water, high resolution images (32-46 µm) were acquired of excised stem segments. Rapidly acquired images showed greater signal in the xylem of healthy stems than in galled stems, suggesting different wood/water interactions. In the 10-mo old plants, the cambium was contiguous between healthy and galled regions. Water transport disruption occurred at the the interface between galled and healthy regions, but in the center of the gall, the secondary xylem appeared water-filled. At two years of age, differences in water distribution patterns were apparent between galled and healthy stems, and between a galled stem which appeared otherwise symptomless and a galled stem from a tree showing initial symptoms of wilt. This study has demonstrated the utility of MRM in non-destructively studying changes in anatomy and functional physiology with fusiform rust gall formation in pine.

A707

ROOT ANATOMY AND MICROFLORA OF PINES ATTACKED BY THE SOUTHERN PINE BEETLE.

C.H. Walkinshaw, N.J. Hess and T.J. Perry. USDA Forest Service, Box 5500, Pineville, LA 71361.

Roots of Gulf Coastal Plain pine species were collected from forty paired plots of both southern pine beetle (SPB), Dendroctonus frontalis, attacked trees and non-attacked trees. No significant differences were found in mean diameter, basal area, radial growth, and site indices between the SPB and non-SPB plots. Forty-one genera of fungi were identified from root cultures. The most prevalent were the insect-associated staining species, Leptographium procerum, L. terebrantis, Ophiostoma ips, and Graphium spp. One or more of these fungi were found in 82% of the SPB plots and 35% of the non-SPB plots. Anatomical analysis revealed that fungi in roots from SPB plots colonized internal root tissues. Numerous wounds were found in the cortex of roots. These observations of interactions among insects, fungi and root health should help develop predictive tools and strategies to reduce losses caused by the SPB and root diseases.

A708

SEED-BORNE *Fusarium* species IN UNTREATED EASTERN WHITE PINE SEED.

Cynthia M. Ocamb and Jennifer Juzwik, USDA Forest Service, North Central Forest Experiment Station, 1992 Folwell Ave., St. Paul, MN 55108.

Fusarium species were isolated from two seed lots (200 seeds/lot) of white pine (*Pinus strobus*) from planter hoppers in use at two Wisconsin nurseries. Untreated seeds were placed on a Nash-Snyder medium for 18 days. In the four seed collections, 69, 73, 43.5, and 90 %, respectively, of the seeds yielded *Fusarium* species: *F. acuminatum, F. avenaceum, F. equiseti, F. graminearum, F. moniliforme, F. oxysporum, F. poae, F. polyphialidicum, F.*

proliferatum, F. reticulatum, F. sambucinum, F. semitectum, F. solani, and F. sporotrichioides; however, *F. proliferatum* (57%) and *F. sporotrichioides* (35%) predominated. Thus, infested seeds can introduce these fungi into fumigated nursery beds to potentially cause seedling diseases.

A709

ROOT PATHOGENS ISOLATED FROM TREES IN FOREST HEALTH MONITORING PROGRAM SURVEYS. M. Baldwin, J.A. Carlson, and S.A. Alexander. Department of Plant Pathology, Physiology and Weed Science, VPI&SU, Blacksburg, VA 24061-0330.

The root disease indicator is a component of Forest Health Monitoring, a program jointly sponsored by the U.S. Forest Service and the U.S. Environmental Protection Agency. Sampling for fungal root pathogens was conducted in the 1990-93 Pilot and Demonstration surveys in the southeastern United States. Forest trees were sampled by taking two 2.5 cm^3 wood chips from each of two primary roots located on opposite sides of the tree. Isolations from the chips were made on a general medium plus media specific for *Heterobasidion annosum, Armillaria* spp., and *Leptographium procerum.* Of 80 trees sampled in 1990 in the coastal plain of Virginia, one had *H. annosum* and two had *L. procerum.* In 1991, two of 120 trees sampled in western Georgia had *H. annosum* and three had *L. procerum.* In 1992, sampling of 158 trees in Virginia, North Carolina, South Carolina, and Georgia *detected H. annosum* and *L. procerum* in only a single tree each. No *Armillaria* species were isolated in any year. Data for 1993 will be discussed.

A710

EARLY INFECTION EVENTS OF HYPOXYLON ATROPUNCTATUM ON SHUMARD OAK SEEDLINGS. L.C. Childress, P. Fenn and K.S. Kim. Dept. of Plant Pathology, 217 Plant Sciences, Univ. of Arkansas, Fayetteville, AR 72701.

Asymptomatic infections of Hypoxylon atropunctatum can occur in oaks. The purpose of this study was to establish infections in oak seedlings by ascospore inoculation and determine, by scanning electron microscopy (SEM), whether H. atropunctatum penetrates directly or by natural openings. Greenhouse-grown seedlings were inoculated with an ascospore suspension, given a dew period, then surface sterilized and plated on yeast extract glucose agar. The fungus was isolated from 44% of nodes sampled, but rarely isolated from internodes. Seedlings for SEM were grown in a growth chamber for 5 to 8 wks, inoculated, given a dew period and returned to the growth chamber for up to 14 days when tissue was processed for SEM. Ascospore germination occurred by a longitudinal germ pore. Germ tubes and branched hyphae were uniformly thick. Hyphae entered natural openings in the cuticle and epidermis of stems and petioles, stomatal penetration was not observed. Good evidence for a distinct penetration structure has not been found.

A711

RESPONSE OF MAPLE SAPWOOD TO INJURY AND INFECTION. Walter C. Shortle. USDA Forest Service, PO Box 640, Durham, NH 03824.

The formation of a column boundary layer (CBL) along portions of columns of wound-initiated discoloration (WID) is part of the compartmentalization mechanism of tree defense following injury to maple tree stems. No CBL is found along most of the boundary between WID and sapwood (SW) in many maple trees. When a CBL is observed, it is common to observe rotted wood (RW) within the column of WID. Mobile ion concentrations indicative of early stages of infection were determined on dilute tissue extracts. No increase in mobile ions was associated with WID and the WID/SW boundary lacking a CBL; whereas, RW, infected WID (iWID) associated with RW, and CBL at the iWID/SW boundary had marked increases in mobile ion concentration. This indicated that infection spreading from RW through WID and into SW triggered CBL formation as a hypersensitive type reaction involving rapid cell death and high phytoalexin concentration at the iWID/SW boundary. WID formation is a response to injury; CBL formation to infection.

A712

POTATO PROTEINASE INHIBITOR II GENE IS EXPRESSED BY TRANSGENIC POPLAR TREES. F. Avila[1], J. Martinez[2], M. Kuhl[2], and N. B. Klopfenstein[2]. [1]Department of Plant Pathology and [2]USDA Forest Service, University of Nebraska, Lincoln, NE 68583-0722

Hybrid poplar, Populus alba L. X P. grandidentata Michx. clone 'Hansen', was transformed with chimeric genes containing the coding region of potato proteinase inhibitor II (pin2) using an Agrobacterium binary vector system. The transferred DNA contained either a bacterial nopaline synthase (nos) or cauliflower mosaic virus (35S) promoter linked to a pin2 structural gene and a selectable marker gene, a neomycin phosphotransferase II (NPTII). The presence of the transferred pin2 sequences in poplar has been confirmed using polymerase chain reaction (PCR). ELISA tests detected expression of pin2 in leaves of transgenic poplar. Western blotting analysis detected a 8 Kd and a 12 Kd proteins in transgenic poplar. Proteins of similar sizes were also present in purified pin2 and in potato, but they were absent in untransformed poplar. These results indicate that pin2 was translated and processed in transgenic poplar. Transgenic poplar lines that express pin2 in leaves will be used for future bioassays of pest resistance and possible outplanting.

A713

GIS APPLICATIONS FOR DISEASE RISK ANALYSIS. Cohen, S. D., USDA-APHIS, Hyattsville, Maryland. 20782.

Desktop geographic information system mapping software provides a convenient method to examine data, test spatial analysis queries, and develop specialized applications. Atlas-GIS, a desktop mapping package, was selected for development of GIS applications. Digital Line Graph Data layers from U.S. Geological Survey at 1:100,000 and 1:2 million scales were imported into Atlas-GIS and examined for application suitability. A disease risk analysis application, Eurasian poplar leaf rust (*Melampsora larici-populina*) was constructed to test spatial query abilities and application development. Additional data layers and associated attributes were created for counties reporting rust disease and counties reporting susceptible plant hosts (*Larix occidentalis, Populus tremuloides, Populus trichocarpa*). Selection of counties containing susceptible hosts near disease foci was done by proximity analysis spatial queries. Management issues and the spread of disease were examined with spatial analysis operations such as buffer zones.

A715

ETIOLOGY OF SYCAMORE DIEBACK AND MORTALITY IN THE SOUTHERN UNITED STATES. V. D. Ammon[1], F. I. McCracken,[2] and S. R. Yann.[3] [1]Mississippi State University, Mississippi State, MS; [2]University of Arkansas, Fayetteville, AK; and [3]USFS Southern Hardwoods Laboratory, Stoneville, MS.

Eighteen genera of fungi were isolated from declining sycamores growing on 18 sites in 7 southern states. Seven isolates demonstrated various levels of pathogenicity on inoculated sycamores and 11 were wood saprophytes. Infection rate and disease development varied by season and fungal inoculum. Spring inoculations with Ceratocystis fimbriata platani and Phomopsis scabra produced the greatest incidence of infection and canker development. Only C. fimbriata platani inoculations consistently resulted in mortality. Interactions of C. fimbriata platani with the other pathogenic fungi were neutral.

A716

TAXONOMIC, mtDNA HAPLOTYPE, VCG, AND MORPHOLOGICAL DIVERSITY OF COLLETOTRICHUM SPP. CAUSING FRUIT-ROT OF APPLES. J. C. Correll, D. D. Rhoads[1], and J. C. Guerber. Dept. of Plant Pathology and [1]Dept. of Biological Sciences, University of Arkansas, Fayetteville, AR 72701.

Isolates of Colletotrichum, recovered from apple fruit with typical bitter rot symptoms from Arkansas and North Carolina, were identified to species and characterized for

mitochondrial DNA (mtDNA) RFLP haplotype, vegetative compatibility group (VCG), and colony morphology. Two species, C. gloeosporioides and C. acutatum, were identified among the 273 isolates examined. Monoconidial isolates of C. gloeosporioides could be characterized as either teleomorphic (T) or non-teleomorphic (NT) based on their ability to produce perithecia and ascospores. The C. acutatum isolates could be distinguished into two distinct cultural types, chromogenic (C) and non-chromogenic (NC); the chromogenic isolates produced distinct pink-red color on PDA. The T and the NT isolates of C. gloeosporioides each belonged to a distinct mtDNA RFLP haplotype. The C and the NC isolates of C. acutatum belonged to a third haplotype. Three NC isolates of C. acutatum had a fourth mtDNA haplotype. Four and two VCGs were identified among the T and NT isolates of C. gloeosporioides, respectively. Six and five VCGs were identified among the NC and C isolates of C. acutatum, respectively. A single VCG was recovered from several of the orchards sampled.

A717

RAPD EXAMINATION OF PHYLOGENETIC RELATIONSHIPS IN COLLETOTRICHUM GLOEOSPORIOIDES. W. C. Dyson, D. D. Rhoads, and J. C. Correll[1]. Dept. of Biological Sciences and [1]Dept. of Plant Pathology, University of Arkansas, Fayetteville, AR 72701.

Previously, we examined a collection of Colletotrichum gloeosporioides from diverse hosts for mtDNA RFLPs and total DNA RAPD patterns. These data indicated that there were ten major groups based on the mtDNA RFLP and RAPD patterns. In the current study, the relationships of isolates from this collection were examined by reciprocal hybridizations of RAPD products. Simple matrices, based on hybridization of the amplified products, suggest an evolutionary relationship between isolates of C. gloeosporioides from certain hosts. For example, isolates from apple and a subgroup of lime isolates were related while another distinct group included isolates from avocado, mango, lychee, northern jointvetch (Aeschynomene virginica), and a second lime subgroup. Isolates from Stylosanthes and Ludwigia did not belong to either of the two groups and probably represent distinct lineages.

A718

REGIONAL DNA FINGERPRINT (MGR586) DIVERSITY OF THE RICE BLAST PATHOGEN, MAGNAPORTHE GRISEA, IN ARKANSAS. J. Q. Xia, J. C. Correll, F. N. Lee, and D. D. Rhoads[1]. Dept. of Plant Pathology and [1]Dept. of Biological Sciences, University of Arkansas, Fayetteville, AR 72701.

DNA fingerprinting was used to examine the regional genetic diversity of the rice blast pathogen, Magnaporthe grisea. A total of 517 monoconidial isolates were recovered from seven cultivars from 19 commercial rice fields in nine counties in Arkansas in 1992. Isolates were compared for DNA RFLP similarities (based on Nei and Li's index of genetic similarity). In a previous study, isolates with >80% shared fragments were assigned to a given fingerprint group; separate fingerprint groups had <60% shared fragments. Based on DNA RFLP similarities, four distinct fingerprint groups (MGR586 Group A, B, C, and D) were identified among the 1992 field isolates. All four fingerprint groups were found in nine of the 19 locations; three groups were found in three locations, two groups in four locations, and one group in three locations. Multiple haplotypes were found within each of the fingerprint groups; within a fingerprint group, haplotypes were 1-10% dissimilar. Over 40 haplotypes were identified among the 517 field isolates. However, within a fingerprint group, a single haplotype was predominant (52-73% of the isolates). A single fingerprint group was found on two cultivars, Mars and Millie. Although only a single field of each of these cultivars was sampled, it is possible that certain cultivars grown in Arkansas serve as a "bottleneck" selecting out specific groups in the regional population.

A719

TOXICITY AND SPORULATION PATTERNS OF *ALTERNARIA* ISOLATES FROM JAPANESE PEAR. R. G. Roberts[1] and E. G. Simmons[2], USDA, ARS, Tree Fruit Research Laboratory, 1104 N. Western Ave., Wenatchee, WA 98801[1], and 717 Thornwood Road, Crawfordsville, IN 47933[2].

Alternaria gaisen Nagano (= *A. kikuchiana* Tanaka), the causal agent of black spot of Japanese pear, is often referred to as a "pathotype" of *A. alternata* because these fungi share certain morphological features. To test the hypothesis that these are distinct fungi, leaves and fruit of 'Nijisseiki' (20th Century) pear with black spot symptoms were collected and air-dried in Nagano, Fukushima, and Tottori prefectures (Japan) during July 1990. *Alternaria* isolations made from diseased tissue by EGS were segregated into six groups based upon sporulation patterns observed at 50X, and at higher magnifications for the *A. gaisen* group (Mycotaxon 48:109-140). Toxigenicity of 134 *Alternaria* isolates associated primarily with Japanese pear from Japan, Korea, Taiwan, Australia and the United States was evaluated in a blind assay by spotting juvenile 'Nijisseiki' leaves with conidial suspensions of each isolate. No isolate from the *A. alternata* group was toxigenic, and no isolate from the United States was toxigenic. All isolates in the *A. gaisen* group were toxigenic, as were occasional isolates from other groups. All toxigenic strains originated from Japan, Korea, or Taiwan. The strict correlation of toxicity data with groupings based on sporulation patterns confirms that *A. gaisen* and *A. alternata* are recognizably distinct taxa.

A720

RELATEDNESS AND DIVERSITY OF COLLETOTRICHUM SPECIES. C.L. Trout and D.O. TeBeest, Department of Plant Pathology, University of Arkansas, Fayetteville, Arkansas 72701.

Species within the genus Colletotrichum are commonly identified on the basis of morphological characters. However, morphological characters are imperfect taxonomic criteria in this genus due to the lack of good characters and wide variability often exhibited within and between species. Isolates representing eight species of Colletotrichum (C. coccodes, C. graminicola, C. lindemuthianum, C. malvarum, C. orbiculare, C. trifolii, C. truncatum and C. gloeosporioides), eight host specific forms of C. gloeosporioides (apple, avocado, citrus, mallow, mango, Aeschynemone, Hakea and Stylosanthes) and Glomerella cingulata (teleomorph of C. gloeosporioides) were examined for relatedness and diversity using molecular markers. A ribosomal DNA (rDNA) probe and a glutamate dehydrogenase (GDH) probe from Neurospora crassa, and a glyceraldehyde-3-phosphate dehydrogenase (GPD) probe from Glomerella cingulata were used for restriction fragment length polymorphism (RFLP) analyses. Initial data suggest that these probes may be useful molecular markers for phylogenetic analysis of Colletotrichum.

A721

USING VCGS TO DETERMINE GENETIC DIVERSITY OF *FUSARIUM MONILIFORME* IN 24 MAIZE SEED LOTS. Cheryl L. Campbell & John F. Leslie. Department of Plant Pathology, Kansas State University, Manhattan, Kansas 66506-5502, USA.

Nitrate-nonutilizing (nit) mutants were generated in 408 isolates of *Fusarium moniliforme* (=*Gibberella fujikuroi*) recovered from seed of two maize cultivars grown at 12 locations in 8 states. Vegetative compatibility tests were used to determine genetic diversity and population similarities within and between locations, seed lots and cultivars. At least 4 and as many as 12 VCGs were found at each site in each seed lot for cultivars 3475 and 3377, for a total of 215 VCGs. Genetic diversity (#VCGs/#isolates) ranged from 0.25 to 0.80 in cultivar 3377, and 0.27 to 0.69 in 3475. Comparisons revealed 20 multimember VCGs (mVCGs) were common between seed lots of the two cultivars, 22 and 24 mVCGs were found only in 3377 and 3475, respectively. Isolates from widely separated sites were members of the same VCG. At least 3 and as many as 10 VCGs at each location were represented at other sites; 62% of mVCGs were recovered at more than one location. Genetic diversity at the 12 sites ranged from 0.30 to 0.70. After all possible pairings, 154 VCGs, including 88 single member VCGs and 6 HSI strains were identified. Although all 24 seed lots contained both the A+ and A- mating types, isolates within a VCG tended to be of only one mating type. Of the mVCGs we examined, 34% contained only A+ strains, 61% contained only A- strains, and 4% contained strains of both mating types. This latter 4% consisted of 5 VCGs, 2 of which contained only a single differing strain in a large VCG. The other 3 VCGs were notable in that each consisted of two distinct groups, each a different mating type, linked by only one strong positive pairing reaction. Pairing reactions were otherwise very weak or produced a dark line of pigmentation beneath the agar with no aerial mycelium.

A722

GENETIC VARIABILITY OF LAGERSTROEMIA TO POWDERY MILDEW (ERYSIPHE LAGERSTROEMIAE). G. R. Johnson and A. M. Townsend. USDA, ARS, U.S. National Arboretum, 3501 New York Ave., NE, Washington, D.C. 20002-1958

Incidence of powdery mildew infection was examined on 37 control-pollinated seedling families of Lagerstroemia (crape myrtle). The families represent a breeding population of slow growing Lagerstroemia hybrids of L. indica, L. fauriei, and L. limii. Mildew was scored as 1=mildew present and 0=mildew absent. Incidence of mildew was shown to be under moderate genetic control, having an estimated heritability of $h^2 = 0.28$. No genetic correlation was found with height or time of flowering. Inbreeding depression occurred for height, but not for mildew resistance.

A723

TEMPORAL AND SPATIAL GENETIC VARIATION IN A PENNSYLVANIA *ALTERNARIA SOLANI* POPULATION. B. J. Christ and D. M. Petrunak. Dept. of Plant Pathology, Penn State University, University Park, PA 16802.

Isozyme analysis at 13 enzyme loci was performed on a population of *Alternaria solani* collected in 1992 from a single field of 'Superior' potatoes in Cambria Co., PA. A total of 49 isolates, that represented six electrophoretic types (ET), were collected over three sampling periods representing early, mid and late early blight disease progression. Of these six ETs, two had been recovered in previous years. Three, five, and two ETs, were recovered at these sampling periods, respectively. There were two predominant ETs (ET 23 and 27) at all sampling periods and they were distinguished by two alleles at the *Pgi* locus. Frequency of ET 23 remained constant (~ 0.50) whereas ET 27 increased from 0.33 to 0.48 from the first to the last sampling period. This increase was associated with displacement of the other ETs. Despite the small sample size, there was greater genetic variation than expected. Frequency and temporal distribution were influenced by the differential fitness of ETs. Spatial distribution of ETs was random across the transect rather than on a gradient. These data may provide insight on sources and dispersal of inoculum.

A724

Altered Specificity of *Rp1* Recombinants.

T.E. Richter[1], A.J. Pryor[2], J.L.Bennetzen[3], S.H. Hulbert[1].
[1]Department of Plant Pathology, Kansas State University; [2]Division of Plant Industry, CSIRO; [3]Department of Biological Sciences, Purdue University.

Recombinant individuals from testcrosses of *Rp1* heterozygotes were screened with 11 different isolates of the rust fungus *Puccinia sorghi*. Several derivatives of

presumptive transposable element insertions in *Rp1* were also screened with the 11 rust isolates. 165 out of 174 derivatives exhibited the expected results of being either susceptible to all of the rust isolates, or had the combined resistance spectrum of the two parental alleles. Nine derivatives, however, showed resistance reactions to the rust that were different from the parental alleles they were derived from. Seven out of the nine derivatives showed resistance to only a subset of the rust isolates that their parental alleles were resistant to. A derivative from the *Rp1*-D allele, in a transposable element background, showed the same resistance spectrum as the parental *Rp1*-D allele, but the resistance was less complete. One derivative, from a *Rp1*-N/*Rp1*-C heterozygote, showed resistance with all isolates screened, even those that were virulent on both parents. This derivative is a candidate for a Mendelian factor that provides race non-specific resistance.

A725

CHROMOSOMAL LOCATION OF WHEAT GENES FOR RESISTANCE TO *PUCCINIA STRIIFORMIS*. X. M. Chen, R. F. Line, and S. S. Jones. Dept. of Plant Pathol. and USDA-ARS, WSU, Pullman, WA 99164-6430.

Wheat cultivars Tyee, Daws, Fielder, Heines VII, Moro, Tres, Nord Desprez, Minister, Vilmorin 23, Hybrid 46, and Clement were reported to have stripe rust resistance genes *Yr*Tye; *Yr*Da1 and *Yr*Da2; *Yr*6 and *Yr*Fie; *Yr*2 and *Yr*HVII; *Yr*10 and *Yr*Mor; *Yr*Tr1 and *Yr*Tr2; *Yr*3a and *Yr*ND; *Yr*3c and *Yr*Min; *Yr*4a and *Yr*V23; *Yr*4b and *Yr*H46; and *Yr*9 and *Yr*Cle, respectively. Each cultivar was crossed with Chinese Spring and 21 monosomic or monotelosomic Chinese Spring lines. F_2 seedlings from monosomic F_1 plants were tested with *P. striiformis* races. The 21 genes in the 11 cultivars were confirmed and chromosomes for 18 of them were determined. *Yr*2 and *Yr*6 are on chromosome 7B; *Yr*3a, *Yr*3c, *Yr*9, and *Yr*10 are on 1B; *Yr*4a and *Yr*4b on are 6B; *Yr*HVII and *Yr*Min are on 4A; *Yr*Tye, *Yr*Fie, and *Yr*Tr2 are on 6D; *Yr*Da1 is on 1A; *Yr*Da2 is on 5D; *Yr*Mor is on 4B; *Yr*Tr1 is on 3A; and *Yr*V23 is on 2B. This is the first report that Chromosomes 1A, 3A, 4A, 4B, 5D, and 6B have *Yr* genes.

A726

INHERITANCE OF RESISTANCE TO *FUSARIUM GRAMINEARUM* IN EIGHT WHEAT CULTIVARS. G-H. Bai [1], G. Shaner [1], and H. Ohm [2], [1] Department of Botany and Plant Pathology, and [2] Department of Agronomy, Purdue University, West Lafayette, IN 47907.

Characterization of inheritance of resistance to scab in wheat is important for development of resistant cultivars. In this study, F_1, F_2, and both backcross populations from the crosses of six resistant cultivars to two susceptible cultivars were quantitatively evaluated for resistance to spread of *F. graminearum* within a spike. Area under disease progress curve (AUDPC) was calculated based on the proportion of scabby spikelets recorded at 3-days intervals from 3 to 21 days after inoculation. Resistance to scab in the six resistant cultivars was conditioned by one to three genes. Narrow sense heritability was high (average = 0.56). A simple additive-dominance model fit the segregation data from eight crosses, and an epistatic model with an additive x additive component fit data from two other crosses. Dominance and epistatic components were significant in a few crosses; they increased resistance in some crosses, but decreased resistance in others. However, the additive component comprised the major portion of genetic variation, so it should be possible to accumulate different genes to enhance resistance to scab in wheat.

A727

THE POSITIVE ACTIVATOR OF PHENAZINE ANTIBIOTIC PRODUCTION IN *PSEUDOMONAS AUREOFACIENS* STRAIN 30-84 HAS HOMOLOGY TO OTHER TRANSCRIPTIONAL ACTIVATORS DEPENDENT ON AN AUTOINDUCER. L. S. Pierson, III, D. W. Wood, and V. D. Keppenne. Department of Plant Pathology, University of Arizona, Tucson, AZ 85721.

A positive regulatory gene, *phz*A, was identified that stimulates expression of the phenazine biosynthetic genes in the biological control bacterium *Pseudomonas aureofaciens* strain 30-84. Transposon mutagenesis using Tn*5*lac demonstrated that *phz*A is divergently transcribed from the *phz* biosynthetic genes and that a functional *phz*A gene is required for phenazine gene expression. The nucleotide sequence of *phz*A revealed an open reading frame of 722 nucleotides which could encode a protein of *ca.* 27 kd (PhzA). The deduced amino acid sequence of PhzA shows strong homology to several other positive transcriptional activators, including LasR of *Pseudomonas aeruginosa* and LuxR of *Vibrio fischerii*.

A728

DIFFERENT WMV-2 RESISTANCE GENES ARE EXPRESSED AT DIFFERENT DEVELOPMENTAL STAGES IN PROGENY OF THE TMG-1 CUCUMBER. T. Wai and R. Grumet, Horticulture Department, Michigan State University, East Lansing, MI 48824.

The Chinese cucumber line TMG-1 is resistant to the potyvirus watermelon mosaic virus 2. Two recessive genes were found to be involved in this resistance (Wai and Grumet, 1991, abstract). The parents, F_1, F_2, and BC progeny of a cross between TMG-1 and a susceptible line WI-2757 were monitored for symptom expression and virus accumulation by ELISA. Ratios obtained from backcross progeny differed depending on whether cotyledons or true leaves were inoculated. Cotyledon-inoculated progeny segregated 1:1 as would be expected for a single recessive gene. In addition, this gene appears to be linked to the *F* (femaleness) locus in linkage group I. Segregation ratios for true leaf-inoculated progeny were 5 resistant: 3 susceptible. The true leaf segregation ratios for both BC's and F_2's were consistent with the presence of an additional gene (dominant) acting in concert with the second recessive gene. These results suggest that the two resistances are expressed differently: one at the cotyledon stage, the second only at the true leaf stage.

A729

ULTRASTRUCTURE OF STREPTOMYCES SCABIES INFECTION OF IN VITRO CULTURED POTATO TUBERS. B. Stein, R. Hammerschmidt, Dept. Botany and Plant Pathology and D. Douches, Dept. Crop and Soil Sciences, Michigan State University, East Lansing, MI 48824

Common scab of potato caused by Streptomyces scabies has been studied with light microscopy but not with electron microscopy. To gain a greater understanding of the infection process, Atlantic and Russet Burbank tubers, produced by in vitro tissue culture, were inoculated with S. scabies and examined by electron microscopy. Growth of S. scabies occurred over the tuber surface where periderm was forming but not on primary tissue near the tuber apex. This growth appeared only on the outer surface of the tuber at first. This suggests that exudates from the senescent epidermal cells facilitated bacterial growth. Infection occurred by the direct penetration of outer cell walls of senescent epidermal cells. Stomata and lenticels were also infected. Penetration of cells was proceeded by what appeared to be enzymatic dissolution of walls. New periderm formed in uninfected tissue under the sites of infection.

A730

ROLE OF THE PHYTOTOXIN CORONATINE IN THE INFECTION OF *ARABIDOPSIS THALIANA* BY PATHOVARS OF *PSEUDOMONAS SYRINGAE* . Shalu Mittal and Keith R. Davis. Department of Plant Biology and Ohio State Biotechnology Center, The Ohio State University, Columbus-Ohio 43210.

Coronatine is a phytotoxin produced by several *Pseudomonas syringae* pathovars and is thought to be an important virulence factor. Recent studies have shown that coronatine-producing isolates of *P. s.* pvs. *tomato* and *maculicola* infect *A. thaliana.* To determine the importance of coronatine in the virulence of these strains on *A. thaliana*, we have examined plants infected with *Pst* DC3000 (Cor+), *Pst* DC3661 (Cor-), *Psm* 4326 (Cor+) and *Psm* 795 (Cor-). Plants were inoculated using either hand infiltration or by dipping methods. The results from hand-infiltrated plants suggest that coronatine is not required for the establishment and multiplication of these strains in the host but does enhance the severity of the disease symptoms. In contrast, in the dipping experiments, the Cor- strains are not able to establish infections as evidenced by very little multiplication *in planta* and no disease symptom development. The results indicate that coronatine plays a critical role during the initial stages of infection under more natural conditions of low bacterial inoculation densities.

A731

PHENOLIC PRODUCTION AND THE ATTACHMENT OF *PHYTOPHTHORA CAPSICI* ZOOSPORES TO WOUNDED ROOTS OF *CAPSICUM ANNUUM*. J.R. Sollars, M.E. Waugh, K. Onsurez, C.L. Biles and C.M. Liddell. New Mexico State University, Department of Entomology, Plant Pathology, and Weed Science, Box 3BE, Las Cruces, NM 88003, U.S.A.

Experiments were conducted to determine whether increased phenol production corresponds with *Phytophthora capsici* zoospore attachment to wounded pepper roots. Roots of seedlings were wounded and inoculated with zoospores 0, 48, 96 and 120 h later. Attachment of zoospores at 0 h was significantly higher than that observed at 96 and 120 h, and the same region of a non-wounded control. Attachment to 120 h old wounds was not different than non-

wound controls, suggesting a wound-repair mechanism. Total phenol production of the root tips increased significantly 24 h after wounding, but 48 h levels were not different than the 0 h controls. A further survey of common phenols indicated that *P. capsici* isolates were inhibited by caffeic acid, coumarin, ferulic acid, hydroquinone, hydroxybenzoic acid, and vanillin. We hypothesize that a wound repair mechanism inhibits zoospore attachment and wound induced phenols may play a role in structural or biochemical inhibition.

A732

BASE-SOLUBLE PROTEINS FROM CORN AS CELL HEMOLYSINS AND INHIBITORS OF MYCELIAL GROWTH OF ASPERGILLUS FLAVUS.
J. N. Neucere, USDA, ARS, Southern Regional Research Center, P.O. Box 19687, New Orleans, LA 70179.

Recent studies showed that a diversity of polypeptides and proteins within cereal grains are implicated in resistance to fungal infections. In this study, the base-soluble proteins from Aspergillus flavus resistant (Yellow Creole) and susceptible (Huffman) genotypes of corn were investigated by in vitro studies. Bioassays of fungal growth inhibition in liquid and solid media showed lethal activity in both varieties with disparity in potency dosage. Cell-surface interactions by red blood cell agglutination assays showed hemolysis by proteins extracted from the susceptible genotype but no adverse reaction by protein from the resistant genotype. Cathodic PAGE of native proteins showed over six protein bands with differences in quantity of individual components. Native anodic PAGE showed diffused banding patterns with two bands present in the susceptible genotype not detected in the resistant genotype. SDS-PAGE showed four distinct bands in Yellow Creole that were absent in Huffman. Further characterization of individual protein bands and direct correlation with antifungal activity remains for future studies.

A733

A SCALE FOR ASSESSING INFECTION PHENOTYPES OF BARLEY INFECTED WITH COCHLIOBOLUS SATIVUS. T. G. Fetch, Jr. and B. J. Steffenson. Dept of Plant Pathology, North Dakota State University, Fargo, ND 58105

An informative rating scale is essential for characterizing infection phenotypes when conducting studies on host-parasite genetics. From the evaluation of a diverse group of barley accessions to Cochliobolus sativus (spot blotch pathogen) at the two leaf stage, a new rating scale for infection phenotype was developed. Nine distinct infection phenotypes are described, based on lesion size and type (amount and extent of necrosis and chlorosis). Infection phenotypes from 1 to 4 are indicative of host resistance and are characterized by necrotic lesions ranging from <0.5 mm to <3 mm in diameter. Infection phenotype 4 is associated with either a chlorotic halo or a restricted chlorotic ring (<0.5 mm) surrounding the lesion. Infection phenotypes 5 to 6, an intermediate response, are typified by necrotic lesions 3-6 mm in length with 0.5-0.75 mm chlorotic rings. Infection phenotypes from 7 to 9 are indicative of host susceptibility and are characterized by large necrotic lesions ranging from 6-10 mm in length and chlorosis expanding both longitudinally (1-2 mm) and transversely (0.75-1.5 mm) from the infection point. This scale will aid in the evaluation of barley genotypes for resistance to spot blotch, and will be a valuable tool for use in genetic studies where classification of infection phenotype is required.

A734

COMPLEMENTARY GENETIC INTERACTION IN FUSIFORM RUST DISEASE ON SLASH PINE. Doudrick, R. L. and Nelson, C. D. USDA Forest Service, Southern Forest Experiment Station, Gulfport, MS 39505.

Rooted cuttings of 60 slash pine clones were artificially inoculated with one of two single urediniospore derived cultures of Cronartium quercuum f. sp. fusiforme. Using the forced air system, basidiospores were applied to succulent tips on multiple shoots of individual cuttings. Presence and absence of galls at nine months post-inoculation were recorded as fusiform rust disease phenotypes. Cuttings were considered infected if a gall developed on at least one inoculated shoot. A modified gene-for-gene analysis that allowed for segregation at pathogenicity loci was developed and applied to these data. Specificity between five complementary gene pairs in host and pathogen were postulated for the disease interaction. Genotypes were proposed for each culture and clone. Further inoculation experiments involving these cultures and clones and genetic test crosses of both will be required to verify the complementary gene pairs and genotypes.

A735

VIRULENCE ASSOCIATION OF *PUCCINIA STRIIFORMIS* IN NORTH AMERICA. X. M. Chen, R. F. Line, and H. Leung. Dept. of Plant Pathol., WSU and USDA-ARS, Pullman, WA 99164-6430.

New virulences of *P. striiformis* are often first detected in a simple race with a narrow virulence spectrum. Complex races that have the new virulence plus many previously existing virulences appear subsequently. To understand the evolution of the complex races, virulence factors in 50 North American races were analyzed under the hypothesis that virulence factors are randomly associated. Of 91 pairs of virulence factors that are theoretically possible, 57 pairs (63%) deviated from the expected frequencies. Among the pairs that deviated, virulence factors in 25 pairs did not occur together in a single race; in 19 pairs one of the virulence factors always occurred with another; and in 13 pairs virulence factors occurred together more often than expected. Thirty-four pairs (37%) did not deviate from the expected frequency based on random association, suggesting that somatic recombination may have occurred in the pathogen.

A736

ACTIVITY OF PHENYLALANINE AMMONIA-LYASE, *O*-METHYL TRANSFERASE AND CINNAMYL ALCOHOL DEHYDROGENASE IN *POPULUS TREMULOIDES* RESISTANT AND SUSCEPTIBLE TO *HYPOXYLON MAMMATUM*. Bruna Bucciarelli[1], M.E. Ostry[2], N.A. Anderson[1], C.P. Vance[3], Dept. of Plant Pathology[1], USFS-NCFES[2], USDA-ARS Dept. of Agronomy and Plant Genetics[3], U. of Minnesota, St. Paul, MN 55108.

Enzyme activity of phenylalanine ammonia-lyase (PAL), *O*-methyl transferase (*O*-Met) and cinnamyl alcohol dehydrogenase (CAD) were evaluated over a 4 day period in green branches of glasshouse-grown canker resistant and susceptible *Populus tremuloides*, wounded (WD) and wound-inoculated (WI) with mycelium of *Hypoxylon mammatum*. Results suggest that the WD and WI resistant genotype expressed higher activity for all three enzymes relative to the susceptible genotype. Maximum activity for these enzymes appeared to occur within the first 24 hours of challenge in the resistant genotype. By comparison, the susceptible genotype showed few discernible trends in activity upon wounding and/or inoculation. The presence of the pathogen increased levels of PAL and CAD activity in the resistant, but not the susceptible genotype.

A737

ALKENAL EFFECTS ON AFLATOXIN PRODUCTION IN THE DEVELOPING COTTON BOLL. H. J. Zeringue, Jr., SRRC, ARS, USDA, 1100 Robert E. Lee Blvd., PO Box 19687, New Orleans, LA 70179-0687

Carpel surface damage to the cotton boll results in the production of volatile alkenals, including trans-2-hexenal. The antagonistic properties of this compound to aflatoxin production was determined on intact 30-day post-anthesis developing cotton bolls grown under greenhouse conditions and inoculated with an aflatoxigenic strain of *Aspergillus flavus*. Before fungal inoculation, the bolls were pretreated by a) drilling 3 mm holes through the carpel surface of the boll, b) placing a glass wool plug over the drilled hole, and c) wrapping the boll with parafilm. 2.2, 4.3, 6.5, or 8.6 μ mole amounts of trans-2-hexenal were injected onto glass wool in 3 different treatment systems, 1) 24 hrs before, 2) 24 hrs after, or 3) simultaneously with a spore suspension of *A. flavus*. The fungal suspension was placed under the glass wool. Three weeks after treatment, the bolls were harvested from the plants and the seeds were assayed for aflatoxin. The results suggest that alkenal pre-treatment of the cotton boll initiates stronger antagonistic property to aflatoxin formation in the cottonseed of the developing cotton boll.

A738

HISTOLOGY OF INFECTION OF FIELD BINDWEED BY *PHOMA PROBOSCIS*.
D. K. Heiny, Department of Plant Pathology, University of Arkansas, Fayetteville, AR 72701, U.S.A.

Disease development in field bindweed (*Convolvulus arvensis*) infected with *Phoma proboscis* is optimum at 20-24 C, but limited at 32 C (*Phytopathology* 81:905-909 [1991]). To compare effects of temperature on fungal development and tissue response, field bindweed plants inoculated with conidia of *P. proboscis* were incubated for varying intervals at 24 C or 32 C prior to sectioning for light microscopy. Following direct penetration of epidermal cells in the stem at 24 C, *P. proboscis* hyphae progressed into the interior of the stem, gradually advancing downward. Outer cell layers of the cortex ultimately desiccated and collapsed. Death of cells did not appear to occur in advance of the hyphae by more than one or two cell layers. Bud primordia within roots developed in response to infection of hypocotyls. Pycnidia developed in dead tissue within 5 days after removal from a 24-hour dew treatment. Field bindweed grew rapidly at 32 C compared to growth at 24 C, and slow development of *P. proboscis* at the higher temperature resulted in greater resistance to disease. Inoculated shoot tips at 32 C had only limited hyphal intrusion in meristematic tissue compared to tissue at 24 C.

A739

NEW RACES OF *PUCCINIA STRIIFORMIS* IN NORTH AMERICA, 1988-1992. Roland F. Line, Abdul Qayoum, and Xianming Chen. USDA-ARS, Washington State University, Pullman, WA 99164-6430.

To monitor stripe rust of wheat (*Puccinia striiformis*), trap plots consisting of commercial cultivars, breeding lines, and cultivars that differentiate races are annually planted at sites in North America, and rust collections are evaluated on differential cultivars under controlled conditions. As of 1987, 39 races had been identified. Since 1988, 12 new races have been detected, three in 1989, three in 1990, one in 1991, and five in 1992. Ten were from eastern Washington and Oregon and two were from northwestern Washington. New virulences with ability to attack newly released cultivars Tres, Hyak, and Madsen first appeared in races with a narrow virulence spectrum. In subsequent years, six races similar to previous races but virulent on Tres were detected. Resistance of Hyak or Madsen from *Aegelops ventricosa* was no more durable than other types of race-specific resistance. Reassortment of virulences was common, even in the absence of a sexual cycle.

A740

RAPID, TRANSIENT INDUCTION OF A PEROXIDASE GENE BY COMPATIBLE *RHIZOBIUM MELILOTI* IN *MEDICAGO TRUNCATULA*. Bonnet, D., Howell, M., Nony, E., and Cook, D. Department of Plant Pathology and Microbiology, and the Crop Biotechnology Center, Texas A&M University, College Station, TX 77843.

Invasion of legume roots by rhizobia is a bacterial infection that leads to formation of a root nodule and nitrogen fixation. Despite the beneficial aspect of this symbiosis, and the tolerance of infection by the plant, close inspection indicates that the plant's response to rhizobial infection may be comparable to responses to pathogenic microorganisms. From a subtractive cDNA library that is enriched for genes expressed during initiation of nodules on *Medicago truncatula*, we have identified a clone that has up to 77% homology to known plant peroxidase genes, several of which are pathogen-induced. In addition to its function, this putative peroxidase differs from previously identified early nodulins because it is transiently expressed during nodule initiation. We have named the gene *rip*1 (*Rhizobium*-induced peroxidase). *rip*1 expression is nearly maximal within six hours of inoculation with compatible *Rhizobium meliloti*, and is turned off by three days post inoculation. Data from Southern blot analysis indicates that *rip*1 is a single copy gene. We hypothesize that this peroxidase is involved in cell wall modification during rhizobial infection.

A742

COMPARISON OF MICROSOMAL MEMBRANE PROTEINS FROM HEAT TREATED AND CALCIUM INFILTRATED GOLDEN DELICIOUS APPLES USING POLYACRYLAMIDE GEL ELECTROPHORESIS. Moline, Harold E., USDA, ARS, PQDI, HCQL,; 10300 Baltimore Avenue, Beltsville, Md 20705-2350.

Changes in microsomal membrane proteins were compared in heat-treated (4 days; 38 C), calcium-infiltrated (3 min; 103 kPa) fruit, and nontreated fruit during the storage life of Golden Delicious apples. Transitory increases in 70 k/da heat-shock proteins were observed in all treatments, although the increases were greater in heat-treated fruit. A similar transitory increase was observed in low molecular wt. proteins (18.5 kDa) following treatment. A 25 kDa protein fraction in heat-treated fruit remained elevated throughout the storage period, while a similar increase in calcium-treated fruit lasted only 20 weeks. An increase was observed in the 45 kDa protein fraction at 20 weeks which remained elevated for the remainder of the storage period. The relationship of these changes with resistance to decay is discussed.

A743

EFFECT OF FUMONISIN B_1 ON VIRULENCE OF *FUSARIUM* SPECIES ISOLATED FROM TOMATO PLANTS. J.O. Kuti, Dept. Agronomy and Resource Sci, Texas A&M Univ-Kingsvile, TX 78363 and H.K. Abbas, USDA-ARS, SWSL, Stoneville, MS 38776.

Effect of fumonisin B_1 (produced by *Fusarium moniliforme* isolated from jimsonweed) on virulence of five *Fusarium* species (*F. equiseti*, *F. moniliforme*, *F. oxysporum*, *F. solani* and *F. tricinctum*) isolated from diseased tomato plants was investigated. All isolates caused leaf blight on inoculated leaves of tomato cultivar 'Roma' but varied in their virulence. Addition of 5 - 50 μM of fumonisin B_1 to conidia suspension (10^4 conidia ml^{-1}) inoculated on leaves of 4-week -old tomato seedlings accelerated disease development and stimulated rate and amount of sporulation of *F. moniliforme* and *F. solani* but not of the other *Fusarium* species. The preliminary results indicate that fumonisin B_1 may play a role in pathogenicity of *F. moniliforme* and *F. solani* on tomato plants.

A744

ORGANIZATION OF THE GENE CLUSTER OF *PSEUDOMONAS SYRINGAE* PV. *MORSPRUNORUM* RESPONSIBLE FOR NECROSIS IN CHERRY AND HYPERSENSITIVITY IN TOBACCO. L.Z. Liang and A.L. Jones, Dept. of Botany & Plant Pathology, Michigan State University, East Lansing, 48824.

Strain PM7 of *Pseudomonas syringae* pv. *morsprunorum* produces necrotic lesions in cherry plantlets and induces a hypersensitive response in tobacco. Six of 1,300 (0.5%) kanamycin-resistant mutants from random Tn*5* mutagenesis could not elicit the hypersensitive response (*hrp* mutants), and five of these mutants did not produce lesions in cherry plantlets. Plasmid pPM419, isolated from a genomic DNA library of wild-type strain PM7, restored the ability to cause a hypersensitive response in three mutants. Restriction enzyme analysis of pPM419 revealed a 27-kilobase (kb) fragment of genomic DNA from *P.s. morsprunorum* PM7. Tn*3*-spice saturation mutagenesis of clones pPM419 and pPM41, followed by marker exchange into the genome of PM7, indicated a 22-kb DNA fragment from *P.S. morsprunorum* PM7 contains the genes for elicitation of necrotic lesions in cherry plantlets and the hypersensitive response in tobacco. Complementation studies revealed that the *hrp* region is organized into seven putative complementation groups, designated *hrpA* to *hrpG*.

A745

Karyotype Analysis of Race 8 and Race 14 Strains of *Ustilago hordei* . Mourad Abdennadher and Dallice Mills, Genetics Program and Department of Botany and Plant Pathology, Oregon State University, Corvallis, Oregon 97331-2902.

Ustilago hordei (Pers.) Lagerth causes covered smut on barley and has a physiologic race structure that obeys the gene-for-gene model. Race 8 strains are pathogenic only on the universally susceptible cultivar, Odessa. Race 14 strains, which were derived from race 8 strains through inbreeding, are pathogenic on all differential cultivars. That race 14 arose by deletion mutation or segregation of heterozygous loci on chromosomes present in multiple copies in race 8, is being investigated. A preliminary estimate of the minimum number of chromosomes in a race 8 strain and a race 14 strain was obtained by hybridization of a telomere-specific probe to Southern-blotted *Eco*RI fragments from CHEF fractionated chromosomal bands, and shown to be 21 and 18, respectively. Using fragments from chromosome-specific libraries as hybridization probes, chromosome length polymorphisms were determined to range from 0 to 90 kilobase pairs (kb) for the first eight linkage groups analyzed. Three chromosomes in the race 14 strain were estimated to be 30 to 90 Kb smaller than the homologous chromosomes in the race 8 strain. The remaining five chromosomes vary by less than 20Kb.

A746

EFFECT OF *TOX5* GENE DISRUPTION ON THE VIRULENCE OF *GIBBERELLA ZEAE*. R.H. Proctor, T.M. Hohn, and S.P. McCormick. Mycotoxin Research Unit, USDA/ARS, NCAUR. Peoria, IL 61604.

The production of trichothecene mycotoxins contributes to the virulence of some species of *Fusarium*. *Gibberella zeae* (anamorph *F. graminearum*) is an important pathogen of cereals and a trichothecene producer. To determine if trichothecenes contribute to the virulence of *G. zeae*, we generated toxin-non producing mutants of the fungus through transformation-mediated disruption of the trichothecene biosynthetic gene (*Tox5*) encoding trichodiene synthase. *Tox5*$^-$ mutants exhibited reduced levels of virulence on seedlings of wheat variety 'Wheaton', but wild type virulence on seedlings of other wheat, corn,

oat, and rye varieties. Head scab also developed more slowly on 'Wheaton' plants inoculated with *Tox5*⁻ mutants compared to those inoculated with the wild type. These results suggest that trichothecenes contribute to the virulence of *G. zeae*, but that the effect may be limited to particular host genotypes.

A752

EFFECTS OF PINE BARK ON SOIL ENZYME ACTIVITY, MICROFLORA, AND NEMATODE POPULATIONS. N. Kokalis-Burelle, R. Rodríguez-Kábana, C. F. Weaver, and P. S. King, Department of Plant Pathology, Auburn University, Auburn, AL.

Evaluation of enzyme activities and microbial populations may help define the mechanisms involved in decomposition of organic amendments and biocontrol of pathogens. Powdered pine bark was added to nematode infested soil at 0, 5, 10, 15, 20, 25, 30, 35, 40, 45, and 50 g/kg. Total fungal populations were correlated with increasing levels of pine bark. Two predominant fungal species, Penicillium chrysogenum and Paecilomyces variotii, were isolated from amended soil. Total bacterial populations were not affected by pine bark. Soil enzyme activities were correlated with pine bark rates at all sampling times. Trehalase activity was correlated with total fungal populations, and with the predominant fungal species, but was not related to bacterial populations. Heterodera glycines juveniles in soybean roots and cysts/g root, declined with increasing levels of pine bark.

A758

EVALUATION OF SOYBEAN CULTIVARS FOR RESISTANCE TO RENIFORM NEMATODE. J. J. Cornelius and G. W. Lawrence. Department of Plant Pathology and Weed Science, Mississippi State University, Mississippi State, MS 39762.

Soybean cultivar maturity groups IV and V were evaluated for resistance to Rotylenchulus reniformis. The test was conducted in the greenhouse using an initial inoculum level (Pi) of 2,000 juveniles and vermiform adults (JVA) per pot. Host susceptibility was evaluated using Oostenbrinks Rf value (final population/initial population). Twenty-two group IV and thirty-four group V soybean cultivars were evaluated. Maturity group IV had 9 resistant cultivars and 13 susceptible cultivars. Reproductive factors ranged from 0.13 for Hartz 4464 to 3.48 for Ring Around 451. Maturity group V had 20 resistant cultivars and 14 susceptible cultivars. Reproductive factors ranged from 0.15 for Rhodes and Hartwig to 5.56 for RVSL 77. Results indicate that soybean cultivars are available with resistance to R. reniformis.

A760

Revised Edition of the Pictorial Key to Genera of Plant-parasitic Nematodes. W.F. Mai, P.G. Mullin, H.H. Lyon, K. Loeffler, and T.L. Clark. Department of Plant Pathology, Cornell University, Ithaca, NY 14853

A fifth edition of the Pictorial Key to Genera of Plant-Parasitic Nematodes is currently in preparation. This edition embodies several improvements to the fourth edition (published in 1975 and currently out of print). More than 200 genera of known or suspected plant-parasitic nematodes have been described since the fourth edition was published. Two genera, *Globodera* and *Bursaphelenchus*, have been added to the updated fifth edition. In addition, two substitutions have been made: *Paratrichodorus* has been inserted in place of *Trichodorus*, and *Tetylenchus* has been dropped in favor of *Merlinius*. Other plant-associated genera are listed in an "Addendum" rather than in the body of the key, since we considered that the inclusion of a large number of additional genera would result in an unwieldy key that would be too confusing for beginning students to use. A single reference has been included for many of these additional genera. Approximately 2500 references are listed in the revised "Selected References" section, and the list of general references has been expanded to include more recent publications. Three relatively recent classification outlines have also been included in this edition.

A766

EFFECT OF THE MICROFLORA OF APPLE WOUNDS ON THE ESTABLISHMENT AND PERFORMANCE OF THE ANTAGONIST CANDIDA OLEOPHILA. J. Mercier* and C.L. Wilson, USDA/ARS/AFRS, 45 Wiltshire Road, Kearneysville, WV 25430. *Visiting Scientist

The interactions between the biocontrol agent Candida oleophila and the natural microflora of apple wounds were studied on fruit stored at 4°C. Non-infected wounds were found to be colonized mainly by yeasts (mostly Sporobolomyces roseus), Aureobasidium pullulans, and Gram-negative bacteria (mostly Erwinia spp.). Thus, a number of isolates from representative taxa were mixed and co-inoculated with C. oleophila to evaluate their effect on the establishment and performance of C. oleophila against grey mold rot. The natural microflora did not affect the growth of C. oleophila at the wound site in any way. Furthermore, the control of grey mold rot by C. oleophila was either not affected or was improved by the presence of natural microflora. It is concluded that the presence of natural microflora has a neutral effect on C. oleophila and can even be beneficial for the biocontrol of storage diseases of apples.

A767

EFFECTIVENESS OF SPOROBOLOMYCES ROSEUS AGAINST POSTHARVEST DISEASES OF APPLE WITH VARIOUS APPLICATION METHODS. W. J. Janisiewicz, D. Peterson, and B. Bors, USDA, ARS, Appalachian Fruit Research Station, Kearneysville, WV 25430.

Pink yeast Sporobolomyces roseus, isolated from pear fruit, was applied to wounded Golden Delicious apple by drop inoculation of the wounds, dip or spray to control blue-mold (Penicillium expansum) and gray-mold (Botrytis cinerea). Reduction in incidence of blue-mold was from 100% to 0% and gray-mold from 78% to 0% on apples drop-inoculated with suspensions containing 7.9×10^6 and and 6.3×10^5 cfu/ml of the yeast, respectively, and then challenged with the pathogen at 10^4 conidia/ml. In dip application, reductions were from 33% to 0% for blue-mold and from 92% to 4% for gray-mold with yeast concentrations of 7.9×10^6 and 5.3×10^6 cfu/ml, respectively. On apples sprayed with S. roseus mixed with both pathogens (each at 10^4 conidia/ml) and stored for six months at 1 C, less than 1% of fruit developed lesions in the antagonist/pathogen treatment as compared to 14% in the control and 9% for Mertect. Wounds were readily colonized by S. roseus and the populations increased by two orders of magnitude in drop and spray applications after 48 hrs. at 18 C and 3 months at 1 C, respectively.

A768

Whisker mold, a new postharvest disease of citrus in Texas caused by fungus, *Penicillium ulaiense*. M. Skaria, C.G. Eayre*, and J. Fucik, Texas A&I University Citrus Center, Box 1150, Weslaco, TX 78599, and *USDA-ARS, 2301 S. Intl. Blvd, Weslaco, TX 78599.

During the 1992-93 citrus harvest season, citrus packers in the Lower Rio Grande Valley of Texas had a high incidence of sour rot, green mold, and blue mold, caused by *Geotrichum candidum*, *Penicillium digitatum* and *P. italicum*, respectively. While sampling air and fruit in packinghouses for these pathogens, we isolated a Penicillium which produced whiskers characteristic of *P. ulaiense*, a new postharvest pathogen reported in California recently by Drs. G. Holmes, J. Eckert, and J.Pitt. *P. ulaiense* produces blue-gray spores on synnemata. A Texas isolate was confirmed by them as *P. ulaiense*, cause of whisker mold. Whisker mold disease was reproduced on a grapefruit inoculated with pure culture, fulfilling Koch's postulates. Wounding is required for infection.

A769

DIFFERENCES IN AFLATOXIN PRODUCTION BY *ASPERGILLUS FLAVUS* IN CORN GENOTYPES. B.Z.Guo[1], J.S.Russin[1], R.L.Brown[2], T.E.Cleveland[2] and N.W.Widstrom[3]. [1]Dept. of Plant Path. & Crop Physio., LSU Ag. Ctr., Baton Rouge, LA 70803; [2]UADA/ARS/SRRC, New Orleans, LA 70179; [3]Insect Biology & Management Lab, USDA/ARS, Tifton GA 31793.

In view of the importance of the genetic approach for the prevention of aflatoxin accumulation in corn, an attempt was made to study the interactions of corn genotypes and the aflatoxin producing fungus, *Aspergillus flavus*. A laboratory technique was used to screen thirteen corn hybrids and one inbred for susceptibility to aflatoxin production. Intact kernels and kernels wounded in the endosperm using a $20_G1/2$ needle were inoculated by immersion in a conidial suspension (10^6/ml) of *A. flavus*. Corn genotypes varied in levels of aflatoxin production and were separated into three groups based on patterns of toxin production in wounded and non-wounded kernels. The first group was characterized by very high levels of toxin in both wounded and non-wounded kernels and the second group supported significantly lower levels of toxin production in non-wounded kernels. A third group was characterized by drastic reduction in levels of toxin production regardless of wound. These results suggest two possible mechanisms that may be involved in limiting toxin production in corn kernels. Physical factor(s) associated with intact kernels and physiological/biochemical factor(s) are hypothesized and will be pursued in the future.

A770

THE ROLE OF THE PERICARP OF CORN KERNELS TO REDUCE INFECTION AND AFLATOXIN PRODUCTION BY *ASPERGILLUS FLAVUS*. B.Z.Guo[1], J.S.Russin[1], T.E.Cleveland[2], R.L.Brown[2] and N.W.Widstrom[3]. [1]Dept. of Plant Path. & Crop Physil., LSU Ag. Ctr., Baton Rouge, LA 70803; [2]USDA/ARS/SRRC, New Orleans, LA 70179; [3]Insct Biology & Management Lab, USDA/ARS, Tifton, GA 31793.

The outer layers of corn kernels have been extensively studied. The pericarp is the outmost layer of corn kernels and is made up of several layers of cells differing in their degree of degradation and cell-wall thickness. It affords considerable protection against invasion of the kernel by pathogens. Previous studies suggest that a physical barrier associated with intact kernels is important in preventing infection and subsequent toxin accumulation. To test this hypothesis, we initiated three studies to examine the role of the cutinized pericarp as a barrier to *A. flavus* penetration. Immersing intact corn kernels in 1M KOH for 10 min. resulted in increased fungal growth and aflatoxin production compared to water-treated kernels. In a second study, *A. flavus* infection and aflatoxin production were reduced by diisopropyl fluorophosphate (DFP), a specific inhibitor of fungal cutinase. A third study showed that *A. flavus* can grow on purified cutin as the sole carbon source. These lines of primary evidence suggest that the pericarp on intact kernels plays an important role to reduce fungal growth and toxin production by *A. flavus*.

A771

EFFECT OF DDVP ON THE PRODUCTION OF FUMONISINS ON A SYNTHETIC SOLID MEDIUM. J.-P Chen, Y.-C. Xu, and C.J. Mirocha. Univ. of Minn., St. Paul, MN 55108.

One ml suspension of *Fusarium moniliforme* culture, NRRL 13569, was evenly distributed on the surface of a synthetic agar (1.5%) medium in each 10 cm petri dish. DDVP (2,2-dichlorovinyl dimethyl phosphate) was applied into the culture at day 0, 1, 2, 3, 4, 5, 7 after inoculation. Cultures were incubated at 22-25°C and harvested daily from 1 to 14 days. Cultures treated with DDVP were harvested at 2, 3, 5 or 7 days after treatment. Quantitative analysis was done using fumonisin *o*-phthalaldehyde derivatives by HPLC. The major products obtained were fumonisin B_1, B_2 and B_3 with optimal yields of 330, 250 and 250 µg/petri dish, respectively. Fumonisin production reached a plateau 7 to 10 days after inoculation. DDVP applied at day 0 to day 3 inhibited fumonisin production 40 to 80% while there was no inhibition in later applications. Merits of this method are: short production time, simple extraction and clean-up, and enhanced purity. In addition, it provides a unique way to study the biosynthesis of fumonisins by *F. moniliforme*.

A772

PURIFICATION OF FUMONISINS B_1, B_2, AND B_3 FROM CULTURES OF FUSARIUM MONILIFORME ON CORN. J.B. Sutherland, M. Holcomb, C.E. Cerniglia, H.C. Thompson, Jr., and A.J. Williams. National Center for Toxicological Research, Food and Drug Administration, Jefferson, AR 72079.

The fumonisins are mycotoxins produced by Fusarium moniliforme that have been shown to be carcinogenic. Cultures of F. moniliforme were grown on corn and extracted with an acetonitrile/water solution, which was filtered, concentrated, and extracted with chloroform. The aqueous phase was applied to an Amberlite XAD-2 column (2.5 x 24 cm), which was washed with water. The fumonisins were eluted with methanol, concentrated, and dissolved in water. They were applied to a Whatman LRP-2 C_{18} column (2.5 x 24 cm), washed with water, eluted with acetonitrile/water, and concentrated. The fumonisins were separated by HPLC with a semipreparative C_{18} column and a complex gradient of acetonitrile and 1% aqueous acetic acid. Fumonisins B_1, B_2, and B_3 could be detected with a fluorescence detector after derivatization with fluorescamine.

A773

INFLUENCE OF MODULAR STORAGE ON ASPERGILLUS FLAVUS CONTAMINATION OF SEED COTTON IN MISSISSIPPI. W. E. Batson, Jr., J. Caceres, and Debra Newman. Mississippi State University, Mississippi State.

Aspergillus flavus infection and subsequent aflatoxin contamination of seed has long been recognized as a problem for the cottonseed industry in arid Western Arizona and Southern California. Recently, the cottonseed industry in the United States determined that aflatoxin contamination can occur sporadically over a wide area of the cottonbelt. Superficial field populations of A. flavus have been identified on seed cotton in the mid-south. This study reports A. flavus infection and aflatoxin contamination of seed in moduled seed cotton and describes a procedure for repeated access to a module for sampling over time. A. flavus contamination of seed cotton began to increase within a contaminated module in less than one week of storage.

A774

QUANTITATIVE ASSESSMENT OF *ASPERGILLUS FLAVUS, FUSARIUM MONILIFORME*, AND ASSOCIATED MYCOTOXINS IN MAIZE FLOURS FROM COSTA RICA. O. M. Viquez and R. A. Shelby. Department of Food Science, Alabama A.& M. University, Normal, AL 35762, and Department of Plant Pathology, Auburn University, AL 36849.

Forty seven lots of Costa Rican maize flour were selected, representing the major maize growing regions, harvest dates, and methods of storage. All flours were from indigenous open pollinated white cultivars and were intended for local consumption, mainly as tortillas. Lots were tested for total aflatoxins and fumonisins by HPLC of the fluorescent derivatives. Fungal populations in the flours were measured by plating serial dilutions on rose bengal salt agar for *A. flavus*, and acidified potato dextrose agar for *F. moniliforme*. Mean aflatoxin levels were high (17.22 ppb) as were *A. flavus* populations in the grain. Correlation of aflatoxin with *A. flavus* and fumonisin with *F. moniliforme* were low, suggesting that presence of viable fungus was not an indicator of toxin levels. Aflatoxin and fumonisin were positively correlated, suggesting that poor harvest and storage conditions may cause simultaneous accumulation of both toxins.

A775

EFFECTS OF NATURALLY OCCURRING AROMATIC COMPOUNDS ON BACTERIAL POPULATIONS IN SOIL. E. M. Bauske, J. W. Kloepper, and R. Rodríguez-Kábana. Auburn University, AL., 36849.

The effects of furfural, benzaldehyde, and a 1:1 combination of furfural-benzaldehyde on soil bacteria were determined in a greenhouse experiment. All treatments were applied at a rate of 1 ml/kg soil. The soil was sampled at 0, 1, 14, 21, and 28 days after treatment, plated on 5% Tryptic Soy Agar, and colonies were enumerated. The untreated control averaged 5.94 log $CFUg^{-1}$ soil throughout the study. After one day, bacterial populations were reduced by addition of the compounds. Populations increased in treated soils after one week and remained higher than populations in untreated soil throughout the study. Twenty three isolates were randomly sampled from benzaldehyde and control treatments and identified using fatty acid analysis and Microbial Identification System (Microbial ID Inc., Newark, DE). Genus richness and evenness of the sample were determined by direct counts of genera and use of modified Hill's ratio, respectively. Richness and evenness were reduced by application of benzaldehyde. One day after benzaldehyde application *Bacillus* spp. predominated and from 7 to 28 days, *Pseudomonas* spp. predominated.

A776

CHARACTERIZATION OF SUPPRESSIVENESS OF HAIRY VETCH-AMENDED SOILS TO *THIELAVIOPSIS BASICOLA*. B.L. Candole and C.S. Rothrock, Dept. of Plant Pathology, Univ. of Arkansas, Fayetteville, AR 72701.

Populations of *Thielaviopsis basicola* and the incidence of black root rot on cotton seedlings were reduced in soils amended with hairy vetch as a green manure in field and controlled environmental studies. A nylon fabric technique was used to characterize this suppressiveness under laboratory conditions at amendment levels of 0%, 0.25%, and 0.75% (w/w). Chlamydospore germination in amended and non-amended soils was less than 5% and microscopic examination showed no germ tube lysis. Chlamydospore viability was assessed by recovering nylon fabrics containing chlamydospores and determining chlamydospore germination on TB-CEN medium. Viability was reduced by an average of 28% at the 0.25% amendment level within 48 hrs. Germination also was less than 5% on nylon fabrics containing chlamydospores suspended over both amended and non-amended soils in petri dishes. Chlamydospore viability was significantly reduced at the 0.25% amendment level. Ammonia was detected in the atmospheres of soils with 0.25% and 1.0% amendment levels in additional experiments. Chlamydospore germination was inhibited in saturated atmospheres of petri dishes containing as low as 0.3 ul/ml ammonium hydroxide. These results suggest that the suppression of *T. basicola* in hairy vetch-amended soils was due to a volatile factor, possibly ammonia.

A777

RELATIONSHIP BETWEEN POT INDUCED SOIL SUPPRESSIVENESS TO TAKE-ALL OF WHEAT AND POPULATIONS OF FLUORESCENT PSEUDOMONADS. A. Sarniguet and P. Lucas. INRA, station de pathologie végétale, centre de recherches de Rennes, BP 29 35650 Le Rheu, France.

Soil suppressiveness was induced in pot experiments after successive plantings of wheat when inoculum of Gaeumannomyces graminis var tritici (Ggt) was added at every cycle. Without infestation with Ggt there was no reduction of soil conduciveness. Fluorescent pseudomonads from the rhizoplane dramatically increased on attacked roots at the first crop compared to those on healthy plant. The subsequent plantings also increased pseudomonad populations on healthy plants but to a lesser extent compared to attacked plants. In contrast, the population of total aerobic bacteria increased slightly after several crops. Successive plantings and soil infestation with the pathogen also induced qualitative changes in pseudomonad populations. Root necrosis caused by the pathogen are of prime necessity to quantitative and qualitative changes in fluorescent pseudomonad populations, which are related to the build up of soil suppressiveness. This study is continuing using molecular and biochemical approaches in order to better describe the population changes correlated with antagonistic activity.

A778

SOIL-INCORPORATION OF A GREEN OAT CROP IN THE GREENHOUSE AND FIELD TO CONTROL *APHANOMYCES COCHLIOIDES*. C.E. Windels, J. Nielsen, and R.A. Kuznia. NWES, Univ. Minn., Crookston 56716.

Soil from 7 fields infested with *A. cochlioides* was sown to oat or left fallow in the greenhouse at 18 C for 4 wk. Oat plants were cut into pieces (1-2 cm) and mixed into soil in which oat had grown. Fallow and oat-amended soils were kept moist at 25-27 C for 3 wk and then planted to sugar beet. After 4 wk, root rot indices (0-100 scale) were significantly lower in the 7 field soils where oat had grown (16, 17, 22, 28, 37, 53, 68) compared to fallow soil (82, 97, 60, 88, 93, 99, 100), respectively. In 4 field trials, oat was sown in alternate strips with fallow soil in August 1991 and incorporated 15 cm deep 6 wk later. Sugar beet cv Maribo Ultramono ([MU] susceptible to *Aphanomyces*) and ACH 205 (tolerant) were sown in May, 1992. There were no differences in disease, stand, or yield in oat-amended and fallow plots. ACH 205 had less disease and higher yields than MU in 3 of 4 fields. Thus, suppression of *Aphanomyces* by a oat crop needs to be understood to be effectively managed in the field.

A779

CONTROL OF PHYTOPHTHORA DAMPING-OFF IN BEDDING PLANTS WITH ALUMINUM. D. M. Benson, Department of Plant Pathology, North Carolina State University, Raleigh 27695-7629.

A peat:vermiculite potting mix was limed at 3 kg/m^3 and seeded 3 days later in 81-cell plug trays with snapdragon *(Antirrhimum majus)*, vinca *(Catharanthus roseus)*, or petunia *(Petunia x hybrida)*. Seeds were covered with additional mix infested with *Phytophthora parasitica* and germinated under mist. Aluminum sulfate was applied as a 150 ml drench/640 cm^2 tray at 0, 10, 25, or 50 meq Al/100 cm^3 mix. Four days after seeding, exchangeable aluminum was 0, 0, 0.5, and 2.0 meq Al/100 g at the four rates of aluminum, respectively, [pH range 5.6-5.1]. For snapdragon and vinca, all rates of aluminum controlled (P=0.05) pre-emergence damping-off (0-21%) compared to the infested controls where damping-off was 48 and 56%, respectively. At 25 or 50 meq Al/100 cm^3 mix, no damping-off occurred on snapdragon or vinca. For petunia, damping-off was 58, 27, 19, and 2% for 0, 10, 25, or 50 meq Al/100 cm^3 mix, respectively.

A780

ENDOPHYTE-MEDIATED TOLERANCE OF SEEDLING DISEASES. K.D. Gwinn, A. M. Gavin, and C.A. Blank. Dept. of Entomology and Plant Pathology, The University of Tennessee, Knoxville 37996.

Endophyte-mediated tolerance of tall fescue, perennial ryegrass, and other selected fescues to seedling diseases caused by Rhizoctonia zeae, R. solani, or Pythium aphanidermatum was evaluated. Soilless medium (30g) was mixed with pathogen-colonized seed in a Magenta™ box. Twenty seeds from each of two seed lots (one with high endophyte infestation level and one with low endophyte infestation level) were planted. Treatments for each seed lot were control pathogen-colonized medium. Experiments (10 replicates/treatment) were repeated twice. When tall fescue seed lots with low endophyte infestation level were used, seedling losses due to Rhizoctonia zeae were significantly greater than when seed lots with a high endophyte infestation were used. Other pathogen-grass combinations are being evaluated.

A781

CROP RESIDUE AND GREEN MANURE EFFECTS ON *APHANOMYCES EUTEICHES*-DISEASE INCIDENCE ON PEA. J.L. Williams, F.L. Pfleger, and J.A. Percich. Department of Plant Pathology, University of Minnesota, St. Paul, MN 55108.

Effects of eight rotation crops and of *Aphanomyces euteiches* disease incidence on a subsequent pea (*Pisum sativum*) crop were studied in the greenhouse. Fresh pea vine weights and inoculum potential values (percent of *A. euteiches* infected pea seedlings in a modified rolled towel assay) were obtained for each crop treatment. Mature oat residue and 5-wk-old oat green manures incorporated into *A. euteiches*-infested soil significantly increased fresh pea vine weight by 50-70% and 46-66%, respectively. Oat green manure effectively reduced inoculum potential by 54-95%. Five-wk-old manure of sweet corn and rapeseed also increased pea vine weight and reduced inoculum potential values. Mature soybean residue significantly reduced disease incidence over pea residue. Inoculum potential values negatively correlate with pea vine weights.

A782

SPATIAL PATTERNS OF *PYTHIUM ULTIMUM* ZOOSPORE ENCYSTMENT ON PEA ROOTS. L. M. Dandurand, G. R. Knudsen, and D. J. Schotzko. PSES Department, University of Idaho, Moscow, ID 83844.

Variability among colonization sites may influence encystment patterns of zoosporic pathogens. We used geostatistics to quantify spatial patterns of zoospores on pea roots. Plants were grown in sand (20 C, 5 d), then roots were exposed to zoospore suspensions of *P. ultimum* var. *sporangiiferum* (150, 1500, or 15000 zoospores/g of sand) for 3 hr. Roots were stained with 0.05% trypan blue and zoospore counts were made in 83 µm x 83 µm sample units, over the visible root surface. Spatial coordinates and number of zoospores were recorded for each sample unit. Spatial statistics were calculated for each inoculum density level, for the following directions: omnidirectional, 45^0, 90^0, 135^0, and 180^0. The range of spatial influence and nugget (estimated random and/or measurement error) were determined. At the lowest inoculum density, no spatial structure was evident in any direction; i.e., cysts were uniformly distributed over the root surface. Spatial structure became evident at the intermediate and high densities, particularly in the 180^0 direction (along the root axis) (e.g., intermediate: range ≈ 296 µm, nugget ≈ 0.35; high: range ≈ 344 µm, nugget ≈ 0.24). Further investigations are aimed at quantifying the spatial structure of zoospore encystment in the presence or absence of known biocontrol agents.

A783

FIELD COLONIZATION OF COMMON BEAN (*PHASEOLUS VULGARIS*) BY *BACILLUS SUBTILIS* STRAIN GB03. W.F. Mahaffee[1], E.J. Sikora[1], L. Kuykendall[2] and J.W. Kloepper[1], [1]Dept. Plant Pathology, Biological Control Institute,[2]Alabama Agriculture Extension Service, Auburn University, 36849.

Colonization of *Bacillus subtilis* strain GB03, active ingredient of Kodiak®, on common bean (*Phaseolus vulgaris*) was monitored in the field from planting until harvest. Five "on farm" field trails were established in Chambers county, AL with two lima bean cultivars ('Jackson Wonder', at two planting dates, and 'Henderson Bush') and two snapbean cultivars ('Strike' and 'Seville'). Treatments consisted of fungicide-treated seed, fungicide-treated seed plus Kodiak®HopperBox, and fungicide-treated seed plus Kodiak®Concentrate. Sampling was done at planting, V1, V4, R5, R7, and R9 developmental growth stages. Seed populations of GB03 on Kodiak®Concentrate-treated seed were higher than Kodiak®HopperBox-treated seed in all experiments at planting. Rhizosphere populations on Kodiak®Concentrate-treated seed remained as much as 1 log cfu/g higher than on Kodiak®HopperBox-treated seed; however, there was no difference in final stand count among treatments. Effects of treatments on yield and grade will be discussed.

A784

ISOLATION AND CHARACTERIZATION OF AN ALUMINUM RESISTANT MUTANT OF *THIELAVIOPSIS BASICOLA*. U. J. Harrison and H. D. Shew. North Carolina State University, Raleigh, NC 27695.

Thielaviopsis basicola is sensitive to aluminum (Al $^{3+}$) in vitro. Aluminum suppresses mycelial growth and germination and production of spores of *T. basicola*. During testing of an aluminum sensitive isolate of *T. basicola*, a spontaneous mutant resistant to aluminum was isolated. The Al-resistant isolate is identical in appearance and pathogenicity to the parental isolate but is not inhibited by levels of aluminum found in suppressive soils. The mutation was stable following repeated transfers in culture and when reisolated from inoculated plants. Populations of the Al-resistant isolate increased 5x following inoculation of burley tobacco plants in soil suppressive to black root rot, whereas populations of a wild-type isolate were only 1.2x of the initial population. All isolates of *T. basicola* that have been obtained from suppressive soils have been sensitive to aluminum. This mutant is being used to study the mechanism of suppression to *T. basicola* found in soils.

A785

VIRULENCE DIVERSITY IN FUSARIUM OXYSPORUM F.SP. SPINACIAE AND SCREENING FOR FUSARIUM WILT RESISTANCE. M.B. Fiely, J.C. Correll, and T.E. Morelock[1]. Dept. of Plant Pathology and [1]Dept. of Horticulture and Forestry, University of Arkansas, Fayetteville, AR 72701.

A worldwide collection of Fusarium oxysporum f.sp. spinaciae isolates has been examined for vegetative compatibility group (VCG) diversity and consists of three VCGs (VCG 1, 2, and 3). The three pathogenic VCGs have been recovered from throughout the U. S. (AR, CA, NY, OK, SC, TN, and WA), Canada, Japan, and Sweden. Representative isolates from each VCG were tested for virulence on spinach seedlings (cv. Grandstand) in a greenhouse pathogenicity test. Two-week-old seedlings were inoculated by injecting 10 ml

of a conidial suspension (10^6 conidia/ml) into the soil at the base of each plant. Seedlings were rated for disease 7 to 21 days after inoculation. Seedlings were rated as healthy, symptomatic (wilted), or dead. Isolates in VCGs 1 and 3 were more virulent than isolates in VCG 2. Although VCG 2 isolates killed spinach seedlings, they consistently took longer to kill seedlings than the VCG 1 and 3 isolates. A screening procedure using different inoculum concentrations and different temperatures is being evaluated to examine cultivar resistance.

A786 Withdrawn

A787

EFFECTS OF TILLAGE REGIMES AND FARMING SYSTEMS UPON VAM FUNGI. D.D. Douds, Jr., L. Galvez, R. R. Janke, and M. A. Wagoner: USDA-ARS ERRC, 600 E. Mermaid Lane, Wyndmoor, PA 19118, USA (DDD & LG) and Rodale Research Center, 611 Siegfriedale Rd., Kutztown, PA 19530, USA (RRJ & MAW).

A field experiment was initiated in 1988 to study the interaction of tillage regimes and farming systems. Conventional and low-input maize/soybean/wheat rotations were established with tillage treatments ranging from no-till to moldboard plowing. Sampling for VAM fungi began in spring 1992. Soil cores were removed to a depth of 27cm and divided into 9cm sections. Spores were isolated from this soil, quantified, and characterized. Field soil was used for an infectivity assay in the greenhouse using *Paspalum notatum*. Farming system had a significant effect upon VAM fungi. Soil in low-input agriculture had greater populations of VAM fungus spores. Tillage regime had little effect upon populations. Tillage X depth interaction terms frequently were significant for different types of spores. Upper sections of soil, and those from the low-input plots, produced greater colonization in the greenhouse assay than soil from deeper sections or conventionally farmed plots.

A788

POSSIBLE NEW ANASTOMOSIS GROUP OF RHIZOCTONIA SOLANI (THANATEPHORUS CUCUMERIS). C. S. Rothrock[1], D. E. Carling[2], S. A. Winters[1], P. M. Kinney[1] and K. Brainard[2]. [1]University of Arkansas, Fayetteville and [2]University of Alaska Fairbanks, Palmer.

Isolates of Rhizoctonia solani were isolated from rice seedlings at Stuttgart or soybean seedlings at Colt, AR and from soil in 1991 and 1992. Both fields had histories of soybean and rice production. Isolates from the two locations anastomosed with one another, but failed to anastomose with other described anastomosis groups, with the exception of a very low frequency with isolates in AG-8. Thanatephorus cucumeris was confirmed as the teleomorph. These uncharacterized isolates were pathogenic on cotton and radish when hypocotyls were inoculated or when cotton or radish was planted in soil infested with chopped potato-soil or sand-cornmeal inoculum. Isolates also produced symptoms on wheat and rice and to a lesser degree on soybean and potato. These isolates were slightly less pathogenic than an isolate of AG-4 on cotton and much less pathogenic than this isolate in infested soil on soybean, radish, wheat, or rice, or an isolate of AG-3 on potato. These uncharacterized isolates had a mean daily radial growth rate of 20 mm on potato dextrose agar at 25 C. This group also was auxotrophic for thiamine. Cultures were dark brown in color, with radiating aerial hyphae and a slight annular growth pattern.

A789

DEVELOPMENT OF FATTY ACID LIBRARIES TO TRACK *STREPTOMYCES* SPP. IN SOIL. J. H. Bowers, L. L. Kinkel, R. K. Jones, and N. A. Anderson. Dept. of Plant Pathology, University of Minnesota, St. Paul 55108.

Gas-liquid chromatography of cellular fatty acids was used to distinguish among introduced suppressive strains (PonR and PonSSII), indigenous pathogenic strains, and saprophytic strains of *Streptomyces* spp. isolated from soil, potato roots, and tubers from field plots. The objective was to determine the relative proportion of each ecological group over time. Randomly selected isolates from 1991 and 1992 experimental field plots were isolated, grouped according to morphology, and stored at 4 C. Libraries of fatty acid profiles were developed for the suppressive strains using the Microbial Identification System (Microbial ID, Inc., Newark, DE). Strains PonR and PonSSII could be distinguished from one another using principle components analysis. Dendrogram analysis separated PonR and PonSSII with a Euclidean distance of 9.14. These two strains also could be distinguished from a random collection of pathogenic *S. scabies* isolates (library developed by T. C. Ndowora) with a Euclidean distance of 20.66. The major fatty acid for both suppressive strains is 15:0 anteiso (22-27% of the total fatty acid content). The strains differed in relative proportions of 15:0 iso and 16:0 iso. Preliminary analyses indicated that introduced strains and pathogenic strains were recovered at very low frequencies and only comprised <1 and 3%, respectively, of total *Streptomyces* in 1991 samples.

A790

A COMPARISON OF VEGETATIVE COMPATABILITY GROUPS AND GENOMIC VARIATION OF PHYMATOTRICHUM OMNIVORUM. J. L. Riggs, and S. D. Lyda, Dept. of Plant Pathology and Microbiology, Texas A&M University, College Station, TX 77843.

Eighty *Phymatotrichum omnivorum* isolates from nine different hosts throughout Texas were characterized using Restriction Fragment Length Polymorphic (RFLP) DNA and Random Amplified Polymorphic DNA (RAPD). Twenty random dexamer oligo-primers were screened with the RAPD technology and over 50 probes from a *P. omnivorum* genomic library and several conserved gene probes were used in the RFLP screenings. RAPDs revealed a greater degree of genomic variation than RFLPs. Using seven RAPD primers, the 80 isolates could be separated into seven distinct groups. Variation was greater among populations collected from throughout the state, than the variation of isolates collected from within a single site of infestation. Intra- and inter- pairings of RAPD groups were screened for vegetative compatibility (VC). Visual observations of the pairings confirm that *P. omnivorum* isolates segregate into distinct groups and there was a correlation between VC and RAPD groups.

A791

GAZANIA spp.: AN ALTERNATE HOST OF LETTUCE MOSAIC POTYVIRUS (LMV) IN THE SALINAS VALLEY. F. M. Zerbini Jr.[1], S.T. Koike[2], and R.L. Gilbertson[1]. [1]Department of Plant Pathology, University of California-Davis and [2]UC Cooperative Extension, Salinas, CA.

A survey of weeds and ornamental plants was made in the Salinas Valley of California in order to determine whether alternate hosts infected by lettuce mosaic potyvirus (LMV) were prevalent. Based on detection using ELISA, a high incidence of LMV was detected in various plantings of the ornamental plant *Gazania*, with 33 out of 44 samples testing positive. Initial efforts to sap-transmit LMV from *Gazania* to lettuce or *Nicotiana benthamiana* plants were unsuccessful. However, LMV was transmitted to both species using aphids (*Myzus persicae*). The precise role of LMV-infected *Gazania* as a potential inoculum source for LMV outbreaks in the Salinas Valley is unknown, and field experiments are being conducted to assess the role of this alternate host in the epidemiology of the disease.

A792

EFFECT OF IMIDACLOPRID ON NON-FLIGHT MOVEMENT OF *RHOPALOSIPHUM PADI* (L.) (HOMOPTERA: APHIDIDAE) AND THE SUBSEQUENT SPREAD OF BARLEY YELLOW DWARF VIRUS. C. Gourmet[1], A. D. Hewings[2,3], and F.L. Kolb[1]. [1]Department of Agronomy, [2]USDA ARS Crop Protection Research Unit, and [3]Department of Plant Pathology, University of Illinois, Urbana, IL 61801

Barley yellow dwarf luteoviruses (BYDV) cause the most economically important viral disease of cereals worldwide. In many parts of the world, including the American Midwest, the bird cherry-oat aphid, *Rhopalosiphum padi* (L.) is the most important vector and the PAV serotype occurs most frequently. In some regions, insecticides are used to reduce the abundance of vectors on small grains. However, insecticide application can result in an increase in aphid activity and a subsequent increase in disease incidence. The objective of this study was to compare transmission characteristics of viruliferous *R. padi* after access to oats treated with different rates of Imidacloprid, a seed-treatment insecticide. Studies with BYDV-PAV-IL in the field indicate an inverse relationship between insecticide rate and disease spread. Preliminary greenhouse studies suggest that after access to treated plants, the behavior of *R. padi* is altered. The aphids walked and fed atypically, often abandoning the host plant. Virus transmission was determined by visual symptoms and the enzyme-linked immunosorbent assay (ELISA); spread from a focus of infestation to individual oats planted in a grid was measured.

A793

INTERACTIONS OF POTATO VIRUS X, POTATO VIRUS Y AND POTATO LEAFROLL VIRUS WITH VERTICILLIUM WILT IN POTATOES. Ernest E. Banttari, Neil A. Anderson, Javier Plasencia, and German Hoyos, Department of Plant Pathology, University of Minnesota, St. Paul, MN 55108.

The effects of primary infection with potato virus X (PVX), potato virus Y (PVY), PVX + PVY or potato leafroll virus (PLRV) of potato cultivars, 'Kennebec', 'Krantz', and 'Reddale' on vascular colonization by *Verticillium dahliae* were compared in field tests at Grand Forks, ND, and St. Paul, MN. Plants grown from tubers infected with viruses the previous season were used in these experiments.

The interactions of PVX, PVY, and PVX + PVY and vascular colonization of the hosts by *V. dahliae* were variable from experiment to experiment, but plants infected with PVX, generally, had higher Verticillium colonization indexes than non-infected plants. PVX + PVY reduced vascular colonization by *V. dahliae* in Krantz and Kennebec, but increased it in Reddale. PLRV and PLRV + PVX caused increases in vascular colonization in all three cultivars.

A794

CHARACTERIZATION OF TOMATO SPOTTED WILT VIRUS (TSWV) RESISTANCE IN THE TOBACCO CULTIVAR 'POLALTA'. B. S. Kennedy and M. T. Nielsen. Department of Agronomy, University of Kentucky, Lexington, KY 40546.

Tomato spotted wilt virus (TSWV), a thrip-vectored virus, is widespread in Europe and is becoming an increasing concern to commercial tobacco producers in the southern United States. A tobacco cultivar, Polalta, from Poland reportedly has resistance to TSWV. The resistance may have resulted from an interspecific hybridization between *Nicotiana alata* Link and Otto and *N. tabacum* L. Experiments were conducted to characterize the resistance in Polalta and to examine the inheritance of this resistance. Polalta was crossed with TSWV-susceptible burley tobacco genotypes 'TN 86' and KY 90119, and the resulting F_1 plants were selfed and backcrossed to the parents. Parental, F_1, F_2 and backcross generations (BC_1) were grown in the greenhouse and six-week-old plants were inoculated mechanically with a local isolate of TSWV. Plants were evaluated visually for symptom development and by PAS-ELISA ten days after inoculation. The burley parental lines expressed typical TSWV symptoms including large, necrotic lesions on the inoculated leaf followed by systemic necrotic patterns and leaf malformation. Disease symptoms were much less severe on the Polalta parent and the F_1 plants, and were limited to small pin-point necrotic lesions on the inoculated leaves, however, systemic symptoms were not present on these plants. Segregation patterns in the F_2 generation and in the backcrosses to the burley parents suggested two genes controlled the TSWV resistance. Some phenotypically abnormal plants, thickened leaves and irregular veination, were noted in the F_1 and segregating generations. However, these abnormalities did not appear to be associated with resistance to TSWV.

A795

SELECTING SUBCLOVER FOR RESISTANCE TO CLOVER YELLOW VEIN VIRUS. M. R. McLaughlin and T. E. Fairbrother, USDA-ARS, Forage Research Unit, Mississippi State, MS 39762.

Research to screen the U.S. germplasm collection of subclover (*Trifolium subterraneum*) for resistance to clover yellow vein virus (CYVV) was begun. Ten plants each of 246 PI lines were grown in the greenhouse during the winter and spring of 1991-'92. Seedlings were mechanically inoculated with CYVV-Pratt and evaluated 21-28 days later. Initial DAS-ELISA confirmed an absolute correlation of symptoms with infection, so later evaluations were based on symptoms. Symptomless plants were reinoculated and reevaluated up to five times. Of all plants tested, over 98% were infected. Symptomless plants (45 from 22 lines) were allowed to make seed and classified taxonomically as var. *yanninicum* (1), var. *oxaloides* (7), and var. *flagelliforme* (14) (Zohary & Heller, Israel J. Bot. 14:112-134). Progeny of symptomless plants were screened in summer 1992 as described and six plants in four lines were selected. We conclude little resistance to CYVV exists in the U.S. collection.

A796

BARLEY YELLOW DWARF VIRUS RESISTANCE IN OAT MEASURED BY ELISA. R. Ranieri, G. Shaner, R. M. Lister. Botany and Plant Pathology Department, Purdue University, W. Lafayette, IN 47907, USA.

A large population of oat accessions (approx. 400) that was selected based upon low yellow dwarf symptom scores was screened for resistance and evaluated for tolerance against barley yellow dwarf virus (BYDV). Plants were inoculated with the P-PAV isolate of BYDV and quantitative ELISA was used to identify resistance in oat plants in early growth stages of development. The plants were then transplanted in the field for tolerance evaluation. Some accessions proved to be resistant based on the ELISA scores. Ogle and Clintland 64, used as tolerant and sensitive checks, four accessions from the initial germplasm screening, and four new Purdue oat lines selected for low symptom expression were included in a series of experiments to record virus content fluctuation over time. The data were consistent with those of the large screening: the genotypes selected for low symptom score also showed low ELISA values. Resistance and tolerance to P-PAV in oat seem to be highly correlated. Purifications of P-PAV demonstrated that Ogle yielded less virus than Clintland 64. In these two cultivars ELISA values and virus content were highly correlated.

A797

OCCURRENCE OF A MECHANICALLY TRANSMISSIBLE VIRUS IN *TROPAEOLUM TUBEROSUM* FROM ECUADOR. T.A. Evans[1], V.D. Damsteegt[2], S. Soria[3], R. Vega de Rojas[3], A.S. Stone[2], R.L. Jordan[4], and S.L. Kitto[1]. [1]University of Delaware, Newark, [2]USDA-ARS, Frederick, MD, [3]AMDE Corp., Ambato, Ecuador, and [4]USDA-ARS, Beltsville, MD.

Tropaeolum tuberosum or mashua is a tuber crop indigenous to the Andean highlands and of economic value to its native peoples. Initially, 10 accessions of mashua were tested for the presence of virus. Bioassays on an herbaceous host range detected at least one mechanically transmissible virus in each accession. All isolates reacted positively with universal potyvirus monoclonal antibody in indirect ELISA tests and 600-800 nm flexuous rods were visible in negatively-stained leaf dip preparations. A survey of 46 accessions of mashua maintained at INIAP's Santa Catalina Research Station, Quito, Ecuador and 8 grower's fields for the presence of virus using both bioassay and indirect ELISA was carried out in April, 1993. Thirty accessions and plants from 8 grower's fields tested positive for potyvirus. Further characterization of isolates and attempts to eliminate virus from mashua via shoot tip culture will be discussed.

A798

SEROLOGICAL CHARACTERIZATION OF A SOYBEAN STRAIN OF TOBACCO MOSAIC VIRUS. L.L. McDaniel[1]; M.L. Maratos[1]; J.E. Goodman[1] and S.A. Tolin[2]. [1]American Type Culture Collection, Rockville, MD 20852 and [2]VPI & SU, Blacksburg, VA 24061.

Properties of a strain of tobacco mosaic virus (TMV-S) isolated from Glycine max were compared with those of two TMV isolates of the common strain, one TMV strain infecting Phaseolus vulgaris (TMV-B), and an isolate of sunn-hemp mosaic virus (SHMV). The virion capsid protein subunits of the common and soybean strains were 21 kDa. The capsid proteins of TMV-B and SHMV, after reduction and alkylation, migrated as doublets and more rapidly than capsid proteins of the common and soybean strains in acrylamide gels containing sodium dodecyl sulfate. The TMV-S genome is monopartite (6.4 kb) and has a profile of dsRNA that is identical to that of the common strain. Host symptomatology, serological assays, particle morphology, and whole virion gel electrophoresis demonstrated that TMV-S is closely related to the common strain of TMV, but only distantly to SHMV and TMV-B, which should be regarded as an SHMV isolate.

A799

POSSIBLE ASSOCIATION OF TWO SOIL-BORNE VIRUSES WITH VASCULAR NECROSIS OF SUGARBEET. Hsing-Yeh Liu, J. E. Duffus, and G. C. Wisler. USDA-ARS, 1636 East Alisal Street, Salinas, CA 93905.

Two soil-borne viruses have been isolated recently from sugarbeet roots with vascular necrosis in the Imperial Valley of California. The infectious agents are mechanically transmissible. One of these viruses is isometric and approximately 25 nm in diameter. It contains a single species of single-stranded RNA of approximately 3.70 kb and a single capsid protein of approximately 31.0 kDa. Purified virus was infective and had an A_{260}/A_{280} ratio of 1.66. An antiserum to the purified virus had a titer of 1/512 in immunodiffusion tests. The particle morphology, protein coat subunits, and nucleic acid size are similar to those of tobacco necrosis virus (TNV). However, no serological relationship to TNV has been demonstrated in immunodiffusion and western blot analyses. Another spherical virus isolated from necrotic sugarbeet roots was serologically related to tomato bushy stunt virus. The distribution, economic importance, and the relationship of these viruses to the increasing vascular necrosis syndrome in the Imperial Valley is not known.

A800

SEROLOGICAL COMPARISONS OF BEET NECROTIC YELLOW VEIN VIRUS (BNYVV) WITH OTHER ROD-SHAPED VIRUSES OF SUGARBEET. G.C. Wisler, H.-Y. Liu, and J.E. Duffus, USDA-ARS, Salinas, CA 93905.

Five BNYVV isolates (three from California, one from Nebraska, and one from Idaho) and eight other rod-shaped viruses isolated from sugarbeet (two from Texas, five from Nebraska, and one from Idaho) were compared in western blot analyses. Those antisera which reacted only to BNYVV were to; (1) the C-terminus of the BNYVV capsid protein (CP), (2) the 14-kDa and 75-kDa nonstructural proteins (courtesy K. Richards) and, (3) four monoclonal antibodies to the CP of BNYVV (courtesy G. Grassi and L. Torrance). An antiserum to the 25-kDa nonstructural protein (K. Richards) reacted with four of the BNYVV isolates, but not with one which had been maintained by mechanical transmission for several years. An antiserum to the whole virion of BNYVV reacted stongly with homologous BNYVV isolates (MW of c. 22-kDa), but weakly with the eight other rod-shaped viruses of sugarbeet, with a MW of c. 24-kDa. In reciprocal tests, antisera to the two viruses from Texas reacted strongly with all eight rod-shaped isolates (c. 24-kDa), but weakly with the five BNYVV isolates (c. 22-kDa). An antiserum to the 42-kDa nonstructural protein (K. Richards) reacted with all BNYVV isolates (MW c. 42-kDa) and the eight other rod-shaped virus isolates (MW c. 43-kDa). All BNYVV isolates produced characteristic chlorotic local lesions on *Chenopodium quinoa*. Thus, BNYVV appears to be distinct from the other rod-shaped viruses of sugarbeet tested, based on the MW of the CP and reactivity with antisera to the 14-, 25-, and 75-kDa proteins.

A801

PREVERNALIZATION INCIDENCE OF WHEAT STREAK MOSAIC VIRUS IN SOUTH DAKOTA WINTER WHEAT. M. A. C. Langham[1], D. J. Gallenberg[1], and J. E. Powell[2]. [1]Plant Science Department, South Dakota State University, Brookings, SD 57007; [2]Forest Insect & Disease Research, USDA-FS, Washington, DC 20090-6090

During each of the 1991 and 1992 fall growing seasons, sixty-five winter wheat fields throughout the winter wheat production areas of South Dakota were surveyed to determine the incidence and severity of wheat streak mosaic virus (WSMV). Fifty random plants were collected from each of the fields and frozen until analysis. Extracted plant sap was analyzed for WSMV infection with Protein-A ELISA. In 1991, WSMV was widespread throughout the state with WSMV detected in over 40% of the fields in the survey. WSMV was detected throughout the state; however, areas of

concentration occurred in the western region. The incidence of WSMV-infected plants in individual fields ranged from 2% to 38%. Lower levels of WSMV were detected in 1992.

A802

EXPRESSION OF IMMUNOGLOBULIN TO THE N PROTEIN OF TOMATO SPOTTED WILT VIRUS IN TOBACCO. K. D. Chenault (1), J. L. Sherwood (1), R. S. Nelson (2), M. B. Hein (3). (1) Department of Plant Pathology, Oklahoma State University, Stillwater, OK 74078, (2) Samuel Roberts Noble Foundation, Ardmore, OK 73402, (3) Scripps Research Institute, LaJolla, CA 92037.

The genome of tomato spotted wilt virus (TSWV), the type member of the genus Tospovirus in the family Bunyaviridae, consists of three linear ssRNA molecules. The nucleocapsid (N) protein, produced from the S RNA may also be involved in the regulation of viral nucleic acid replication. cDNAs coding for the H and L chains of a MAB to the N protein were produced by first strand cDNA synthesis followed by PCR cloning. DNAs coding for the H and L chain were inserted into binary vectors for Agrobacterium mediated plant transformation. Regenerated plants contained either H or L chain constructs as shown by PCR or Southern blot analysis. Northern blots of R_0 plants indicated H and L transcripts of the predicted size. Plants transformed with the L construct are producing L chain protein at 1 to 42 μg/mg plant protein. Additional transformants are being made to facilitate H chain production. In addition, with a single-chain antibody construct, the effects of leader sequences that differ in hydrophilicity are being examined.

A803

BIOLOGICAL AND MOLECULAR CHARACTERIZATION OF POTYVIRUSES FROM *Passiflora*. D. Benscher, S.S. Pappu, C.L. Niblett, E.P. Rybicki[1], and J. Bird[2], Plant Pathology Dept., Univ. of Florida, Gainesville, Fl 32611-0680, [1]Microbiology Dept., Univ. of Capetown, Rondebosch, 7700, South Africa, [2]Dept. of Crop Protection, Univ. of Puerto Rico, Rio Piedras, PR, 00928.

Potyviruses were isolated from *Passiflora edulis* and *Passiflora ligularis* from Colombia, Thailand, Dominican Republic (DR), and Puerto Rico (PR). These viruses were compared to known potyviruses of *Passiflora* from Australia and South Africa, two strains of soybean mosaic virus (SMV), watermelon mosaic virus 2, and two strains of bean common mosaic virus (BCMV NL-8 and US1) by host range and nucleotide sequence homology in the coat protein gene and the 3' non-coding region (3'NCR). Host range, nucleotide sequence, and dot blot hybridization data indicate that the isolates from Colombia were very similar to SMV. Coat protein sequence data also show a high homology between the DR and South African isolates, and the PR and BCMV-US1 isolates, whereas the Thailand isolate remains distinct.

A804

DETECTION OF TEV PROTEINS IN INFECTED TOBACCO ROOTS. Willem G. Langenberg[1], Ernest Hiebert[2] and Dan E. Purcifull[2], USDA/ARS and Department of Plant Pathology, University of Nebraska-Lincoln, 68583[1], and University of Florida, Gainesville, 32611[2]

The appearance of several proteins coded for by the potyvirus tobacco etch virus (TEV) was studied in infected tobacco root tips. Antibodies to TEV structural protein, cylindrical inclusion (CI) protein, nuclear inclusion proteins a and b (protease and polymerase) were used as primary probes on ultrathin sections, followed by labelling with goat anti-rabbit IgG attached to colloidal gold. Unequal concentrations and distribution of target protein (few early CIs and no nuclear inclusions) were observed in the earliest stages of infection. CI protein was detected first followed by structural protein and then by the nuclear inclusion proteins. The differential detection of the TEV proteins was unexpected because potyviral proteins are expressed in equimolar amounts by processing of a large polyprotein.

A805

CHARACTERIZATION OF A γ-TUBULIN GENE FROM *COCHLIOBOLUS HETEROSTROPHUS*. K. Beickman and M. Perlin. Univ. of Louisville, Louisville, KY 40292.

γ-Tubulin is a recently discovered member of the tubulin family which is localized to the centrosome or spindle pole body (in fungi) and may play a role in nucleation of microtubules. The protein is highly-conserved in amino acid sequence, even across kingdoms, with 60-80% identity observed. Here, we report the characterization of the γ-tubulin gene from the ascomycete, *Cochliobolus heterostrophus*, the causative agent of southern corn leaf blight. With degenerate primers which had successfully amplified the γ-tubulin gene from *Ustilago violacea*, a phytopathogenic basidiomycete, the polymerase chain reaction (PCR) was used to amplify the corresponding region from *C. heterostrophus*. The DNA sequence of the resulting ca. 1 kb fragment revealed several introns. The predicted protein sequence showed 80% identity with that from *Aspergillus nidulans*, a saprophytic ascomycete, and 75% identity with the γ-tubulin from *U. violacea*. This information is currently being used in phylogenetic analyses.

A806

SEQUENCE AND FUNCTIONAL ANALYSIS OF LIGHT ENHANCED CLONE cLE6 OF *CERCOSPORA KIKUCHII*. T.M. Callahan, M. Ehrenshaft, and R.G. Upchurch, USDA/ARS and Dept. Plant Pathology, N.C. State Univ., Raleigh, NC 27695-7616.

Cercospora kikuchii, a fungal pathogen of soybean, produces the red phytotoxic polyketide, cercosporin. Light is a positive regulatory cue for the induction of cercosporin synthesis. We previously used a light/dark subtractive hybridization approach to isolate cDNA clones whose message accumulation is light-enhanced and parallels the accumulation of cercosporin in a light-grown culture. DNA sequence analysis of the most strongly light enhanced clone, cLE6, reveals an ORF of 1818 bp that predicts a polypeptide of 606 amino acids with a molecular weight of 65,424 and a pI of 5.08. We have continued the characterization of cLE6 by constructing a disrupted, bialaphos-resistant tagged version of the genomic analog of cLE6 and are in the process of assessing the phenotypes of the wild-type transformants containing this plasmid.

A807

ISOLATION AND SEQUENCE OF THE *CERCOSPORA NICOTIANAE* PHYTOENE DEHYDROGENASE GENE. M. Ehrenshaft and M. Daub. Dept. of Plant Pathology, NCSU. Raleigh 27695-7616.

Cercospora nicotianae synthesizes the photosensitizer cercosporin which produces highly toxic singlet oxygen when illuminated. Since carotenoids are known to be efficient quenchers of singlet oxygen we are investigating their role in *Cercospora* resistance to cercosporin autotoxicity. Using conditions of lowered stringency we identified a *Cercospora* genomic clone which hybridized strongly to the *Neurospora crassa* carotenoid biosynthetic gene for phytoene dehydrogenase. Comparison of the deduced amino acid sequences indicated that the *Cercospora* gene product has a nearly 60% identity with the *N. crassa* polypeptide and an approximately 30% homology with phytoene dehydrogenase proteins isolated from prokaryotes. The putative *Cercospora* polypeptide also contains both the 5' and 3' amino acid consensus sequences found in other microbial phytoene dehydrogenases. Southern hybridization analysis suggests that the *Cercospora* phytoene dehydrogenase gene is present as a single copy.

A808

ELECTROPHORETIC KARYOTYPE OF *CERCOSPORA KIKUCHII*. R. C. Hightower, T. M. Callahan, and R. G. Upchurch, USDA/ARS and Department of Plant Pathology, North Carolina State University, Raleigh, NC 27695-7616.

An electrophoretic karyotype of *Cercospora kikuchii* was obtained using contour-clamped homogenous electric field gel electrophoresis. Chromosomes were fractionated into eight bands, with two of these bands migrating as a doublet. The estimated sizes of the *C. kikuchii* chromosomes range from 2.0 to 5.5 Mb, based on migration relative to the chromosomes of *Schizosaccharomyces pombe* and *Saccharomyces cerevisiae*. Using this determination of chromosome number and size, the total genome size of *C. kikuchii* is estimated to be 28.4 Mb. In addition, several genes were assigned to chromosomal bands by hybridization of contour-clamped homogenous electric field gel blots with various radiolabeled probes including tubulin, ribosomal DNA, and light-enhanced cDNAs isolated previously from *C. kikuchii*.

A809

CONSERVATION OF GENES ENCODING PECTIN DEGRADING ENZYMES AMONG BACTERIA, FUNGI AND PLANTS. M.C. Ho[1], M. P. Whitehead[1], T.E. Cleveland[2] and R.A. Dean[1]. [1]Dept. of Plant Pathology and Physiology, Clemson University, SC 29634. [2]USDA, ARS, Southern Regional Research Center, New Orleans, LA 70124.

Genes encoding pectin degrading enzymes have been isolated from numerous organisms including bacteria, fungi and plants. We have isolated and sequenced a pectate lyase gene, *pel*A, from *Aspergillus nidulans* and two

polygalacturonase genes, *pec*A and *pec*B, from *A. flavus*. Comparisons of nucleic acid and deduced amino acid sequences with other genes encoding pectin degrading enzymes revealed highly conserved regions. In general, fungal polygalacturonases were found to be greater than 60% identical whereas lyases were less than 25% identical. Degenerate oligonucleotides were designed based on the nucleotide sequence from the most conserved regions in the genes. These oligonucleotides have been used as primers for PCR cloning of other pectinase genes from fungi. The nucleic acid sequence has been determined and used to construct phylogenetic relationships.

A810

IN VITRO TRANSCRIPTION ANALYSIS OF THE *PDA*1 GENE OF *NECTRIA HAEMATOCOCCA* Yijun Ruan and David C. Straney. University of Maryland, College Park, MD 20742

The *Pda*1 gene in *Nectria haematococca* encodes pisatin demethylase, an enzyme which detoxifies pisatin, phytoalexin of pea. Expression of the *PDA*1 gene is induced by pisatin. In order to understand the transcriptional regulation of the *PDA* gene, we have established an *in vitro* transcription system for *N. haematococca*. Whole cell extracts from pisatin-induced mycelium produce a transcript from the *PDA*1 promoter on a plasmid template. The transcript is accurately initiated at an *in vivo* start site and its formation is dependent upon the *PDA*1 promoter's TATAA box. The transcript is produced by RNA polymerase II based on its inhibition with αamanitin and Pol II-specific monoclonal antibody. Significantly, extract from uninduced mycelium does not produce this specific transcript, indicating that regulation is preserved in the extract and allowing us to dissect the DNA and protein components of the pisatin regulation of transcriptional control.
Maryland Agriculture Experiment Station Contribution Number 8677

A813

A SECOND ENDOGLUCANASE CLONED FROM *MACROPHOMINA PHASEOLINA*. Haiyin Wang and Richard W. Jones, Botany & Plant Pathology, Lilly Hall, Purdue Univ., West Lafayette, IN 47907.

The soilborne fungus *M. phaseolina* infects over 500 plant species. It remains latent in the tissue until the host is stressed. The active infection is accompanied by extensive decay of root and stem tissue. We have begun to clone the genes encoding cellulases of *M. phaseolina* in an effort to determine the differential expression of cell wall degrading enzymes during latent and active infection. Enzyme activity assays in nondenaturing gels indicate three possible endoglucanases from this fungus. Endoglucanase clones were derived from cDNA expression libraries. Clone selection was made after overlay with carboxymethylcellulose and Congo Red staining. Previously we reported the cloning of an endoglucanase encoding a 1.4 kb mRNA. We now report cloning of a second endoglucanase gene which encodes a 1.8 kb mRNA. This endoglucanase clone has no 5' or 3' terminal homology to the previously cloned endoglucanase, and neither endoglucanase has terminal homology to other reported cellulases. Sequence data are being used to design primers for PCR analysis of transcriptional activity *in planta*.

A814

CHARACTERIZATION OF THE *A* MATING-TYPE ALLELE OF *USTILAGO HORDEI*. John E. Sherwood and Shirley Gerhardt. Dept. Plant Pathology, Montana State University, Bozeman, MT 59717.

The *Ustilago hordei A* mating-type allele was cloned from a lambda library using heterologous probes encoding the *a* locus of *U. maydis*. Five phages with overlapping inserts contained sequences with homology to *mfa1* and *mfa2* (genes encoding the mating factors from the *U. maydis a1* and *a2* alleles), and *pra1* (the pheromone receptor gene from *a1*). No phage with homology to *pra2* were identified. Two *Bam*H1 fragments with homology to the *U. maydis* probes were subcloned and transformed into both mating types of *U. hordei*. One fragment caused severe cell deformation with structures resembling conjugation tubes when transformed into *a* cells, but not *A* cells. The culture supernatant from that transformant induced similar morphological changes in both mating types of *U. hordei*. The culture supernatant from *A* cells, but not *a* cells, transformed with the second fragment, which did not cause morphological changes in the transformants, induced conjugation tube-like structures when added to either mating type of *U. hordei*.

A815

MOLECULAR CLONING AND DNA SEQUENCING OF THE *THIELAVIOPSIS BASICOLA* CALMODULIN GENE. Michael Hood, C.O. Opperman, H.D. Shew, North Carolina State University, Raleigh, NC 27695.

Soils suppressive to black root rot of tobacco caused by *Thielaviopsis basicola* are characterized by pH > 5.0 and exchangeable aluminum levels greater than 0.5meq Al/100g soil. Al and calmodulin antagonists inhibit germination and production of spores and mycelial growth of *T. basicola*. High levels of Al cause conformational changes in calmodulin, a highly conserved calcium regulating protein, and thereby inhibit vital cellular processes. In order to examine the role of calmodulin in the suppression of *T. basicola* by Al, the calmodulin gene was cloned and sequenced. Degenerative DNA primers were prepared from amino acid sequences of calmodulin from other organisms, and a portion of the calmodulin gene was amplified by subsequent PCR. A fusion protein made from the calmodulin gene of *T. basicola* will be a valuable tool in preparing calmodulin-specific antibodies for use in immunohistological investigation of Al effects on pathogenesis by *T. basicola*.

A816

DIFFERENTIATION OF THE SOYBEAN SUDDEN DEATH SYNDROME PATHOGEN, *FUSARIUM SOLANI*, FROM OTHER ISOLATES OF *F. SOLANI* BASED ON CULTURAL MORPHOLOGY AND MITOCHONDRIAL DNA RFLPs. J.C. Rupe, J.C. Correll, and P. Yount, University of Arkansas, Fayetteville.

Twenty isolates of *Fusarium solani* were examined for cultural morphology on PDA, for ability to cause sudden death syndrome (SDS) symptoms on soybean in greenhouse pathogenicity tests, and for mitochondrial DNA RFLPs. Isolates producing SDS symptoms had slow growing, appressed, slimy, blue colonies that sometimes stained the medium a dark maroon and produced masses of macroconidia, but few microconidia. Eleven SDS isolates were collected from SDS-infected soybeans growing in a number of Arkansas fields while the 9 non-SDS isolates were isolated from a variety of other hosts. SDS isolates were homogeneous with respect to mtDNA RFLPs and distinct from the non-SDS isolates. The non-SDS isolates were heterogeneous for mtDNA RFLPs. Thus, the SDS pathogen appears to be genetically distinct from other *F. solani* isolates.

A817

MAPPING AND CROSSHYBRIDIZATION OF MITOCHONDRIAL DNA FROM SEVERAL SPECIES OF PYTHIUM. Joseph Prenger and Frank N. Martin, Plant Pathology Dept., University of Florida, Gainesville, Florida 32611

Mitochondrial DNA (mtDNA) of several representative species of the genus Pythium have been mapped. The goal is to identify probes and polymorphisms to be used for isolate identification and phylogenetic studies, particularly with respect to morphologically similar species. The mtDNA of Pythium spp. contains a large inverted repeat (IR) which stabilizes the DNA from mutations. The relative stability makes it possible to make intra- and interspecific comparisons of conserved and highly variable restriction sites. Several probes have been produced by subcloning different regions of the IR. Preliminary evidence indicates that portions of a highly conserved region hybridize to all species examined, while less conserved regions differentiate between a number of species. Data on the relationship of these conserved and variable regions relative to coding regions will be presented and their utility for species identification discussed.

A818

UNIVERSAL PCR PRIMERS THAT ALLOW INTERGENERIC DIFFERENTIATION OF ASCOMYCETES AND THEIR APPLICATION TO VERTICILLIUM. K.-N. Li, T. L. German, and D. I. Rouse. Department of Plant Pathology, University of Wisconsin, 1630 Linden Dr. Madison, WI 53706

Identifying specific DNA probes is important in applying molecular technology to develop rapid and sensitive methods to detect microbes. A pair of PCR primers, NMS1 and NMS2, were designed and tested with thirty isolates of ascomycetes. The primers amplified with high stringency a region of about 600 base pairs (bp) in the mitochondrial small rRNA gene. The exact size of the fragment depends on the species. The sizes and RFLP patterns of the amplified region differentiated the isolates at or below intergeneric level. This locus from 3 plant pathogenic species of *Verticillium*: *V. dahliae*, *V. albo-atrum*, and *V. tricorpus* were sequenced. Two bp were different between *V. dahliae* and *V. tricorpus* and one bp between *V. dahliae* and *V. albo-atrum*. A pair of PCR primers, VMSP1 and VMSP2, was designed and used to amplify a region of 140 bp within the locus. Tests of the primers with DNA from more than sixty *Verticillium* and other fungal isolates showed that these primers allowed differentiation among the three *Verticillium* species.

A819

INTRASPECIFIC RELATIONSHIPS OF *RHIZOCTONIA SOLANI* DERIVED FROM NUCLEAR RIBOSOMAL DNA (ITS-5.8 S and 18 S rDNA) POLYMORPHISM. Z. L. Liu, Formosa Plastics Corp., P. O. Box 69, La Ward, TX 77970; L. L. Domier, USDA-ARS-MWA and J. B. Sinclair, Dept. of Plant Pathology, Univ. of Illinois at Urbana-Champaign, Urbana, IL 61801-6704.

Previously, we reported restriction analysis of the and 5.8S ribosomal RNA gene region (ITS-5.8S rDNA) of isolates *R. solani* in AG 1 to AG 10 and AGBI. We have extended these investigations to include DNA polymorphism of 18S rRNA gene of 25

intraspecific groups (ISGs) representing the 11 AGs. A PCR-based restriction-enzyme mapping strategy was developed and used to map the 18S region in each of the isolates. Four types of DNA restriction maps were constructed in the 18S rDNA region for the 25 ISGs. Most ISGs shared map type I. ISGs 2E and 9 had map II and differed from most ISGs by one restriction site. ISG 5C had map type III and differed from most ISGs by two sites. ISGs 10A and B had map type IV and showed site variation for five restriction enzymes. Phylogenetic analyses using variation of restriction sites in the ITS-5.8S and 18S regions and the lengths of the spacer fragments showed that the ISGs were genetically distinct units, but closely related, except for ISGs 10A and B (AG 10). A comparison of the 25 ISGs in relation to the 11 anastimosis groups is presented.

A820

ISG-SPECIFIC POLYMORPHISM IN THE NUCLEAR INTERNAL TRANSCRIBED SPACER (ITS) AND 5.8S rDNA REGIONS OF *RHIZOCTONIA SOLANI*. Z. L. Liu, Formosa Plastics Corp., P. O. Box 69, La Ward, TX 77970; L. L. Domier, USDA-ARS-MWA and J. B. Sinclair, Dept. of Plant Pathology, Univ. of Illinois at Urbana-Champaign, Urbana, IL 61801-6704.

The restriction-fragment-length polymorphism in the ITS-5.8S rDNA region was studied in 30 isolates of *R. solani* representing 9 AGs (AG 3 through 10 and BI). DNA restriction maps were constructed for each isolate using a PCR-based mapping technique and digestion with two restriction enzymes. Based on these maps, the isolates were divided into 14 genetically distinct groups . The sizes of the amplified DNA fragments varied from 0.63 to 0.74 kilobase pairs among the 14 intraspecific groups (ISGs). Among the 30 isolates, two ISGs were identified within AG 3, two in AG 4, three in AG 5, and two in AG 10. Two isolates of AG 6, one of AG 7, one of AG 8, six of AG 9 and two of AG BI, formed separate groups. Using the variation of the restriction enzyme sites in this region as group characteristics, we propose that each of the 14 groups is an independent ISG.

A821

DOUBLE-STRANDED RNA (dsRNA) DETECTION USING NON-ISOTOPIC NUCLEIC ACID PROBES. N. Bharathan, J. Jones, M. Singh-Lapp, and D. M. Nelson. Department of Math and Natural Science, Northern State University, Aberdeen, SD 57401.

The genetic relatedness of double-stranded RNA (dsRNA) from field isolates of *Rhizoctonia solani* belonging to four anastomosis groups (AG's) were studied using ECL detection systems (Amersham). DsRNA used as probes were denatured and directly labeled with the enzyme horseradish peroxidase. Homologous dsRNA sequences up to 1 ng were very easily detected in dot-spot hybridization. All hybridizations were done under high-stringency conditions. Experiments are currently being done to use effectively non-isotopic dsRNA detection in gel-blot filter - hybridization and compare its sensitivity to 32p labeled dsRNA. Preliminary studies using ECL detection system suggests little or no sequence - homology among dsRNAs from isolates in AG-3, AG-5 and AG-9.

A821a

VIRAL GENES CARRIED ON TWO DOUBLE-STRANDED RNA'S (DSRNA) FROM *RHIZOCTONIA SOLANI*. D. K. Lakshman AND S. M. Tavantzis. Department of Plant Biology and Pathology, University of Maine, Orono, ME 04469-0118.

Field isolates of *R. solani* contain numerous, genetically diverse dsRNA elements. No apparent relationship exists between the mere presence of dsRNA and virulence. Specific dsRNAs, however, are associated with expression of virulence in *R. solani*. We study a genetic model that is anticipated to unveil the biological role of dsRNA in *R. solani*. From parental virulent strain Rhs 1AP, conversion to hypovirulence correlated with the "appearance" of 3 novel dsRNAs (25 kb, 3.7 kb and 1.2 kb) in subculture Rhs 1A1, and reduced vigor coincided with the loss of 2 dsRNAs (23 kb and 6.5 kb) in subcultures Rhs 1A2 and 1A3. The 5 dsRNAs were cloned and shown to be genetically unrelated to one another. Sequence data analysis suggests that 2 dsRNAs, 23 kb and 3.7 kb, carry genes of viral nature. To our knowledge, this is the first report on cloning and sequencing dsRNA from *R. solani*.

A822

COMPARISON OF *PHYTOPHTHORA CITROPHTHORA* AND *P. PARASITICA* ISOLATES BY PCR AMPLIFICATION WITH SPECIES-SPECIFIC AND ARBITRARY PRIMERS. T. Ersek, J.T. English, and J.E. Schoelz, Dept. of Plant Pathology, Univ. of Missouri, Columbia, 65211.

Isolates of *P. citrophthora* and *P. parasitica* were compared by PCR amplification of genomic DNA. The 24-bp oligonucleotide primers derived from chromosomal DNA specific for the respective species amplified a 650-bp fragment and a 1000-bp fragment of *P. citrophthora* and *P. parasitica* DNA, respectively. Various isolates of either species were indistinguishable with these primers. Arbitrarily chosen 10-bp primers, however, could detect DNA polymorphisms (RAPD markers) that relate to phenotypic variation of isolates within these species.

A823

ASSESSMENT OF THE REPLICATIVE DEPENDENCE AND BIOLOGICAL INFLUENCE OF THE SATELLITE RNA OF PEA ENATION MOSAIC VIRUS (PEMV). S.A. Demler, L. Nooruddin, D.G. Rucker and G.A. de Zoeten. Department of Botany and Plant Pathology, Michigan State University, East Lansing MI 48824-1312.

The helper virus of the satellite of PEMV is composed of two unrelated and autonomously replicating RNAs. The objective of this study was to dissect the relative contribution of each RNA species to the subsistence and transmission of this satellite RNA. Infectivity assays demonstrated that the replication functions encoded by RNA 2 are solely responsible for the replication of the satellite RNA. In contrast, structural functions encoded by RNA 1 are responsible for the encapsidation of both the satellite RNA and RNA 2. Although this trilateral arrangement is comparable in many respects to the groundnut rosette disease complex, we found no evidence of satellite intervention in aphid transmission. Consistent with the symptom modulation associated with satellite elements, the PEMV satellite RNA is capable of suppressing symptom intensity in specific host plants.

A824

RNA 2 OF THE LS-CMV DETERMINES THE PRESENCE OF RNAS 4a AND 5. Lee Zhang and Peter Palukaitis, Dept. of Plant Pathology, Cornell University, Ithaca, NY 14853.

The genome of cucumber mosaic virus (CMV) consists of 3 RNAs, namely RNA 1, RNA 2 and RNA 3 as well as a CMV subgenomic RNA 4. CMV can be divided into two subgroups, I and II. One of the common feature of subgroup II strains of CMV is that the generation of RNA 4a and 5 in the infected plants, while the strains of subgroup I CMV do not have RNAs 4a and 5. Full-length cDNA clones of a subgroup II strain LS-CMV, were constructed and infectious RNAs were made from these clones and the pseudorecombinants constructed between infectious transcripts of the LS-CMV cDNA clones and the Fny CMV (a subgroup I strain) cDNA clones (which were constructed previously in this laboratory) were used to map which RNa of the subgroup II strains conditioned for the presence of RNAs 4a and 5. We found that the generation of RNA 4a and 5 was correlated with the presence of RNA 2 from LS-CMV. The genomic seqeunces encoding RNAs 4a and 5 were delimited by nucleic acid hybridization and partial sequence determination. These data will be presented.

A825

COMPLETE NUCLEOTIDE SEQUENCE OF THE CFH STRAIN OF BEET CURLY TOP VIRUS. D. C. Stenger, Department of Biological Sciences, Northern Illinois University, DeKalb, IL 60115.

The complete nucleotide sequence of the hypervirulent CFH strain of the geminivirus beet curly top virus (BCTV) has been determined. The circular DNA genome of BCTV-CFH consists of 2927 nucleotides and shares extensive sequence homology with the biologically distinct California strain. Analysis of the CFH nucleotide sequence indicated that the rightward open reading frames (ORFs) R1, R2, and R3 were highly conserved (>95% conserved amino acid homology), while the leftward ORFs L1, L2, L3, and L4 shared less conserved amino acid homology (78.8%, 66.5%, 86.7%, and 56.7%, respectively) with the corresponding California strain ORFs. The CFH DNA sequence also contained a unique 12.5 kd ORF (R4); however, there is no evidence to suggest that R4 is expressed. Regions of the genome which have diverged may account for differences in pathogenicity among BCTV strains.

A826

FURTHER EVIDENCE THAT PEPPER MOTTLE AND POTATO VIRUS Y ARE DISTINCT POTYVIRUSES: SEROLOGY AND NUCLEOTIDE SEQUENCE OF THE COAT PROTEIN GENE AND 3' UNTRANSLATED REGION OF PEPPER MOTTLE VIRUS NC165. Ramon Jordan and Frank Turano°, USDA-ARS, PSI and NRI°, BARC-West, Beltsville, Maryland

The sequence of the 3' 1344 nucleotides of the genome of a strain of pepper mottle potyvirus (PepMoV-NC165) was determined. The sequence contains 255 nucleotides of the NIb gene, the complete coding region of the viral coat protein, followed by a 3' non-coding region of 270 nucleotides and poly(A) tail. The degree of homology (ignoring gaps) between the coat protein and 3' non-

coding sequences of PepMoV-NC165 with 28 potyviruses (including 7 strains of potato virus Y; PVY) ranged from 49 to 67%, while a sequence identity of 88% was found with the California strain of PepMoV (PepMoV-C). Optimal alignment of the 3′ non-coding regions between PepMoV-NC165 and PepMoV-C gave a 97% identity, whereas the degree of homology with the other potyviruses ranged between 36 and 44%. When the deduced amino acid sequence of the coat protein coding region of PepMov-NC165 was compared, a sequence identity of 92% was found with PepMoV-C. However, the degree of homology with the coat proteins of the other potyviruses ranged from 52 to 75%. Serological analysis using a panel of potyvirus-specific and group cross-reactive monoclonal antibodies also revealed significant differences between the two strains of PepMoV and other potyviruses, including PVYs.

A827

ANALYSIS OF THE BINDING OF CUCUMBER MOSAIC VIRUS-FNY AND TOBACCO MOSAIC VIRUS-U1 MOVEMENT PROTEINS TO NUCLEIC ACIDS. Qiubo Li and Peter Palukaitis, Department of Plant Pathology, Cornell University, Ithaca, NY 14853.

The movement protein from cucumber mosaic virus (Fny strain) and tobacco mosaic virus (common strain) were expressed in *E. coli* via an expression vector pET11a. In gel retardation assays, the two expressed proteins behaved similar with respect to binding to single-stranded RNA (ssRNA). Neither protein showed a difference in binding to the homologous RNA vs a heterologous RNA. In a competition binding assay, it was found that ssDNA was an effective competitor against the binding of ssRNA by both movement proteins. Antisera have been made against both expressed proteins and will be used to further characterize the RNA-protein complexes formed in competition binding assays involving both movement proteins.

A828

CLONING AND EXPRESSION OF WHEAT SOILBORNE MOSAIC VIRUS (WSBMV) PUTATIVE CELL-TO-CELL MOVEMENT PROTEIN GENE. X. M. Zhu, J. L. Sherwood, and R. E. Pennington. Department of Plant Pathology, Oklahoma State University, Stillwater, OK 74078-9947.

RNA 1 of Wheat Soilborne Mosaic Virus encodes the putative movement protein (Wilson, personal communication). WSBMV RNA was extracted from virions purified from naturally infected wheat. The putative movement protein gene was amplified using reverse transcription followed by PCR and the gene cloned into the expression vector pET11b. Two clones were obtained; one representing the full length movement protein resulting in a 37 kD product in *Escherichia coli*, and a second clone missing the 247 nucleotides at the 3'end and producing a 32 kD protein. The full length clone was sequenced and compared with the sequence of the Nebraska isolate of WSBMV. Antiserum against the 32 kD protein is being produced to localize the putative movement protein in WSBMV infected plants.

A829

A SEQUENCE COMPARISON OF SELECTED GEMINIVIRUSES NATURALLY INFECTING WEEDS AND CROPS IN FLORIDA. A. M. Abouzid and E. Hiebert, Department of Plant Pathology, University of Florida, Gainesville, FL 32611.

Segments of DNA-A (AL1 1978- 496 AR1, about 1.1 kb) and of DNA-B (BL1 2039- 2 CR, about 0.6 kb) were amplified by PCR and cloned from naturally, geminivirus infected weeds (*Sida* spp), collected from North/Central (Gainesville) and South Florida (Homestead) and from infected beans north of Gainesville. The common regions of the selected geminiviruses were compared by alignment with previously sequenced tomato mottle virus (TMoV), and with a geminivirus (MaGV-FL) infecting *Macroptilium lathyroides* in Homestead. The resulting alignment of A component segments showed a high sequence homology (94%) of the Sida isolate from the Gainesville area with TMoV and with the geminivirus infecting beans. The Sida from Homestead showed a high homology in A component to the MaGV-FL. The sequence comparisons with the B component intergenic segment (BL1-CR) revealed a high similarity between viruses infecting the same host. These studies may provide information regarding the origin of the new geminiviruses infecting crops in Florida.

A830

Correlation Between Arrangement of Integrated Virus Coat Protein Transgenes and Level of Virus Resistance J.Russell McMaster, David M. Tricoli, Kim J. Carney, Paul F. Russell, Hector D. Quemada, Maury L. Boeshore, David W. Groff, Keisha Hadden, and Jon P. Hubbard Asgrow Seed Company, Kalamazoo, MI 49001

Commercial squash production in many important growing areas is severely reduced by virus disease. Two of the most important viruses that affect squash production are zucchini yellow mosaic virus (ZYMV) and watermelon mosaic virus II (WMVII). Traditional sources of resistance to these viruses have not been incorporated into commercially acceptable squash varieties. To provide commercially acceptable resistance to ZYMV and WMVII, we engineered ZYMV and WMVII coat protein genes (CP) into proprietary squash varieties. Plants engineered with ZYMV and WMVII coat protein genes exhibit resistance to ZYMV and WMVII under field conditions. We characterized a ZYMV/WMVII-resistant squash line (ZW-20) that was transformed with engineered CP genes. During virus challenges of ZW-20 squash plants under field conditions we observed two levels of virus resistance: resistant and moderately resistant. The two levels of resistance segregated in Mendelian fashion. Southern blot analysis revealed that each level of virus resistance strictly correlates with specific integrated T-DNAs that include CP genes. One set of T-DNA CP gene insertions (ß) is correlated with near immunity to both ZYMV and WMVII, and a second set of T-DNA CP gene insertions (α) is correlated with moderate resistances. It remains unclear why different T-DNA CP insertions into the same plant are correlated with different levels of virus resistance.

A831

HOST RANGE IN RELATION TO BASE SEQUENCE IN A VARIABLE REGION OF THE COAT PROTEIN OF SEVERAL STRAINS OF SUGARCANE MOSAIC VIRUS S. G. Jensen, J. S. Hall, and J. E. Partridge USDA-ARS Lincoln, NE and University of Nebraska, Lincoln, NE 68583

Five strains of sugarcane mosaic virus (SCMV) were tested for their infectivity to sugarcane. The variable region of the coat protein identified by Frenkel, et al (J. Gen. Virol. 72:237, 1991) was PCR cloned and sequenced. The results were compared with published data for SCMV (Australia) which infects sugarcane and SCMV-MD-B from Iowa which does not infect sugarcane. SCMV strains A, D, E, and Hawaii-4 from USA and Caja 3 from Peru all infected sugarcane. Within the variable region sequence similarities to SCMV- Aust. vary from 91% for SCMV-A to 53% for Hawaii-4. Sequence similarity to SCMV-MD-B varies from 93% for SCMV-E to 54% for SCMV-A. Hawaii-4 differs from SCMV-MD-B (86% similar) in a few base substitutions and a single amino acid deletion. Since Hawaii-4 infects sugarcane while SCMV-MD-B does not, it is questionable if this segment of the viral genome controls infectivity to sugarcane.

A832

CONSTRUCTION OF TOMATO SPOTTED WILT VIRUS (TSWV) REPLICONS. Kathryn E. Richmond and Thomas L. German. Department of Plant Pathology, University of Wisconsin, Madison, WI 53706.

TSWV is a membrane-bound, thrips-transmitted, tripartite negative stranded RNA virus with a host range of over five hundred plant species. Various constructs from the smallest viral genomic RNA (S RNA) have been made to determine the viral sequences necessary for replication. The 5′ and 3′ non-coding regions were obtained by PCR amplification of first strand cDNA from TSWV-infected plant tissue to form a replicon. We have generated constructs of both the replicon and the S RNA lacking the non-structural protein (NSs) gene with and without the β-glucuronidase (GUS) reporter gene. These constructs will be inoculated to TSWV-infected cells to monitor their replication.

A833

CLONING AND EXPRESSION OF THE C-TERMINUS OF THE TOMATO SPOTTED WILT VIRUS (TSWV) L PROTEIN. Scott T. Adkins[1], Tae-Jin Choi[1], John L. Sherwood[2], Diane E. Ullman[3] and Thomas L. German[1]. [1]Dept. of Plant Pathology, Univ. Wisconsin, Madison 53706. [2]Dept. of Plant Pathology, Oklahoma State Univ., Stillwater 74078. [3]Dept. of Entomology, Univ. of Hawaii, Honolulu 96822.

TSWV contains one negative sense (L) and two ambisense (M and S) genomic RNAs. The L RNA encodes the putative viral RNA-dependent RNA polymerase which has a predicted M, of 331.5 kDa. A 1.7 kb clone of the 5′ end of the L RNA encoding 493 amino acids at the C-terminus of the L protein was generated using a primer complementary to eight conserved, terminal bases. The clone was engineered in-frame into a pET (Novagen) bacterial expression vector. A fusion protein of the expected size (57 kDa) was produced and gel purified for polyclonal antibody production. Antibodies will be used for Western blot analysis and immunolocalization of L protein in vectors, plants and virions.

A834

CLONING AND EXPRESSION OF THE TOMATO SPOTTED WILT VIRUS (TSWV) NSM PROTEIN IN E. COLI. Tae-Jin Choi[1], Kelly D. Chenault[2], Diane E. Ullman[3], John L. Sherwood[2] and Thomas L. German[1]. [1]Department of Plant Pathology, University of Wisconsin, Madison, WI 53706; [2]Department of Plant Pathology, Oklahoma State University, Stillwater, OK 74078; [3]Department of Entomology, University of Hawaii, Honolulu, HI 96822.

TSWV is the type member of the *Tospovirus* genus, the only genus containing plant infecting viruses within the family Bunyaviridae. The genome

of TSWV encodes two nonstructural proteins, NSs and NSm which are encoded on the small and middle RNA, respectively. The NSs protein has been detected in the cells of thrips vectors and infected plants, but not in virions. We cloned the gene for the NSm protein of a Hawaiian isolate of TSWV and expressed it in *E. coli* to investigate the presence of the NSm protein in infected thrips and plants and its function during the virus infection processes. Antibody is being elicited in a rabbit for immunoblot analysis and *in situ* localization.

A835

VASCULAR CELL INFECTION BY THE U1 AND MASKED (M) STRAINS OF TOBACCO MOSAIC VIRUS IN *N. Tabacum* cv Xanthi nn. X.S. Ding, M.H. Shintaku and R.S. Nelson, The Noble Foundation, P.O. Box 2180, Ardmore, OK 73402.

The M strain of TMV can replicate and spread cell-to-cell similarly to the U1 strain in inoculated leaves of tobacco but is delayed in accumulation in upper uninoculated leaves. Using immunocytological techniques, we determined that the percentage of mesophyll and bundle sheath cells infected in chlorotic lesions at 3 and 4 days post inoculation (DPI) was similar for M-TMV and U1-TMV but in vascular cells the percentage was significantly less for M-TMV. In systemically infected tissue at 4 and 5 DPI, the percentage of mesophyll, bundle sheath and vascular cells infected was less for M-TMV versus U1-TMV. These results show that M-TMV has difficulty accumulating in the vascular cells of the inoculated leaf, and from previous results this phenotype maps to the open reading frame encoding the 126-kDa protein.

A836

Investigations into the molecular biology of potato leafroll luteovirus by means of agroinfection

Ulrich Commandeur and Robert Martin, Agriculture Canada, 6660 NW Marine Dr., Vancouver, B.C. CANADA V6T 1X2

A major obstacle in the investigation of the molecular biology of potato leafroll luteovirus (PLRV) is the absolute requirement of the aphid vector for virus transmisson. The use of *Agrobacterium tumefaciens* to introduce plant viral genomes into plants, via T-DNA transfer (agroinfection), was limited to pathogens with a circular genome. Recently the cDNA of another luteovirus, beet western yellows virus, was successfully introduced into plants by agroinfection. In this paper we describe the successful introduction of PLRV cDNA into host plants by agroinfection. The recombinant Ti-plasmids contained the cDNA corresponding to the complete PLRV genome flanked by the cauliflower mosaic virus 35S promoter and its corresponding transcription termination signal. Several different procedures were used for agroinfection. The influence of 5'-nonviral sequences on infectivity was tested. Viral mutants were created *in vitro*, and the effects of these mutations with respect to viral replication, movement, encapsidation and transmission will be discussed.

A837

SEQUENCE OF TOBACCO VEIN-BANDING MOSAIC VIRUS COAT PROTEIN GENE. B.B. Reddick and L.F. Habera. Dept. of Entomology and Plant Pathology, The University of Tennessee, Knoxville 37996.

Tobacco vein-banding mosaic potyvirus (TVBMV) is a new pathogen of solanaceous crops in North America. Reverse transcription of the 3' end of TVBMV RNA yielded a cDNA, which was cloned into Bluescript plasmid and subsequently sequenced by production of a nested deletion series set. The sequence of TVBMV-CP gene will be compared to other potyviruses of tobacco such as potato virus Y, tobacco etch virus and tobacco vein mottling virus.

A838

DEVELOPMENT OF TRANSGENIC TOBACCO EXPRESSING SMV-CP GENE. T.A. Thompson and B.B. Reddick. Dept. of Entomology and Plant Pathology, The University of Tennessee, Knoxville 37996.

The soybean mosaic virus coat protein (SMV-CP) gene was subcloned into a plasmid, pBI121, containing the CaMV 35S promoter. Agrobacterium tumefaciens, strain LBA4404, was transformed with the modified plasmid containing the SMV-CP gene and cocultivated with leaf discs of burley tobacco lines Va509, Ky14, MS14, and L8. Transgenic plants generated from tissue culture will be screened for the presence of the SMV-CP gene by Southern blot analysis and expression of the SMV-CP gene will be assayed with PAS-ELISA and Northern blot analysis. F_1 plants of lines containing the SMV-CP gene will be evaluated for coat protein-mediated protection against potato virus Y, tobacco etch virus, tobacco vein mottling virus, and tobacco vein banding mosaic virus.

A839

REDUCTION OF ALLELOPATHIC EFFECTS OF LEAVES BY COMPOSTING. R. D. Raabe. Department of Plant Pathology, University of California, Berkeley, CA 94720.

Failure of plants to grow under or near certain plants such as *Eucalyptus* spp. and black walnut is ascribed to as allelopathy. To determine if decomposed leaves of these plants have inhibitory effects, fallen leaves of *Eucalyptus* spp. and black walnut were composted using the rapid composting method. California live oak leaves also were used and in some experiments needles of Monterey pine were used. Seeds of carrot, cucumber, lettuce, and radish were planted in undiluted compost and in U.C. mix as a control. Carrot seeds were inhibited in all composts. Lettuce was inhibited in oak and black walnut compost. Plants were shorter in all composts as compared to U.C. mix. When the composts were diluted with U.C. mix to give mixes of 50% and 25% compost, germination of seeds was equally good except lettuce was inhibited by the 50% black walnut mix. Oven dry weights of lettuce and radish were less in all of the 50% compost mixes than in U.C. mix.

A840

SPIN OUT™ ROOT CONTROL COATING FOR CONTAINER-GROWN NURSERY PLANTS – A NEW USE FOR THE FUNGICIDE/BACTERICIDE COPPER HYDROXIDE. M. A. Crawford and E. H. Mester. Griffin Corp., P. O. Box 1847, Valdosta, GA 31603.

Container production of nursery plants has several advantages over field production including decreased production time, more plants per acre, extended year-round planting season, and decreased shipping costs. One problem associated with container production is the development of a restricted root system which results in circled, girdled, and matted roots where up to 80% of the root mass is located within 1 inch of the container wall. To correct root system deformities, standard practice is to cut the root ball, which can remove greater than 50% of the root system. This results in poor growth known as transplant shock. Spin Out™ root control coating is an effective means of controlling undesirable root growth without compromising top growth. When plant roots contact the Spin Out™ coating on the inner surface of containers, root elongation is inhibited and lateral root growth is stimulated. This results in the development of a more natural, fibrous root system. Spin Out™ represents the first novel use of copper hydroxide since the introduction of Kocide® fungicide/bactericide 30 years ago.

A841

PHYTOTOXICITY OF NICKEL-COATED GRAPHITE OBSCURANT FIBERS. M.C. Sadusky and M. Simini, GEO-CENTERS, Inc., Fort Washington, MD 20744.

Nickel-coated graphite (NCG) obscurant fibers, used for military training, were mixed into soils as either whole fibers or crushed fibers. *Cucumis sativus* L. and *Zea mays* L. were exposed to 0, 0.5, 1, 2, 3, and 4g whole NCG/kg soil, and 0, 2, 4g crushed NCG/kg soil in either a Sassafras sandy loam (field soil) or a potting soil (Baccto^{R}). Plant heights, and fresh and dry weights were taken after 14 days. Foliar injury was measured using a digital imaging analysis system (DIAS). Plant heights and weights were significantly ($p=0.05$) reduced as the concentrations of whole and crushed fibers were increased in both soil types. Foliar injury, estimated by DIAS, increased ($p=0.05$) as fiber concentrations increased. Growth reduction and injury were greater in the potting soil vs. field soil, and in cucumber vs. corn.