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Abstracts of Presentations

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A1

BIOLOGICAL ACTIVITIES OF VARIOUS FUMONISINS IN JIMSONWEED AND MAMMALIAN CELL CULTURES. H. K. Abbas,* W. C. A. Gelderblom, M. E. Cawood and W. T. Shier. SWSL, ARS, USDA, Stoneville, MS 38776, USA; MNR/MRC, South African Medical Research Council, Tygerberg 7505, South Africa; and Department of Medicinal Chemistry, Univ. of Minnesota, Minneapolis, MN 55455, USA.

Fumonisins A_1, A_2, B_1, B_2 and B_3 and their hydrolysis products, aminopentols AP_1 and AP_2 were tested for toxicity on plant and animal bioassay systems. The plant bioassay system employed jimsonweed (Datura stramonium L.) leaves and leaf discs and toxicity was detected as electrolyte leakage, photobleaching and quantitative of chlorophyll reduction. Cultured mammalian cell lines detected toxicity as inhibition of cell proliferation. Fumonisins B_1, B_2 and B_3 at 50 µg/ml or less were effective toxins in all plant and animal bioassay systems examined, except 3T3 mouse fibroblasts, whereas fumonisins A_1 and A_2 exhibited little or no activity. However, the hydrolytic degradation products AP_1 and AP_2 exhibited toxicity similar to or greater than B-series fumonisins in all test systems, including substantial toxicity to 3T3 mouse fibroblasts.

A2

PRODUCTION OF THE MYCOTOXIN FUMONISIN B1 BY *Alternaria alternata* f. sp. *lycopersici*. J-P. Chen, W.P. Xie and C.J. Mirocha. Department of Plant Pathology, Univ. of Minn. St. Paul, MN 55108.

Fumonisin B1 (FB1) is a mycotoxin produced by *Fusarium moniliforme* (*Fm*); it causes Equine Leukoencephalomalacia and is suspected of causing esophageal cancer in humans. AAL is a phytotoxin produced by *Alternaria alternata* f.sp. *lycopersici* (*Aa*). FB1 and AAL are structurally and functionally similar but not identical. To determine the biosynthetic derivatives of AAL and FB1, kinetics of toxin production by *Aa* and *Fm* were carried out in liquid cultures for 110 d. Ten ml of the culture medium was sampled, cleaned and analyzed by HPLC at various post-inoculation time intervals. AAL was detected on day 3 in the *Aa* culture, reached its maximum level on days 7-10. In addition to AAL, FB1 was found in the *Aa* culture at 7 d and stayed at a low level until 24 d after which it increased rapidly to the maximum level and became the major dominant compound in culture. Identities of FB1 and AAL were confirmed by TLC, fast atom bombardment MS, ion spray MS and parent-daughter MS/MS. This is the first report on production of FB1 by *Aa*.

A3

ISOLATION AND STRUCTURE IDENTIFICATION OF TWO DERIVATIVES OF THE MYCOTOXIN FUSAROCHROMANONE PRODUCED BY *Fusarium equiseti*. Weiping Xie, Chester J. Mirocha and Yechun Wen, Department of Plant Pathology, University of Minnesota, St. Paul, MN 55108.

Two derivatives of fusarochromanone (TDP-1) were isolated from a rice culture of *F. equiseti* (Alaska 2-2). The structures were identified as 2,2-dimethyl-5-amino-6-(2′-ene-4′-hydroxyl-butyryl)-4-chromone (TDP-Y) and 2,2-dimethyl-5-amino-6-(4′-hydroxyl-butyryl)-4-chromone (TDP-7B) by mass spectral fragmentation analysis. Molecular weights of TDP-Y and TDP-7B were determined as 275 amu and 277 amu, respectively, by electron impact (EI), fast atom bombardment (FAB) and chemical ionization (CI) mass spectra. High-resolution EI mass spectra yielded molecular formulae $C_{15}H_{17}NO_4$ (TDP-Y) and $C_{15}H_{19}NO_4$ (TDP-7B). Hydrogenation of TDP-Y yielded TDP-7B, confirmed by the EI mass spectrum. The structures were confirmed by proton nuclear magnetic resonance and infrared spectra. This work represents a portion of the study of the biosynthesis of TDP-1.

A4

LEUCONOSTOC MESENTEROIDES, A FIRST REPORT OF A COCCOID PLANT PATHOGENIC BACTERIUM OF TOMATO FRUIT. K.E. Conn, J.M. Ogawa, B.T. Manji, and J.E. Adaskaveg, Department of Plant Pathology, University of California, Davis, CA 95616.

A coccoid bacterium, *Leuconostoc mesenteroides* ssp. *mesenteroides*, is reported for the first time as the causal agent of a post-harvest decay of fresh market tomato fruit in California and Mexico. Diseased portions of tomato fruit were watery in texture but otherwise intact. Pathogenicity of the gram-positive, lactic acid-producing bacterium was demonstrated following Koch's postulates on mature-green, P19 tomato fruit harvested from a field test plot. Cell suspensions or decayed tomato fruit tissue were used for inoculum. Symptom development was discernible within 24 hr of inoculation as small, slightly sunken, firm, water-soaked lesions surrounding points of inoculation. After 5 days, lesion diameters increased to 28 mm at 20 C and to 47.7 mm at 33 C. Bacterial populations increased in wound inoculated tissue from an initial population of 2.4×10^4 cfu to a maximum of 2.6×10^8 cfu/0.1 gram tissue after 48 hr at 20C and to 8.1×10^6 cfu/0.1 gram tissue after 24 hr at 33C. Viable cell populations decreased after 5 days to 7.3×10^6 and 1.8×10^4 cfu/0.1 gram tissue for fruit incubated at 20C and 33C, respectively. This bacterium can be isolated from diseased fruit in the field or in post-harvest storage, and has been observed to cause sporadic post-harvest losses.

A5

BIOCONTROL OF BLUE MOLD OF APPLES UNDER COMMON AND CONTROLLED ATMOSPHERE STORAGE. J. P. Stack, T. S. Wright, and S. N. Jeffers, EcoScience Corporation, One Innovation Drive, Worcester, MA 01605, D. Cooley[1] and W. Brameledge[2], Depts. of Plant Pathology[1] and Plant & Soil Science[2], Univ of Massachusetts, Amherst 01003.

Four apple cultivars (McIntosh, Empire, Delicious, Golden Delicious) were harvested from the Univ. of Mass. Horticultural Research Center and used to evaluate the effectiveness of biocontrol organisms against blue mold, *Penicillium expansum*, Link ex. Thom., (Pe). Fruit (60 fruit/replicate, 5 replicates/treatment) of each cv were wounded and subjected to dip treatments (2 minutes) containing diphenylamine (DPA), Pe (2×10^4 conidia/ml), and either one of three biocontrol agents (ESI10, ESI12, ESI11) or thiabendizole formulated as Mertect 340 F, 1.25 ml/L. Nontreated controls (+DPA, -Pe) were also included. Fruit were placed under commercial cold storage (CS; 2° C air) for 4 months or commercial controlled atmosphere storage (CA; 3% O_2, 3% CO_2, 1° C) for 6 months. At intervals, fruit were removed from storage and evaluated for disease incidence (DI, percentage of wounds with lesions) and severity (DS, mean lesion diameter). For Empire after 10 wks CS, or after 19 wks CA, DI was 68% less and DS was 90% less in the ESI10 treatment than the thiabendazole or Pe treatments. Biocontrol of Pe was also achieved on Delicious and Golden Delicious. ESI12 also showed biocontrol potential.

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A6

EFFICACY OF IPRODIONE-WAX/OIL MIXTURES FOR CONTROL OF POST-HARVEST DECAY OF FRUIT CAUSED BY *RHIZOPUS* AND *ALTERNARIA* SPP. J.M. Ogawa, J.E. Adaskaveg, and K.E. Conn, Plant Pathology, UC Davis, CA 95616

A non-fungicidal, water-soluble, food-grade wax/oil mixture significantly enhanced the efficacy of iprodione for control of post-harvest decays caused by *Rhizopus* and *Alternaria* spp. On Fairtime peach or P19 tomato, iprodione (1.2, 2.4, and 4.8 g/L) or DCNA (2.4 and 4.8 g/L) in water alone or in 1 part wax/oil:5 parts water were compared to fruit treated with 1 wax/oil:5 water and non-treated fruit. Fruit were treated, dried, wounded, inoculated with a spore suspension (20,000/ml) of *Rhizopus stolonifer*, and incubated 4 days at 20C, >95% RH. Iprodione and DCNA significantly reduced both incidence of decay and lesion diameter when mixed with wax/oil compared to the two controls. Moreover, iprodione-wax/oil mixtures provided control similar to that of DCNA-wax/oil mixtures. On tomato fruit, iprodione and iprodione-wax/oil mixtures were compared for inhibition of post-harvest development of *Alternaria alternata*. Fruit were harvested, treated with iprodione (4.8 g/L), iprodione-wax/oil, or wax/oil mixtures, and incubated at 25C, >95% RH. After 7 days, iprodione-wax/oil mixtures significantly reduced development of *A. alternata* compared to other treatments, whereas no difference was observed between wax/oil or fungicide-alone treatments. Mixtures of iprodione-wax/oil may be an alternative to DCNA.

A7

EFFECT OF NITROGEN FERTILIZATION ON BROWN ROT (MONILINIA FRUCTICOLA) SUSCEPTIBILITY IN NECTARINES. Themis J. Michailides[1], R. Scott Johnson[2], and D. P. Morgan[1], University of California, [1]Dept. of Plant Pathology, Berkeley and [2]Dept. of Pomology, Davis/Kearney Agric. Center, Parlier, CA 93648.

In the summer of 1990, preliminary experiments indicated that 12.5% of Flavortop nectarines from trees fertilized with 360 kg NH_4NO_3/ha had natural infections by M. fructicola, as compared to only 4.2% and 0% of fruit from trees fertilized with 280 and 112-195 kg/ha, respectively. Similarly, 76-90% of Fantasia nectarines collected from trees that had been fertilized with 280-360 kg NH_4NO_3/ha rates and spray-inoculated (without wounding) with a conidial suspension of M. fructicola were infected and developed 10 lesions per fruit, whereas 62-67% of fruit from trees fertilized with 112-195 kg NH_4NO_3/ha were infected with only 2 to 3 lesions per fruit. Similar results were obtained with inoculated Flavortop nectarines, although incidence and severity of disease were lower than that of Fantasia. After wound inoculation, however, Fantasia fruit from trees fertilized with different levels of NH_4NO_3 were equally susceptible to the fungus. The differences observed in cuticle thickness of fruit fertilized with different levels of N may partially explain the differences in fruit susceptibility to the disease.

A8

EFFICACY OF CHLORINE FOR CONTROL OF POSTHARVEST PATHOGENS OF TOMATOES IN PACKINGHOUSE DUMP TANKS IS INFLUENCED BY WATER TEMPERATURE. J.A. Bartz, Cynthia G. Eayre, A.U.O. Sabaa-Srur, J.K. Brecht, and S.A. Sargent, Plant Pathology and Horticultural Sciences Depts., University of Florida, Gainesville, 32611.

About 50 ppm free chlorine (pH 7.0) prevented most wounded tomato fruit floating in 25°C water from becoming infected after injection of spores of *Rhizopus stolonifer* or *Geotrichum candidum* into the water. Disease incidence in fruit stored 4 days at 20°C averaged 53% in the control and 7% with 50 ppm free chlorine for Rhizopus rot and 37 and 3%, respectively, for sour rot. In contrast, with water at 40°C 10-20 ppm free chlorine led to similar decay reductions. Aqueous cell suspensions of *Erwinia carotovora* (soft rot) were nearly 100-fold more sensitive to free chlorine than were the spore suspensions. Free chlorine levels much less than the recommended 100-150 ppm are adequate to protect tomato fruit in dump tanks and flumes from infection by postharvest pathogens.

A9

INFECTION RATES OF ALTERNARIA ALTERNATA AND PHYTOPHTHORA CAPSICI IN RELATION TO CHILE PEPPER FRUIT MATURITY. C.L. Biles and M.M. Wall, Department of Entomology, Plant Pathology and Weed Science and Department of Agronomy and Horticulture, New Mexico State University, Las Cruces, NM 88003.

New Mexican chile peppers are susceptible to several distinct fruit rots, 2 of which are caused by *Alternaria alternata* and *Phytophthora capsici*. Experiments were conducted to determine the relationship between infection rates of *A. alternata* and *P. capsici* and chile pepper fruit maturity. Fruit were inoculated on the day of harvest with a conidial suspension of *A. alternata* or a zoospore suspension of *P. capsici*. Infection rate of *A. alternata* increased as peppers matured and ripened (0.22 mm/day in mature green and 1.86 mm/day in non-dehydrated red fruit) with the largest lesions occurring on fruit harvested 60 days after flowering. Conversely, the infection rate of *P. capsici* decreased (14.06 mm/day in mature green and 10.72 in non-dehydrated red fruit) as the pepper fruit matured. Young green peppers were most susceptible. Further experiments showed that Alternaria fruit rot severity correlated with total and reducing sugars in the pepper fruit. Alternaria lesion diameters remained small until 60 days after flowering when total and reducing sugars increased 60% and 78%, respectively. The decreased severity of fruit rot caused by *P. capsici* appeared to correlate with increased cuticle thickness.

A10

SOIL POPULATIONS OF ASPERGILLUS FLAVUS GROUP FUNGI IN AGRICULTURAL FIELDS IN ALABAMA, ARIZONA, LOUISIANA, AND MISSISSIPPI. P. J. Cotty, USDA, ARS, Southern Regional Research Center, P.O. Box 19687, New Orleans, LA 70179.

The magnitude and composition of populations of Aspergillus flavus group fungi in agricultural soils from 8 cotton growing areas distributed over 4 states were studied. Over 1,200 isolates from 31 fields were classified by species, toxin producing ability, and strain type. Significant differences in the incidences of species and the S strain of A. flavus were detected among areas, states, and regions. Differences in average toxin producing ability among populations resident in individual fields and areas were also detected. The S strain of A. flavus was detected in all states studied, extending the distribution of this strain throughout several major cotton producing areas. Aspergillus parasiticus occurred infrequently and was not detected in Arizona. The results suggest regional adaptation of A. flavus group populations and demonstrate the existence of naturally established populations of A. flavus with different abilities to produce aflatoxins.

A12

BIOLOGICAL CONTROL OF POSTHARVEST DISEASES OF APPLE. M.L. Gullino, C. Aloi, D. Benzi and A. Garibaldi, DI.VA.P.R.A. - Patologia vegetale, Via Giuria 15, 10126 Torino, Italy.

Two yeasts, screened among hundreds of microrganisms isolated from unsprayed apples, showed a good antagonistic activity against Botrytis and Penicillium rot of apple. The selected strains, classified as Candida sp. and Trichosporon sp., significantly reduced rots caused by both pathogens when applied by dipping fruit in a cell suspension (10^6 cells/ml), both at room temperature and at 4 °C. An interval of at least 12 hours was necessary between treatment with the biocontrol agents and artificial inoculation with the pathogens in order to achieve good control. Some biological characteristics of the two biocontrol candidates and potential for their application under practical conditions will be presented and discussed.

A13

CALCIUM AND CALMODULIN ARE INVOLVED IN APPRESSORIUM DEVELOPMENT OF COLLETOTRICHUM TRIFOLII. V. Warwar and M. B. Dickman. Department of Plant Pathology, University of Nebraska, Lincoln, NE 68583-0722.

We are interested in the developmental sequence of events which culminate in appressorium (infection structure) development, in C. trifolii, causal agent of alfalfa anthracnose. Conditions have been established for induction of appressoria, although races of this fungus consistently differ in the temporal sequence of events. Our initial studies have focused on the relative contributions of calcium and calmodulin from spore germination through appressorial maturation. A range of structurally diverse agents including calcium ionophores, calcium channel blockers, and calmodulin antagonists were tested as effectors for these processes. Data suggests that calcium is important in the overall differentiation process and that calmodulin is essential for the development of appressoria.

A22

MELANIN AND PERITHECIAL DEVELOPMENT IN OPHIOSTOMA PILIFERUM. W. C. Zimmerman, R. A. Blanchette, T. A. Burnes, R. L. Farrell. Repligen Sandoz Research Corportation, Lexington Mass. 02173; University of Minnesota, St. Paul. 55108.

Colorless strains of Ophiostoma piliferum are currently being used with success in large scale industrial applications as a pretreatment of wood chips to remove pitch before mechanical pulping. It is therefore of practical interest to determine what effects melanin deficiency may have on growth, development, dissemination and survival of this fungus. Melanin deficient strains, devoid of pigmentation within hyphae, were unable to produce perithecia when paired with other colorless isolates of the opposite mating type. Melanization and perithecial development were restored on medium supplemented with an extract of spent culture medium derived from a brown variant of O. piliferum. The extract was analyzed by HPLC and shown to contain scytalone, an intermediate of the DHN melanin pathway. Pure scytalone also restored pigmentation and perithecial development. Scanning electron microscopy was used to evaluate the morphology of perithecial development of melanin-deficient and melanin - induced strains of O. piliferum. The role of melanin in perithecial development and the implications of melanin deficiency for dissemination and survival of this fungus will be discussed.

A23

HOST STRESS AND RESISTANCE OF RED PINE TO STAINING FUNGI (*Leptographium spp.*). K.D. Klepzig, E.B. Smalley and K.F. Raffa. Univ. of Wisconsin-Madison, Madison, WI 53706

Red pine seedlings were grown in the greenhouse under "normal" and "shaded" (40% of "normal" light intensity) light conditions for approximately 8 weeks, until growth reduction was apparent in shaded trees (as measured by new shoot and needle length). Trees were then wounded or inoculated with *L. terebrantis*. Extent of colonization within each seedling was measured. Resinous lesions were 20-47% longer in shade stressed than in unstressed trees. This experiment was repeated in the UW biotron facility using *L. procerum, L. wageneri* and *L. terebrantis*. In separate experiments, both phenolic compounds and monoterpene vapors from red pine were found to inhibit growth of *Leptographium spp.* It is proposed that stresses on red pine result in decreased concentrations of defensive compounds and increased susceptibility to pathogens.

A24

EVIDENCE FOR OUTCROSSING IN *GIBBERELLA ZEAE* (*FUSARIUM GRAMINEARUM*). R. L. Bowden and J. F. Leslie, Department of Plant Pathology, Kansas State University, Manhattan, KS 66506.

Fusarium graminearum Group II from wheat head scab is homothallic, yet possesses high diversity in vegetative compatibility groups (VCGs). We hypothesize that this diversity may be maintained by sexual outcrossing. Complementary nitrate assimilation mutants (*nit*1 or NitM) from different VCGs were paired for sexual crosses on carrot agar plates. Ascospores from individual perithecia were plated onto a medium containing nitrate as the sole nitrogen source. Perithecia that produced approximately 25% wild type colonies were considered to be heterozygous. The frequency of heterozygous perithecia at the colony interface ranged from 25 to 63% in different pairings. Recombinant perithecia contained progeny with *nit*1, NitM, and wild type phenotypes in the ratio of 1:2:1. The double *nit* mutant class was indistinguishable from the NitM single mutant class. In one cross, progeny with four different VCG phenotypes were obtained: two parental and two recombinant. This result suggests that the two parental isolates differed at two *vic* loci. Based on these data, we conclude that sexual recombination in *G. zeae* can be induced readily under laboratory conditions and is probably important in the life cycle of this fungus.

A25

PLANT INDUCIBLE NUCLEASE FROM *FUSARIUM SOLANI* AND OTHER PLANT PATHOGENIC FUNGI SIGNAL HOST RESPONSES. Lee A. Hadwiger, David Christian, Dave Gerhold, Becky Allaire. Department of Plant Pathology, Washington State University, Pullman, WA, 99164-6430.

F. solani, F. oxysporum strains isolated from plants, humans, and crustaceans produce and release nuclease following contact with pea endocarp tissue. The 22 K nuclease from *F. solani* f. sp. *phaseoli* (Fsph) causes single-strand breaks in double-stranded DNA and its activity is increased 400-fold by 10 mM Ca^{++}. *Fusarium* nuclease activity is detectable in plant nuclei 2 h post inoculation. Pure nuclease applied to pea endocarp at high conc. prior to inoculum renders tissue susceptible whereas pure nuclease applied at low conc. induces disease resistance response genes (DRRGs) 206, 49 and β-glucanase. The DRRG 49 product accumulates in nuclei of pea cells in and adjacent to those challenged by Fsph. We propose that the *Fusarium* nuclease functions as a signal in activating defense genes by altering host DNA.

A27

DEMONSTRATION THAT MUTATIONS IN CODONS 198 AND 200 OF THE BETA-TUBULIN GENE CONFER RESISTANCE TO BENOMYL IN FUNGI. H. Koenraadt and A. L. Jones, Dept. of Botany and Plant Pathology and the Pesticide Research Center, Michigan State University, East Lansing, MI 48824.

Sequence analysis and allele-specific oligonucleotide analysis revealed that resistance to benomyl in field strains of Venturia inaequalis and other plant pathogenic fungi are associated with mutations in codons 198 or 200 of the beta-tubulin gene. Transformation experiments were initiated to investigate whether mutations in these codons were directly responsible for resistance to benomyl. Transformation of V. inaequalis was hampered by an inability to produce protoplasts. Site-directed mutagenesis was performed on pBT6, a plasmid that contains the beta-tubulin gene of Neurospora crassa and confers resistance to benomyl due to a mutation in codon 167. Codon 167 was altered to the original wild-type codon, and codons 198 or 200 were altered to obtain the mutations observed in V. inaequalis. Transformants of N. crassa were recovered that were resistant to benomyl. The transformation experiments show that amino acids 198 and 200 of beta-tubulin play a key role in resistance to benomyl and sensitivity to structurally related N-phenylcarbamates.

A28

USE OF REPETITIVE SEQUENCES FOR THE IDENTIFICATION OF SPECIES-SPECIFIC DNA FRAGMENTS IN *FUSARIUM OXYSPORUM*. F.C. Leung, D.P. Chandler, X.Z. Shen, A.E. Jarrell, and R.J. Fellows. Pacific Northwest Laboratory, P.O. Box 999, Richland, WA 99352.

Genomic repetitive sequences (low C_{0t}-DNA) from *Fusarium oxysporum conglutinans* (ATCC #16601) and *F. o. lycopersici* (ATCC #16322) have been isolated and used as probes for the identification of species-specific DNA fragments. The use of low C_{0t}-DNA from these *formae speciales* (f. sp.) as probes in the DNA assay permitted the identification of distinctive banding patterns among *F. o. conglutinans, F. o. lycopersici, F. o. pisi* (ATCC# 22554), and *F. o. phaseoli* (ATCC# 18131). Autoradiographic results with *F. o. conglutinans* low C_{0t}-DNA demonstrated specific DNA fragments in the *F. o. conglutinans* lanes that are not shared with *F. o. lycopersici, F. o. pisi*, or *F. o. phaseoli*. These may therefore be considered as f. sp.-specific fragments. Species specific DNA fragments were also obtained when low C_{0t}-DNA from *F. o. lycopersici* was used as a probe. These results demonstrated that the DNA fingerprinting assay can be used for strain identification among *Fusarium oxysporum* species and that the use of low C_{0t} DNA as a probe allows the determination of f.sp.-specific DNA fragments. The biological significance of these f. sp.-specific DNA fragments to the host-specificity of these organisms remains to be determined.

A29

RFLPS PROVIDE EVIDENCE FOR SUBSTANTIAL GENE FLOW BETWEEN CALIFORNIA AND OREGON POPULATIONS OF *SEPTORIA TRITICI*. J. M. Boeger and B. A. McDonald. Department of Plant Pathology and Microbiology, Texas A&M University, College Station, Texas 77843-2132.

Gene flow can have a significant impact on the genetic structure of pathogen populations. The genetic structure of an Oregon population of *Septoria tritici* was compared to that of a California population approximately 750 km distant. The same mitochondrial DNA haplotype predominated in both populations. The frequency and distribution of alleles at ten anonymous, selectively neutral, nuclear RFLP loci were compared for the two populations. The genetic structures of the two populations were nearly identical. The same most common alleles were present in approximately the same frequencies in both populations. Many private alleles were present in the Oregon population, probably as a result of the larger sample size. G_{st} statistics were used to estimate the average effective number of migration events between the two populations. These results indicate that there is a substantial amount of gene flow between the two populations, suggesting that *S. tritici* has the potential for long-distance dispersal. One possible mechanism for this dispersal would be via the ascospores of the teleomorph *Mycosphaerella graminicola*.

A30

GENETIC ANALYSIS AND RAPID MAPPING OF A SPORULATION MUTATION IN *MAGNAPORTHE GRISEA*. Hei Leung and Zhixin Shi. Dept. Plant Pathology, Washington State Univ., Pullman WA 99164

A developmental mutation causing abnormal conidium production (Con$^-$) was found in *Magnaporthe grisea*. The Con$^-$ phenotype was a suppression of normal conidium production at the tip of a conidiophore. Instead of producing a cluster of 4-5 conidia sympodially borne on a conidiophore, the mutant produced an aerial hypha bearing a terminal, elongated conidium (4 x 50 μm). The growth rate and sporulation of the Con$^-$ mutant on culture media were 72% and 2.3% respectively of the wild type. Random spore and tetrad analysis showed that the Con$^-$ phenotype was controlled by a single gene (*Con1*). Con$^-$ mutation was epistatic to a spore morphology mutation (Smo$^-$) previously described (Hamer et al. 1989. Genetics 122:351). Con$^-$ segregants were non-pathogenic to rice suggesting the Con$^-$ mutation also affected pathogenesis. As a first step towards physical isolation of the *Con1* gene, we used RAPD-bulked segregant analysis to generate DNA markers flanking the *Con1* locus. A linked marker 7 cM from the *Con1* locus was obtained.

A31

TRANSFORMATION OF POTATO WITH PEA DISEASE RESISTANCE RESPONSE GENES (DRRGs) 49 CAN ENHANCE DISEASE RESISTANCE. Ming-Mei Chang, L.A. Hadwiger, David Culley. Washington State University, Department of Plant Pathology, Pullman, WA, 99163.

Pea tissue inhibits many of the organisms that are pathogens of potato. Gene 49, one of the pea's major DRRGs, is active in both race-specific and non-host disease resistance response to foreign plant pathogens and produces a 17 Kd protein of unknown function. Potato lines transformed with the structural pea gene 49 driven by the 35S CaMV promoter expressed markedly improved resistance to *Verticillium dahliae* and *Erysiphe cichoracearum* in laboratory tests. In peas, gene 49 is located on chromosome 6, and is a "slave" gene whose expression intensity is dependent on a conventional Mendelian single gene, disease resistance trait controlling resistance to *F. oxysporum* f. *pisi* which is located on chromosome 4. Such slave genes may perform the resistance regulated by the Mendelian trait.

A32

GENETIC VARIABILITY AMONG ISOLATES OF *FUSARIUM* SPP. FROM TOMATO. Q.Wang, C.A.Lévesque, Z.K.Punja, and J.E.Rahe, Dept. of Biological Sciences, Simon Fraser University, Burnaby, British Columbia, Canada V5A 1S6

The extent of genetic variability among *Fusarium* spp., including *F. oxysporum f.sp. lycopersici* (FOL), *F. oxysporum f.sp. radicis-lycopersici* (FORL), and *F. oxysporum* (FO) originating from BC, Ontario, and Alberta, were studied using the polymerase chain reaction (PCR) and DNA restriction fragment length polymorphism (RFLP). From sequences of the 3' end of the 28S and the 5' end of the 18S ribosomal genes of some plant and all fungal species available in the EMBL database, two primers were designed to amplify the rDNA intergenic spacer (IGS) with PCR. The PCR-amplified rDNA products (2.8 kb) from each isolate of *Fusarium* spp. were digested with different restriction enzymes and RFLP's were observed. From these polymorphisms, FOL, FORL, and FO isolates were distinguishable from each other when the PCR-amplified IGS was digested with HinfI or HaeIII. With other restriction enzymes (AluI, DdeI, TaqI, HpaII, EcoRI, HindIII), FOL and FORL were not differentiated from each other, but were different from FO. No differentiation of FOL and FORL was achieved by digestion of the PCR-amplified rDNA internal transcribed spacers or the mitochondrial rDNA genes with the enzymes AluI, DdeI, or TaqI.

A34

GENETIC SIMILARITY WITHIN AND AMONG *ACREMONIUM* SPECIES. M. Q. Sevilla and D. D. Pope. Department of Plant Pathology, University of Georgia, Athens, Georgia, 30602.

The genetic similarity of 34 isolates representing five different *Acremonium* and one *Epichloe* species was investigated using random amplified polymorphic DNAs (RAPDs) generated by the polymerase chain reaction (PCR). Seventeen putatively identical *A. coenophialum* isolates from a single field in Georgia were genetically similar but not identical. *A. coenophialum* isolates from diverse geographic locations showed greater genetic variation than the Georgia isolates. Following cladistic analysis, *A. coenophialum* isolates clustered tightly and were readily distinguished from the other *Acremonium* and *Epichloe* species. In a separate study, *in vitro* ergovaline production in *A. coenophialum* was measured. Isolates E11 and E2 from Georgia produced high and low levels of ergovaline, respectively. Their different RAPD patterns suggest that ergovaline production may be under genetic control.

A36

ISOLATION AND CHARACTERIZATION OF MATING-RELATED cDNA CLONES FROM COLLETOTRICHUM GLOEOSPORIOIDES. C.R. Cisar and D.O. TeBeest. Department of Plant Pathology, University of Arkansas, Fayetteville, AR 72701.

Colletotrichum gloeosporioides isolates from northern jointvetch and pecan are sexually compatible. To study the genes involved in mating a subtracted cDNA library was prepared using RNA isolated from a mating culture and RNA isolated from each of the parents. The library was screened using radiolabeled cDNA probes prepared from parental and mating cultures (differential screening). Those cDNA clones corresponding to genes expressed only in a mating culture (i.e mating-specific), or expressed in only one of the parental cultures (i.e. parent-specific), or expressed by both parents but not in a mating culture are being characterized further.

A38

XANTHOMONAD DIVERSITY ON GERANIUM LEAVES AS DETERMINED BY MONOCLONAL ANTIBODIES (MABS), CARBON SOURCE UTILIZATION PATTERNS, FATTY ACID ANALYSES (FAME) AND PATHOGENICITY. J. B. Jones, A.R. Chase, N. C. Hodge, and R. E. Stall. Univ. of Florida, Gainesville.

Xanthomonads were isolated from geraniums grown in diverse geographic areas and compared by various tests. Seven of the 69 strains did not react with a *Xanthomonas campestris* pv. *pelargonii* (Xcp) specific MAb. With FAME a single homogeneous group formed including all but six of the seven strains which did not react with the MAb. Carbon utilization patterns on GN microplates (Biolog, Inc., Hayward, CA) grouped the Xcp strains fairly closely; however, strains which were outliers by FAME and serology were also outliers with Biolog. Select strains which were outliers in Biolog, FAME and MAb tests were confirmed by pathogenicity and/or population studies in geranium leaves not to be Xcp.

A39

CHARACTERIZATION OF *XANTHOMONAS CAMPESTRIS* STRAINS FROM ARALIACEAE. A. R. Chase, N. C. Hodge, R. E. Stall, and J. B. Jones. Dept. of Pl. Pathology. University of Florida, Gainesville, FL 32611.

Strains of *Xanthomonas campestris* were collected from araliaceous hosts: *Hedera helix* (35), *Polyscias* spp. (22), *Brassaia actinophylla* (5), *Dizygotheca elegatissima* (1), *Fatshedera* sp. (1) and *Schefflera arboricola* (2). Strains from each host caused similar symptoms on *H. helix, Polyscias, S. arboricola*, and *B. actinophylla*. Highest bacterial population densities occurred when plants were injected with strains isolated from that plant. Activity on crystal violet pectate medium was high for all strains from *S. arboricola, D. elegantissima, B. actinophylla* and *Fatshedera* but low for all strains from *H. helix* and *Polyscias*. Cellular fatty acids (FA) and carbon source utilization (CSU) profiles segregated *H. helix* and *Polyscias* strains into two distinct, homogeneous groups. Other strains were placed in a third heterogeneous group. Strains from *H. helix* should be remain *X. campestris* pv. *hederae* while *Polyscias* strains may belong to a separate pathovar and those from other araliaceous hosts may belong to other pathovars.

A40

INDUCTION OF NECROGENESIS BY *Agrobacterium tumefaciens* ON GRAPE STEM EXPLANTS. X. Pu and R.N. Goodman. Department of Plant Pathology, University of Missouri, Columbia, MO 65211

Agrobacterium tumefaciens A281 has been reported to be supervirulent on many plant species. However, it induces a necrogenic rather than tumorigenic response on several grape cvs. The necrosis observed in this *A. tumefaciens*-grape system is cultivar specific, and Ti plasmid related. The T-DNA of A281 is required; however, the *vir* genes are not critical for necrogenesis. Exogenous auxin (NAA) increased and kinetin decreased the necrogenesis. Suppression of necrosis by abscisic acid was apparent one week after inoculation with A281. GUS activity in transformed cells can be detected 48 hours after inoculation. Hence, transformation preceded necrogenesis suggesting that necrogenesis induced by A281 was a result of an aborted transformation. Reactivation of tumorigenesis from the necrogenic state was also observed. It appears that plant hormones regulate the induction of necrogenesis that limits tumorigenesis.

A41

ECOTYPE CONVERSION OF PSEUDOMONAS CEPACIA. C. F. Gonzalez and V. A. Valadez. Department of Plant Pathology and Microbiology, Texas A&M University, College Station, TX 77843

The phytopathogen Pseudomonas cepacia produces endopolygalacturonase (Peh), whereas strains of soil and clinical origin do not. The enzyme is considered a pathogenicity factor because isolates that do not produce Peh are unable to increase in population size and cause disease in plants. Mobilization of the 200 kb Peh-encoding plasmid (pPEC321) from P. cepacia strain PCO253 into cured derivatives restored pathogenicity. Transfer of pPEC321 into non-phytopathogenic strains of P. cepacia of soil and clinical orgin enabled them to grow in plants and cause disease symptoms. A cloned 5.3 kb SstI fragment of pPEC320 that contains the PehA gene restored Peh activity to cured derivatives and was expressed in non-phytopathogenic strains of P. cepacia. The cloned pehA gene was mobilized into P. aeruginosa and studies showed that the gene was expressed. However, Peh accumulated in the periplasm indicating lack of export. The pehA locus is being further defined by restriction enzyme and deletion analysis.

A42

OPTIMIZATION OF CORONATINE BIOSYNTHESIS BY PSEUDOMONAS SYRINGAE PV. GLYCINEA IN A DEFINED MINIMAL MEDIUM. D.A. Palmer and C.L. Bender. Dept. of Plant Pathology, Oklahoma State University, Stillwater, OK 74078-9947

Pseudomonas syringae pv. glycinea (PG4180), which causes bacterial blight of soybeans, produces the polyketide phytotoxin coronatine. The effects of environmental and nutritional factors on coronatine production by PG4180 cultures were examined by varying the components of a defined minimal medium (HS). The quantity of coronatine synthesized by PG4180 remained constant or was reduced when the carbon source, nutrient levels (glucose, NH_4Cl, phosphate, Mg, SO_4), amino acid supplements, pH, or osmolarity of the basal medium was varied. The addition of millimolar levels of KNO_3 or micromolar levels of $FeCl_3$ significantly enhanced coronatine production. The yield of coronatine was maximized after a 6-day incubation at 18°C at 280 RPM. Based on these results, we have designed a modified minimal medium which results in maximal yields of coronatine *in vitro*.

A43

GLUTATHIONE S-TRANSFERASE ACTIVITY IN RHIZOSPHERE-COMPETENT BACTERIA. R. M. Zablotowicz, R. E. Hoagland, E. A. Milus*, and C. R. Rothrock*. USDA-ARS, SWSL, Stoneville, MS 38776; *Univ. Arkansas, Dept. Plant Path., Fayetteville, AR 72701.

Glutathione S-transferases (GST) are a family of enzymes catalyzing glutathione conjugation with electrophillic substrates, as a major mechanism of detoxification. A collection of rhizosphere-competent bacteria were assayed for GST activity using chlorodinitrobenzene (CDNB) as substrate. Approximately 70% of these strains (both Gram-positive and Gram-negative bacteria) exhibited CDNB-GST activity ranging from 0.5 to 3.0 nmoles/mg protein/min. Most Gram-negative strains tested rapidly metabolized the CDNB-GST conjugate to a yellow product (A_{405}), thus GST activity based upon CDNB may be underestimated. Strains of Pseudomonas (P. cepacia, P. fluorescens, and P. putida) with GST-alachlor activity have also been identified. The relationship between GST activity and herbicide biotransformation by rhizosphere bacteria is being investigated.

A44

POPULATION DYNAMICS OF A TN5-INDUCED NON-LESION FORMING MUTANT OF *P. SYRINGAE* PV. *SYRINGAE* ON BEAN PLANTS IN THE FIELD. S.S. Hirano, D.K. Willis, and C.D. Upper. Dept. of Plant Pathology and USDA/ARS Plant Disease Resistance Unit, Univ. of Wisconsin, Madison, WI 53706.

The population dynamics of a mutant of *P. syringae* pv. *syringae* that has been rendered unable to cause bacterial brown spot by insertion of Tn5 into *lemA* (Willis *et al.*, MPMI 3:149-156, 1990) was examined relative to its pathogenic parent on bean plants in the field. Strains were applied alone or in combination to replicated field plots. When the strains were applied individually, population sizes of the parent were 10- to 100-fold larger than the mutant (e.g., 5.31 vs. 3.71 log CFU/leaflet) at 1-2 wk post-inoculation. However, when applied together, the population dynamics of the mutant and parent were not significantly different and mimicked those exhibited by the mutant applied alone. A mutation in a regulatory gene that is required for lesion formation has resulted in a mutant that appears to affect the population dynamics of the parent; the parent, however, does not appear to affect the mutant.

A45

Effect of leaf age and position on bacterial colonization of *Chicorium endivia*. M.A. Jacques, C.E. Morris and L.L. Kinkel, INRA, Station de Pathologie Végétale, BP 94, 84143 Montfavet, France and Dpt of Plt Pathology, U. of MN, St Paul, MN 55108.

Packaged sachets of ready-to-use (RTU) scarole (*Chicorium endivia*) are composed of leaves from the heart of the plant because storage decay is thought to occur most frequently for outer leaves. This work quantified the effect of leaf age or position on colonization of scarole by bacteria suspected to decay leaves in RTU sachets. Newly emerged scarole leaves were marked at 6 times (6 age classes) during growth of a fall crop in southern France. Between 11 September and 10 December, populations of total, fluorescent, and pectolytic bacteria were quantified biweekly on each of 30 leaves from every age class present on each sampling date, and soft rot and necrosis were evaluated in storage of experimentally prepared sachets in which all leaf age classes were separated. Leaf age and position significantly influenced both bacterial populations and leaf decay. Outer (older) leaves supported significantly greater bacterial populations and had higher levels of soft rot and necrosis than young leaves on all sampling dates. On newly emergent leaves, populations of fluorescent and pectolytic bacteria, but not total bacteria, increased significantly with date of leaf emergence.

A46

ISOZYME COMPARISONS OF STRAINS OF XANTHOMONAS ORYZAE ORYZAE FROM DIFFERENT AREAS OF THE WORLD. M. R. Bonde, G. L. Peterson, and D. E. Griffin. USDA/ARS, Foreign Disease-Weed Science Research Unit, Bldg. 1301, Ft. Detrick, Frederick, MD 21702.

Fifty-eight strains of *X. o. oryzae*, including isolates from Australia (2), Burma (1), Columbia (1), India (10), People's Republic of China (12), the Philippines (22), and Texas, USA (10), were compared by isozyme analysis in horizontal starch gels. Fourteen strains of *X. campestris oryzicola*, including strains from the Philippines (12) and People's Republic of China (2), were included for comparison. Seventeen enzymes, with a total of 22 putative isozyme loci, were resolved. No variation was detected in any of 22 strains of *X. o. oryzae* from the Philippines or 10 from India. Slight variation was detected (CS = 0.96, maximum CS possible = 1.00) in the 10 Texas strains tested. The 12 strains from the PRC separated into two groups (10 and 2, respectively) each with no variation. Strains from Australia, Burma, and Columbia were similar to the Philippine strains. Huge differences, however, were observed when comparing either geographic groups of *X. o. oryzae* or *X. o. oryzae with X. c. oryzicola*.

A48

DETECTION OF *CLAVIBACTER MICHIGANENSIS* SUBSP. *SEPEDONICUS* IN FIELD SAMPLES VIA DNA/DNA HYBRIDIZATION. J. Drennan, A. Westra, L. Delserone, *N. Gudmestad, *A. Oleson, A. Collmer, and S. Slack, Cornell University, Ithaca, NY 14853 and *North Dakota State University, Fargo, ND 58105

Clavibacter michiganensis subsp. *sepedonicus* (*Cms*), the cause of bacterial ring rot, was detected in field-grown potatoes using a 1.078 kb repeated *Cms* sequence (Mogen et al., Phytopath. 80: 90-96) as a probe in DNA hybridizations. The susceptible cv. Russet Burbank and tolerant cv. Belrus were inoculated with 0, 10^2, or 10^9 cfu of *Cms* strains SS43 or SS13 and sampled at 30, 60, and 90 days after planting (DAP). Stem and petiole samples were processed by centrifuging stem sections, grinding frozen tissues, or directly blotting cut sections on membranes. Generally, detection rates increased as the season progressed and were higher with Russet Burbank and the more aggressive strain SS43. The best detection rate was 94.7% for directly blotted, underground stems of Russet Burbank (10^9 cfu) at 90 DAP, a rate equal to ELISA. Although overall detection rates were higher for ELISA (18.4% ELISA vs. 11.6% direct blot), a high rate of false positives (53.9%) occurred with petiole tissues at 90 DAP using ELISA, while none occurred with DNA hybridizations. Studies utilizing the polymerase chain reaction for greater sensitivity of detection are currently underway.

A49

Natural colonization of pear flower tissues by bacteria during primary bloom. R. J. McLaughlin and R.G. Roberts, USDA, ARS, Tree Fruit Research Laboratory, Wenatchee, WA 98801, V. O. Stockwell, and J. Loper, USDA, ARS, Horticultural Crops Laboratory, Corvallis, OR 97330, and D. Sugar, Southern Oregon Experiment Station, Medford, OR 97502.

Total bacterial populations on stigmas and nectaries of Bartlett and d'Anjou pear flowers were monitored during primary bloom at one orchard in Washington and two in Oregon. At 5-10% bloom developmentally similar (within 1 day of full petal expansion) flowers were marked and 30-36 of these flowers were sampled every 2-4 days until petal fall. Dilution platings of stigma and nectary tissues were done on four media. Highest recoveries were observed on tryptic soy agar, nutrient-yeast dextrose agar, and King's medium B. At the initial sampling dates, frequency of bacterial recovery ($\geq 10^2$ cfu/tissue) on nectaries ranged from 25-50% and on stigmas from 0-32%. At 6-7 days after the initial sampling 90-100% of both floral tissues were colonized at Medford and Cashmere, only 30% of the stigmas and 50% of the nectaries were colonized at Corvallis. At most sampling dates, bacterial population recovery was lowest at the Corvallis site. Highest mean populations observed were ca. 10^3 cfu/tissue, regardless of tissue or site. FAME anlysis of bacterial strains recovered at high populations ($\geq 10^4$ cfu/tissue) indicated that species composition at each site varied considerably.

A50

CHARACTERIZATION OF COPPER RESISTANCE GENE HOMOLOGS FROM THE CHROMOSOME OF A COPPER-SENSITIVE STRAIN OF *PSEUDOMONAS SYRINGAE* PV. TOMATO. C.-K. Lim and D. A. Cooksey, Dept. of Plant Pathology, Univ. of California, Riverside 92521.

Copper-sensitive strains of *Pseudomonas syringae* pv. tomato, as well as many other pseudomonads, contain chromosomal DNA homologous to the plasmid-borne copper resistance operon (*cop*). To investigate the possibility that the *cop* operon evolved from the chromosomal homolog, a cosmid library of the copper-sensitive strain PT12.2 of *P. s.* pv. tomato was screened by colony hybridization with the *cop* operon as a probe. Two chromosomal regions with homology were found and named homologs 1 and 2. Homolog 1 contained DNA related to the *copA* and *copB* genes, and homolog 2 contained homologs of the *copA* and *copC* genes. Proteins similar to CopA and CopC were produced by PT12.2, but no CopB protein was detected in western blots. Deletion of homolog 1 had no observable phenotypic effect on PT12.2; mutations in homolog 2 are in progress.

A51

REGULATORY MUTANTS DEFECTIVE IN REPRESSION OF THE COPPER RESISTANCE OPERON PROMOTER OF *PSEUDOMONAS SYRINGAE* PV. TOMATO. S. D. Mills and D. A. Cooksey, Department of Plant Pathology, University of California, Riverside 92521

The promoter of the copper resistance operon (*cop*) was previously fused to a promoterless *lacZ* gene in the broad-host-range vector pMP190 to make pCOP38. The *cop* promoter was shown to be copper-inducible using this reporter vector. We previously identified a DNA-binding protein that binds specifically to the *cop* promoter when copper is absent but not when copper is present. We suggested that this protein is a repressor that turns off expression of the *cop* operon in the absence of copper. EMS mutagenesis was used to generate repression-defective mutants that were consitutive in their expression of β-galactosidase from pCOP38 in the presence or absence of copper. Mobility-shift assays were performed on these mutants to determine the effect of these mutations on the ability of the repressor to bind to the *cop* promoter.

A52

IRON ENHANCEMENT OF COPPER-CONTAINING BACTERICIDES TO CONTROL WALNUT BLIGHT DISEASE CAUSED BY *XANTHOMONAS CAMPESTRIS* PV. *JUGLANDIS*. Y. A. Lee, M. Hendson, S. E. Lindow, and M. N. Schroth. Department of Plant Pathology, University of California, Berkeley, CA 94720.

The inhibitory effects of copper-containing bactericides on bacteria are dependent on the free copper ion concentration released in water solution and on plant surfaces. The addition of iron to copper-containing bactericides, such as Kocide 101® and Champion®, decreased pH values and increased the availability of free copper ions in water solution and on walnut leaf surfaces. In addition, iron enhanced the sensitivity of copper-tolerant strains of *X. c. pv. juglandis* to copper ions and showed synergistic effects with copper in inhibiting bacterial growth. Kocide 101® amended with iron significantly reduced leaf epiphytic populations of a copper-tolerant strain of *X. c. pv. juglandis* as compared to Kocide 101® alone. Combination of Kocide 101® with iron significantly reduced bud infestation rates and percentage of blighted leaves.

A53

CONTROL OF WHEAT SCAB WITH FOLIAR APPLICATIONS OF BROMOCONAZOLE AT BROOKINGS, SD IN 1991. G.W. Buchenau and Shaukat Ali. Plant Science Department, South Dakota State University, Brookings, SD 57007.

The fungicide Bromoconazole was evaluated for its efficacy in controlling wheat scab on the spring wheat cultivar Butte 86 under field conditions at a site near Brookings, SD in 1991. Five treatments were tested in a randomized complete block design with four replications. Scab ratings were made in the early dough stage by collecting approximately 50 spikes from each plot and rating each spike for scab on a 0-7 scale. Proportion of spikelets infected was estimated as y=sum(number in class*a)/total spikes*33, where a=number of spikelets typically discolored in each class. There was a highly significant negative linear regression of logit infected florets on bromoconazole dose. The logit regression estimated ED_{50} and ED_{90} values at 0.3 and 1.2 kg/ha. Probit analyses provided similar results.

A54

RPA 400727: A NEW SYSTEMIC FUNGICIDE FOR CEREAL SEED TREATMENT. J.Mugnier[1], M.Chazalet[1], C.J.R.Klittich[2], Rhône-Poulenc Agro, [1]Lyon, France, [2]Stockholm, Sweden.

RPA 400727 is a triazole fungicide which provides excellent control of many seed- and soil-borne and foliar diseases of cereals. It is unique among SBI seed treatments in its crop safety at high rates combined with its long-lasting effect in the plant. RPA 400727 at 2.5-5 g ai/100 kg seed completely controls seed-borne bunts, smuts, *Fusarium roseum*, *Septoria nodorum*, and *Rhynchosporium secalis*. At 120 g ai/100 kg seed, 400727 protects against the early-season development of foliar diseases caused by *Pseudocercosporella herpotrichoides*, *Erysiphe graminis*, *Septoria* sp., and *R. secalis*. Rusts (*Puccinia hordei*, *P.striiformis*, *P.recondita*) are controlled from fall planting till late spring. The control of foliar disease by seed treatment can often substitute for one foliar fungicide application. Also, suppressing early season disease reduces the primary inoculum for epidemics. By reducing inoculum and spray operations, this seed-treatment fungicide is well suited to sustainable agriculture systems.

A55

CONTROL OF FUNGAL BROWN SPOT OF CULTIVATED WILD RICE WITH PROPICONAZOLE. R.F. Nyvall and J.A. Percich, University of Minnesota, 1861 Hwy. 169 East, Grand Rapids, MN 55744 and 495 Borlaug Hall, 1991 Upper Buford Circle, St. Paul, MN 55108.

The foliar fungicide propiconazole (Tilt, Ciba-Geigy Corp.) was evaluated for control of fungal brown spot caused by *Bipolaris oryzae* and *B. sorokiniana* in cultivated wild rice (*Zizania palustris*) in North Central Minnesota in 1991. Field plots consisted of six paddies located in four counties. Each paddy was divided into three areas of 4 ha on which propiconazole (219 ml A.I./ha) was applied at boot stage, boot stage and 14 days later, or not applied. Disease severity was rated (% leaf area infected) before each application of propiconazole and at harvest. One and two applications of propiconazole reduced disease severity by 34% and 59%, respectively, at harvest, compared to the control. Yield increases at two applications varied from 213 kg/ha to 863 kg/ha compared to the control. Yields for one application varied from an increase of 560 kg/ha, 0% increase or a decrease of 246 kg/ha compared to the control.

A56

FACTORS AFFECTING CONTROL OF SOUTHERN BLIGHT OF PEANUT WITH PCNB IN OKLAHOMA. J.P. Damicone and K.E. Jackson, Department of Plant Pathology, Oklahoma State University, and H.A. Melouk, USDA/ARS, Stillwater 74078-9947.

One hundred and twelve isolates of *Sclerotium rolfsii*, sampled from peanut fields with different histories of PCNB usage, were tested *in vitro* for growth response to PCNB. The mean EC-50 was 2.86 ug/ml. A standard deviation of ±1.45 ug/ml included 74% of the isolates. The highest EC-50 was 5.63 ug/ml. This indicates that a high level of PCNB resistance was not present in the population. Percent incidence (y) of southern blight in the field on peanut cv. Florunner declined linearly with increasing rates of PCNB (kg a.i/ha) where $y=25.5-1.4x$ ($P<0.01$, $R^2=0.65$). Reduced rates of PCNB resulted in significantly ($P=0.05$) lower disease incidence compared to no treatment when applied in a narrow (8-cm) band but not in a conventional 30-cm band. Failures to control southern blight with PCNB are likely due to insufficient rate or improper application of PCNB rather than resistance in *S. rolfsii*.

A57

W. McFadden, B.D. Ripley and C.S. Burchat. Residues of ethylenebisdithiocarbamate fungicide on grape. Horticultural Research Institute of Ontario, Vineland Station, Ontario, Canada, L0R 2E0 and Pesticide Lab, Ontario Ministry of Agriculture and Food, c/o University of Guelph, Guelph, Ontario, Canada, N1G 2W1

Ethylenebisdithiocarbamate (EBDC) fungicides have been an integral part of the control program for grape diseases in Ontario. A study was initiated to determine if residue levels of EBDC's were within the tolerated level when sprayed at the recommended pre-harvest interval (45 days). Mancozeb (Dithane or Dikar) was applied to 5 grape varieties. The following treatments were applied (1100 L water/ha) with an over-the-row sprayer: no EBDC sprays; 3 pre-bloom EBDC sprays only; and a program with 45- 30- 14- and 7-day pre-harvest intervals for EBDC. Fruit was collected at harvest and analyzed for residues using the carbon disulphide evolution method. In 1990, residues were below the tolerated limit of 7 ppm in all varieties treated with 3 pre-bloom sprays and with a 45-day pre-harvest interval. In 1991, only grapes sprayed pre-bloom had residues below the tolerated amount; the 45-day pre-harvest application showed 10 ppm EBDC.

A58

DETECTION AND CONTROL OF MONILINIA FRUCTICOLA LATENT INFECTIONS IN PLUMS. J. Northover and R.F. Cerkauskas, Agriculture Canada, Research Station, Box 6000, Vineland Station, Ontario, Canada, LOR 2E0.

Paraquat was effective for detection of latent infections of M. fructicola in immature fruits following severe blossom blight in 5 Niagara Peninsula plum orchards in 1991. Plums were surface-disinfested, treated with paraquat (Can. J. Plant Pathol. 10: 297-310), and incubated individually at 95% RH in 250 ml jars in light (PQL). Non-paraquat-treated samples were incubated in the dark (NPQD) or light (NPQL) at 25C. Paraquat enhanced brown rot development; 40% of fruits rotted after 5, 11 and 14 days in PQL, NPQD and NPQL. High latent infection corresponded 15-52% rot at maturity and 4-95% postharvest brown rot. Symptomless plums dipped in flusilazole and tebuconazole (0.1g ai/L) reduced postharvest rot from 79% to 17 and 14% respectively. A 6 min. dip in water at 51.5C reduced latent infection-induced rot from 95% to 0%.

A59

SO_2 FUMIGANT DOSAGES TO CONTROL *BOTRYTIS CINEREA*. J. L. Smilanick and D. J. Henson. USDA-ARS, 2021 S. Peach Ave., Fresno, CA 93727

SO_2 dosages that killed *Botrytis cinerea* were determined with 25, 50, 100 and 200 ppm SO_2 at high humidity. Minimal dosages required to stop postharvest decay of table grapes were determined. Dosages were expressed as parts per million-hours (ppm-hr), the product of the SO_2 concentration in ppm multiplied by the duration of the fumigation in hr. Sporocidal dosages (ED99 $\pm$ sd) were 78.3 $\pm$ 22.3 ppm-hr at 0C and 20.3 $\pm$ 2.0 ppm-hr at 20C. Mycelia on infected berries at 0C were injured by 10 ppm-hr and killed by 50 to 100 ppm-hr. The rate of infections on inoculated berries after 20, 50, 100, or 200 ppm-hr was 68, 47, 2 and 0%, respectively, compared to controls. For initial fumigation to control spores and subsequent applications to control mycelial growth in storage, 100 ppm-hr is the recommended minimum. Colorimetric dosimeters, used to quantify SO_2 for safety purposes, were tested for dosage measurement during fumigation. When placed inside grape packages before fumigation and examined promptly after, their accuracy was sufficient to determine if SO_2 dosages were adequate for decay control.

A60

FUNGICIDE SEED TREATMENTS FOR SHRUNKEN-2 ('SUPERSWEET') SWEET CORN. D.O. Wilson, Jr., S.K. Mohan, E.A. Knott, University of Idaho R & E Center, Parma, ID, and B. Shafii, College of Agriculture, University of Idaho, Moscow, ID.

Shrunken-2 ('supersweet') sweet corn hybrids show poor seed germination and high seedling mortality in the field due to seed rot and seedling diseases. Fungicide seed treatments were evaluated during 1989 (one seedlot, 30 treatments and 32 locations) and 1990 (three seedlots, 11 treatments and 19 locations) for their efficacy in improving seedling stand (4-5 leaf stage) in the field. Mean stand in untreated controls varied from 7% to 53% (median: 13%). The mixture of captan, thiram, metalaxyl and benomyl (CTMB) was the best treatment over all the locations, years and seedlots (mean stand: 41% to 71%; median: 50%). Comparable results were obtained with TMB, CTM + imazalil, and CTM + thiabendazole. In general, omission of any component from TMB reduced the efficacy of the treatment, and addition of other fungicides to CTMB did not improve the stand. Results indicate that an effective seed treatment for shrunken-2 sweet corn should include a broad-spectrum protectant fungicide, a *Pythium*-specific fungicide, and a systemic fungicide with activity against *Penicillium* and *Fusarium*.

A61

In-Vitro Sensitivity of *Rhizoctonia solani* isolates to fungicides and Control of Pocket Rot of Table Beets with Foliar Sprays. G. Olaya and G. Abawi. Dept. of Plant Pathology, Cornell Univ., Geneva, NY 14456.

Sensitivity of 107 isolates of *Thanatephorus cucumeris* (Anamorph: *Rhizoctonia solani*) obtained from naturally infected root, petiole, and leaf tissues of beets to Benlate, Rovral, Monceren, Rizolex, and CGA-173506 was evaluated in Potato-Dextrose-Agar (PDA) plates. None of the isolates grew at 1 ppm of Rizolex and CGA-173506 or 10 ppm of Rovral. However, they varied in their sensitivity to Monceren and Benlate even at 100 ppm. The efficacy of one spray of Benlate, Rovral, Rizolex, and CGA-173506 at 2.2 kg formulated product/ha applied in 920 L of water either before or after inoculation with *R. solani* was evaluated in plots (2 rows, 5 m long) of 2-mo-old beets cv. 'Ruby Queen'. Plants were inoculated with R. solani-infested soil (100 ml/m row) spread on the crown tissues and were over-head irrigated immediately. All fungicides significantly (P=0.05) reduced the % of infected roots at harvest as compared to the control (21.8%). Sprays applied before inoculations with *R. solani* were more effective. Best control (3.8% infected roots) was obtained with CGA-173506 applied before inoculation with *R. solani*.

A62

EFFECT OF SINGLE AND COMBINED APPLICATIONS OF TWO NATURALLY OCCURRING PHENOLIC COMPOUNDS ON SOIL NEMATODE POPULATIONS. A. Soler, R. Rodríguez-Kábana, C.F. Weaver, and P.S. King. Department of Plant Pathology, Auburn University, Auburn, AL 36849.

The nematicidal properties of selected phenolic compounds associated with aromatic plants were examined in a greenhouse study using a sandy loam soil naturally infested with root-knot (*Meloidogyne arenaria*) and soybean cyst (*Heterodera glycines*) nematodes. Thymol at rates ≥150 mg/kg effectively controlled *M. arenaria* but stimulated juvenile populations of *H. glycines*. Benzaldehyde stimulated *M. arenaria* and *H. glycines* at rates of 0.05 and 0.1 ml/kg, respectively. Combined applications of thymol and benzaldehyde showed a synergistic effect in suppressing *M. arenaria* populations, but *H. glycines* was unaffected or slightly stimulated. These preliminary results suggest that naturally occurring flavours and fragrances could be useful to manage phytonematodes.

A63

MANAGEMENT OF BLACK SHEATH ROT OF RICE *(Oryza sativa)* IN TEXAS. N.G. Whitney, Texas A & M University Agricultural Research & Extension Center, Rt. 7 Box 999, Beaumont, Texas 77713.

Black sheath rot caused by *Gaeumannomyces graminis* var. *graminis* is fast becoming a major disease of rice (*Oryza sativa*) in Texas. The disease is seed borne and has been found on several grasses. Yield parameter studies show that the disease has a high yield loss potential. Research results indicate that early planting and delaying the permanent flood aid in controlling the disease. In fungicide management studies benomyl and propiconazol gave good control while iprodione gave less that adequate control. Research results show that the ascospores of the fungus play a major roll in the disease cycle.

A64

REDUCED SENSITIVITY OF *SCLEROTINIA HOMOEOCARPA* TO DMI FUNGICIDES. J.M. Vargas, Jr., R. Golembiewski and A.R. Detweiler, Department of Botany and Plant Pathology and the Pesticide Research Center, Michigan State University, East Lansing, MI 48824.

Fifteen isolates of *S. homoeocarpa* from golf courses where less than satisfactory control from the DMI fungicides was reported, grew on media amended with 10 μg/ml fenarimol and triadimefon. Only three of these isolated grew in the presence of 10 μg/ml propiconazole, but these three isolates also grew on media amended with 100 μg/ml fenarimol. All 15 isolates were also resistant to benomyl based on their growth on PDA amended with 100 μg/ml benomyl and two of the isolates had reduced sensitivity to iprodione at 10 μg/ml. In spray trials involving label rates of fenarimol, tebuconazole, triadimefon, and propiconazole, control of dollar spot caused by *S. homoeocarpa* was poor compared to control with the standard contact fungicide chlorothalonil. The reduced sensitivity of isolates *in vitro* combined with poor control in field trials provides evidence for the development of strains with resistance to DMI fungicides. Control of these strains will be difficult because of their multiple resistance to benomyl and in some cases to iprodione.

A65

REDUCED SENSITIVITY OF *PENICILLIUM DIGITATUM* TO IMAZALIL, THIABENDAZOLE AND O-PHENYLPHENOL. G. J. Holmes and J. W. Eckert. Department of Plant Pathology, University of California, Riverside, CA 92521.

Sensitivity of *Penicillium digitatum* to imazalil (IZL), thiabendazole (TBZ), and o-phenylphenol (OPP) was determined for 137 isolates collected from California citrus packinghouses from 1986 to 1990. IZL-resistant isolates were identified by their sporulation on IZL-treated lemons. Sensitivity was determined by radial growth on fungicide-amended media. Mean EC_{50} values for IZL-resistant isolates were 0.46($\pm$0.16) ppm, 32.2($\pm$4.2) ppm, and 17.9($\pm$5.0) ppm for IZL, TBZ and OPP, respectively; the corresponding resistance factors (EC_{50} resistant/EC_{50} sensitive) were 15, 215 and 3. For IZL, EC_{50} values based on spore germination were 2-3 times less than those based on radial growth. Multiple resistance to IZL, TBZ and OPP occurred in 63% of the isolates. Frequency of multiple resistance increased from 42% in 1986-89 to 77% in 1990. Unlike TBZ and OPP, IZL-resistant *P. italicum* isolates have not been found in California.

A66

SM-9 FOR CONTROL OF RHIZOCTONIA AND PYTHIUM DISEASES OF TURF GRASS. Robert H. Littrell and Mark Gilmore, Coastal Plain Consulting, Inc., Tifton, Georgia 31794.

Laboratory studies were conducted to determine sensitivity of Rhizoctonia spp. and Pythium sp. to SM-9. Several species representing these genera were inhibited when SM-9 was applied to actively growing mycelium. Golf putting greens of hybrid bermuda grass overseeded with rye grass were treated with SM-9 at 0.32 ml/m^2 to control brown patch and Pythium blight. Diseased areas were treated on 6 November and repeated 2 weeks later. Diseased areas measured immediately after treatment were 13.5% of the total area. Two weeks after treatment the diseased area was only 5.5% of the total area, and on 16 December only a trace of disease was found. Nontreated diseased areas continued to increase in size and intensity. Pathogens involved were Rhizoctonia solani, R. cerealis, and Pythium myriotylum. This study indicates SM-9 is an effective control of fungal pathogens of turf grass.

A67

MUTATIONAL ANALYSIS OF THE COAT PROTEIN GENE OF A TYPE II ISOLATE OF BEAN GOLDEN MOSAIC GEMINIVIRUS. O. Azzam[1], D. R. Russell[2], and D. P. Maxwell[1]. [1]Dept. of Plant Pathology, University of Wisconsin, Madison, WI 53706, and [2]Agracetus, Inc., Middleton, WI 53717.

The genome of bean golden mosaic geminivirus (BGMV) is composed of two circular, single stranded DNA components (DNA-A and DNA-B). Symptom formation by geminiviruses is affected by mutations in the viral coat protein gene (AR1) and other genes encoding nonstructural proteins. Two mutations were introduced into AR1 of the sap-transmissible BGMV isolate from Guatemala. One mutant was a deletion of the NsiI fragment (410 bp) in AR1, and the other mutant was made by reversing this NsiI fragment in AR1. Beans were inoculated using the "particle gun" with wild-type DNA-A and DNA-B or combinations of the two mutants of DNA-A and wild-type DNA-B. By ten days after inoculation, systemic infection occurred in plants with wild-type DNA-A (16 infected/21 inoculated), the NsiI fragment deletion mutant (2/21), or the reverse NsiI fragment mutant (3/21). However, these mutants were not sap transmissible and no coat protein was detected on Western blots. Coat protein was only detected in plants infected with wild-type clones. These results show that coat protein of this type II BGMV is not essential for symptom expression and movement.

A68

GEMINIVIRUS COMPLEXES ASSOCIATED WITH TOMATO AND PEPPER DISEASES IN MEXICO. E. J. Paplomatas[1], P. D. Grieco[1], M. R. Rojas[2], D. P. Maxwell[2], and R. L. Gilbertson[1]. [1]Dept. of Plant Pathology, University of California, Davis, CA 95616. [2]Dept. of Plant Pathology, University of Wisconsin, Madison, WI 53706.

A new whitefly-transmitted geminivirus (TGV-MX1) from diseased tomatoes from Mexico was isolated and characterized. Full-length clones of DNA-A and DNA-B were obtained, and excised monomers or 1.5-mers of these clones were infectious when rub-inoculated onto *Nicotiana benthamiana*. Infected *N. benthamiana* developed few symptoms, but the presence of geminivirus nucleic acids was confirmed by DNA hybridization and PCR. The presence of TGV-MX1 in tomatoes and peppers from Mexico was demonstrated by PCR amplification of a fragment of DNA-A. One tomato plant was simultaneously infected with TGV-MX1 and another geminivirus, based on RFLPs of a PCR-amplified fragment of DNA-A. This mixed infection was confirmed by sequence comparisons of cloned PCR fragments. The second geminivirus (TGV-MX2) is closely related to TGV-MX1, but they have significant nucleotide sequence divergence. These results suggest that a complex of closely related geminiviruses are infecting tomato and pepper plants in Mexico.

A69

PSEUDORECOMBINATION BETWEEN TWO DISTINCT GEMINIVIRUSES: HOST RANGE DETERMINANTS RESIDE ON THE DNA-B COMPONENT. Y.-M. HOU[1], D.P. MAXWELL[2] AND R.L. GILBERTSON[1] Departments of Plant Pathology, University of California, Davis[1] CA 95616 and University of Wisconsin[2], Madison, WI 53706.

An infectious pseudorecombinant geminivirus was made between tomato mottle geminivirus (ToMoV, previously called TGV-FL) and bean dwarf mosaic geminivirus (BDMV) in the common host, *Nicotiana benthamiana*. Both of these geminiviruses possess a bipartite genome (DNA-A and DNA-B), but have distinct host ranges, i.e.,ToMoV does not infect bean and BDMV does not infect tomato, as determined by sap transmission. The presence of the input inocula (ToMoV DNA-A and BDMV DNA-B) was confirmed by characterization of PCR amplified DNA-A and DNA-B fragments from infected plants. Less DNA-B was detected in plants infected with the pseudorecombinant than in those infected with homologous combinations. When the pseudorecombinant was inoculated onto beans and tomatoes, only beans were typical of BDMV infection. This result indicates that host range determinants for infecting bean are associated with BDMV DNA-B but not ToMoV DNA-A. We hope to induce geminivirus evolution by repeated passage of the pseudorecombinant through plants.

A70

CLONING, IDENTIFICATION, AND PARTIAL SEQUENCING OF THE GENOMIC COMPONENTS OF A GEMINIVIRUS INFECTING THE BRASSICACEAE. A. M. Abouzid, E. Hiebert, and J. O. Strandberg.* Dept. Plant Pathology, University of Florida, Gainesville, FL 32611 and Sanford*, FL 32771.

A severe viral infection of cabbage, prevalent in Central Florida, has been reported to be caused by a whitefly-transmitted geminivirus with a broad host range in the Brassicaceae (Strandberg et al., 1992, Plant Disease, 76, in press). A viral replicative, double-stranded DNA was isolated from experimentally infected cabbage, mustard, and *Nicotiana edwardsonii*. Analysis of restriction digests of this dsDNA revealed more than one DNA component. Restriction maps of the full length clones of this dsDNA revealed three distinct DNA components. Hybridization of the clones with labeled tomato mottle virus A and B components under high stringency identified one clone as an A component and the other two as B components. Preliminary sequence analyses of the clones indicate that the cabbage virus has a bipartite genomic organization with a possible heterogeneity in the B component as has been described for squash leaf curl virus (Lazarowitz, 1991,Virology 180, 70). Sequence comparisons indicate that the cabbage virus does not share a close sequence relationship to any known geminivirus.

A71

DOUBLE STRANDED (DS) RNA ANALYSIS OF THE WHITE-FLY COMPONENT OF THE SWEETPOTATO VIRUS DISEASE (SPVD-WF) OF SWEETPOTATO. J. A. Abad, R. D. French and J. W. Moyer. Dept. of Plant Pathology, North Carolina State University, Raleigh, NC 27695-7616.

The white-fly transmitted component of the sweetpotato virus disease (SPVD-WF) is currently the most problematic of the sweetpotato pathogens for the international movement of sweetpotato germplasm. The agent is not mechanically transmitted and has not been purified for antisera production. To aid in diagnosis and experimental procedures we have identified four distinct dsRNA species associated with this agent with Mr's of 6.3 x 10^6, 5.2 x 10^6, 3.0 x 10^6, and 2.3 x 10^6, respectively. The four dsRNA species were isolated from infected but not healthy sweetpotato plants. A mini-prep method to detect the two largest and most abundant dsRNAs from 150 mg of infected tissue was developed. ^{32}P end-labeled dsRNA was assayed to confirm their identity and establish this association with diseased tissue. Complementary DNA cloning from dsRNA is in progress with the ultimate goal of generating riboprobes and/or polymerase chain reaction systems (PCR) for rapid detection of this pathogen.

A72

SECOND SITE SUPPRESSOR MUTATIONS ASSIST IN STUDYING THE FUNCTION OF THE 3' NONCODING REGION OF TYMV RNA. Ching-Hsiu Tsai and Thoe W. Dreher, Department of Agricultural Chemistry, Oregon State University, Corvallis, Oregon 97331-6502

Turnip yellow mosaic virus (TYMV) has a positive-sense single-stranded RNA genome 6.3 kb long. In common with several other plant viruses, including brome mosaic virus (BMV) and tobacco mosaic virus (TMV), the 3'-terminal region of TYMV RNA comprises a tRNA-like structure. The 3' noncoding region of TYMV RNA includes this 82-nucleotide-long tRNA-like structure domain and a short upstream region that includes a potential pseudoknot overlapping the coat protein termination codon. Genomic RNAs with point mutations in the 3' noncoding region that result in poor replication and no systemic symptoms in *planta* were inoculated onto Chinese cabbage plants in experiments designed to obtain second site suppressor mutations. Putative second site suppressor mutations were identified by ribonuclease protection and sequencing, and were then introduced into genomic cDNA clones (Weiland and Dreher, 1989. Nucl. Acids. Res. 17:4675-4687) to permit their characterization. The same techniques has also been used to identified the mutations in a severe symptom strain of TYMV isolated from Chinese cabbage plant which was inoculated with wild type virus transcripts.

A73

SENSE RNA-MEDIATED RESISTANCE TO TOBACCO ETCH POTYVIRUS. J. A. Lindbo and W. G. Dougherty. Department of Microbiology, Oregon State University, Corvallis, OR 97331.

Transgenic tobacco plants expressing tobacco etch virus (TEV) coat protein (CP) gene RNA have been generated. Certain transgenic plant lines, expressing a non-translatable "sense" form of TEV CP transcripts, do not develop symptoms when challenged with TEV transmitted by aphids or mechanically. However, these same transgenic plant lines are susceptible to the related potyviruses potato virus Y and tobacco vein mottling virus. In contrast to the extensively-reported phenomenon of CP-mediated resistance, this resistance is 1) RNA-mediated; 2) virus specific; 3) effective in very young plants; 4) independent of inoculum levels; and 5) expressed in protoplasts as an attenuation of virus replication.

A74

CONTROL OF TRANSLATION OF THREE GENES FROM A SINGLE SUBGENOMIC RNA OF BARLEY YELLOW DWARF VIRUS (BYDV). S.P. Dinesh-Kumar and W. Allen Miller, Department of Plant Pathology/MCDB Program, Iowa State University, Ames, IA. 50011

BYDV has a positive sense, 5.7kb RNA genome encoding at least six open reading frames (ORFs). The 22K (coat protein, CP), 17K and 50K ORFs are all translated from a large subgenomic RNA (sgRNA1) (Dinesh-Kumar et al., 1992, Virology, 187:711-722). The CP and overlapping 17K ORFs are translated by initiation at two out-of-frame AUGs, and the 50K ORF by in-frame readthrough of the CP stop codon. To determine the sequences and/or structures that effect the dual initiation, sgRNA1 was mutated and translated *in vitro*. Preliminary results suggest that both primary and secondary structures control dual initiation, but that the former is more important, supporting Kozak's leaky scanning model. We will present results obtained *in vivo*, using a GUS reporter gene, of the sequences and structures that control dual initiation as well as in-frame readthrough of the CP ORF stop codon. The results should be valuable for researchers who want to optimize expression of CP in transgenic plants to obtain disease resistance.

A75

PARTIAL CHARACTERIZATION OF THE GENOMIC RNA OF A CLOSTEROVIRUS ASSOCIATED WITH THE SWEET POTATO VIRUS DISEASE COMPLEX. S. Winter, A. Purac and R. I. Hamilton, Agriculture Canada Research Station, 6660 NW Marine Drive, Vancouver, BC, Canada V6T 1X2.

Filamentous, closterovirus-like particles of modal length 950 nm were consistently seen in leaf dips of Ipomoea setosa infected with the whitefly-transmitted component (WTA) of the sweet potato virus disease complex (SPVD) from Nigeria. A large dsRNA (MW ca. $6.1x10^6$) and several smaller species were detected in all sweet potato and I. setosa infected with the WTA. Synthesis and cloning of cDNA using dsRNA from WTA-infected sweet potato as template resulted in clones which hybridized specifically with nucleic acid prepared from purified closterovirus as well as with RNA isolated from WTA-infected plants. The size of a ssRNA isolated from purified virus was estimated to be ca. 9 kb by Northern hybridization which, in accordance with the largest dsRNA and the length of closterovirus-like particles, was considered to be virion RNA. These data suggest that a closterovirus together with the aphid-transmitted sweet potato feathery mottle virus are the causal agents of SPVD.

A76

THE EFFECTS OF TRANSIT PEPTIDE-MEDIATED EXPRESSION OF POTATO VIRUS Y COAT PROTEIN IN CHLOROPLASTS. M. Naderi and P. H. Berger. Dept. of Plant, Soil, & Entomological Sciences, Univ. of Idaho, Moscow, ID 83843.

Several investigators have previously demonstrated the presence of virions, pseudovirions, viral RNA, coat protein or other virus gene products associated with or within the chloroplasts of infected plants. In the cases of tobacco mosaic virus (TMV) and potato virus Y (PVY), both coat protein (CP) and genomic RNA have been isolated from chloroplasts of infected tobacco. We have targeted the PVY-CP into the chloroplast stromal compartment and cytosol of transgenic tobacco to investigate the effect of PVY-CP and the development of mosaic symptoms. This was accomplished by using an Agrobacterium-based transformation vector that included an upstream chloroplast-specific transit peptide. Initial results indicate that chloroplast development and/or function is disrupted by the presence of CP. Results will be presented describing the effect, at the ultrastructural level, of CP on chloroplasts from these transgenic plants. Studies are also underway to determine the fate of CP that is expressed with or without an upsteam transit peptide.

A77

TOBACCO PLANTS TRANSGENIC FOR NON-STRUCTURAL GENES OF TOBACCO MOSAIC VIRUS (TMV) ARE RESISTANT TO INFECTION BY TMV AND OTHER TOBAMOVIRUSES. J. Donson, G. Kurath, T.H. Turpen, I.A. Khan & W.O. Dawson. Dept. of Plant Pathology, Univ. of California, Riverside, CA 92521.

Nicotiana tabacum cv. Xanthi plants were made transgenic for nucleotides 69-5463 of TMV-U1. The progeny of self-fertilized transgenic plants were inoculated with high concentrations (100ug/ml) of six tobamoviruses (TMV-U1, TMV-U5, TMV-U2, green-tomato atypical mosaic virus, tomato mosaic virus and ribgrass mosaic virus) and one cucumovirus (cucumber mosaic virus - CMV). After one month, upper, non-inoculated leaves were scored for symptoms, and they were screened for virus by local lesion assay. Transgenic lines resistant to infection by TMV-U1 also showed resistance to the 5 other tobamoviruses, but not to CMV. Experiments will be described looking at the mechanism of this protection.

A78

SEQUENCE ANALYSIS OF GENOMIC RNA SPECIES OF TWO SWEET CLOVER NECROTIC MOSAIC VIRUS STRAINS. Z. Ge and C. Hiruki. Department of Plant Science, University of Alberta, Edmonton, Alberta, Canada T6G 2P5.

The nucleotide sequences of the RNA-2s of two sweet clover necrotic mosaic virus strains (SCNMV-38 and -59) consisted of 1446 and 1449 nucleotides, respectively. Both RNA-2s contained one major open reading frame (ORF) potentially encoding polypeptides of 326 amino acid residues. Both the RNA-2 nucleotide sequences and the P2 amino acid sequences shared a significant degree of homology (>90%). However, the identical nucleotides and amino acid residues were not uniformally distributed in the RNA-2 and their encoded proteins. A highly conserved region and a relatively divergent region were found in the P2 proteins of these strains, which may represent different functional domains such as the conserved region being responsible for cell-to-cell virus movement, and the variable region playing an important role in the development of plant disease symptoms. Determination of the complete nucleotide sequence of SCNMV RNA-1 (about 4 kb) is in progress.

A79

GENETIC AND BIOCHEMICAL ANALYSIS OF RCNMV CELL-TO-CELL MOVEMENT. D. G. Cookmeyer, K. H. Kim, and S. A. Lommel. Dept. of Plant Pathology, Box 7616, NCSU, Raleigh NC 27695-7616.

Deletion and frameshift mutations were engineered in the RCNMV capsid and movement protein genes. Capsid protein mutants infected the inoculated and un-inoculated leaves of *Nicotiana benthamiana* and *N. clevelandii* at temperatures below 20°C. However, systemic symptom development required 3 weeks versus 1 week for wild-type transcripts. Mutant capsid protein was not detected in infected tissue extracts. A 39 amino acid carboxy-terminal deletion of the movement protein was indistinguishable from wild-type virus in its ability to form a systemic infection on systemic host plants. Larger movement protein deletions, however, prevented systemic infection of even the inoculated leaves. Purified RCNMV movement protein from an *E. coli* overexpression system has been used to generate polyclonal antisera that detected the movement protein in infected tissue. *In vitro* and *in vivo* RNA binding studies have been developed to assay alanine scanning mutants which have been constructed to map the various movement protein functional domains.

A80

Biogenesis of Defective Interfering RNAs from Cloned Wild-type and Chimeric Tombusviruses. M. D. Law, J. L. Hyslop, and T. J. Morris. University of Nebraska, School of Biological Sciences, Lincoln, NE 68588-0118.

Small RNA species composed of a mosaic of viral sequence (defective interfering RNAs -DIs) have been identified in tomato bushy stunt virus (TBSV) and cucumber necrosis virus (CNV), both members of the Tombusvirus group. These DIs arise *de novo* after high multiplicity of infection passage. We have analyzed the biogenesis of DIs using DI-free infectious transcripts of TBSV, CNV and TBSV-CNV chimeric mutants. Total RNA was extracted from plants 7, 14, and 21 days after inoculation, converted to DNA, and amplified by polymerase chain reaction using primers specific for the conserved 5' and 3' termini. A small heterogenous population of RNA species were amplified from the plants 7 days after inoculation. Subsequent samples taken 14 and 21 days after inoculation exhibited a more homogenous and larger population of DI species. The generation and the evolution of these DI species will be discussed.

A81

COMPLEMENTATION OF A MOVEMENT-DEFECTIVE MUTANT OF TOBACCO MOSAIC VIRUS BY PEANUT CHLOROTIC STREAK VIRUS, A CAULIMOVIRUS. R.D. Richins[1], J. Donson[2], D.J. Lewandowski[2], A.G.C. Lindbeck[2], W.O. Dawson[2], and R.J. Shepherd[1]. Depts. of Plant Pathology, [1]University of Kentucky, Lexington, KY 40506, and [2]University of California, Riverside, CA 92521.

Tobacco (*Nicotiana tabacum* vars. Xanthi and Xanthi-nc) plants systemically infected with peanut chlorotic streak virus (PClSV), a caulimovirus, supported the systemic infection of a tobacco mosaic virus (TMV) mutant which carried a frameshift mutation in the 30K (movement protein) gene. Complementation occurred only at temperatures which were conducive to the systemic spread of PClSV (*i.e.* >30°C). Tobacco plants inoculated with an extract derived from the PClSV+TMV (30K-) infected plants, but incubated at 22°C failed to develop symptoms, indicating that the TMV mutation had not reverted to wild type. 30K-transgenic tobacco plants were, however, able to support the replication of the mutant TMV at 22°C. Reciprocal movement protein gene exchanges between the two viruses will be discussed.

A82

CONTROL OF TRANSLATIONAL FRAMESHIFTING BY BARLEY YELLOW DWARF VIRUS RNA. Rong Di, Veronique Brault, S.P. Dinesh-Kumar & W. Allen Miller, Plant Pathology Dept., Iowa State University, Ames, IA 50011

The putative polymerase gene of barley yellow dwarf virus (BYDV) is expressed by ribosomal frameshifting during translation (Brault & Miller, PNAS 89, 2262-2266, 1992). Like retro- and coronaviruses, BYDV RNA contains a "shifty heptanucleotide" at which the ribosomes change reading frames at low frequency (1-2% for BYDV), followed by a sequence with significant possible secondary structures. We used mutagenesis, followed by translation *in vitro*, in *E. coli*, and in plant protoplasts to find that the stop codon of the upstream reading frame, and downstream structured regions are required for frameshifting. However the "shifty heptanucleotide" tolerates multiple mutations, suggesting that the simultaneous slippage model for frameshifting (Jacks et al., Cell 55, 447-458, 1988) may need to be modified. Frameshifting appears to be an elegant mechanism by which viruses use minimal genetic information to effect low level expression of proteins, such as the viral polymerase, which are needed only in small quantities.

A83

THE PROCESS OF WHEAT SEED INFECTION BY *PYRENOPHORA TRITICI-REPENTIS*. A. M. C. Schilder and G. C. Bergstrom. Department of Plant Pathology, 334 Plant Science, Cornell University, Ithaca, NY 14853.

Pyrenophora tritici-repentis, incitant of tan spot of wheat, is known to be seedborne. The seed infection process was studied by microscopic observation of the fungus on the flower parts and seeds at 1, 3, 5, 7, and 14 d after spraying a conidial suspension on spikes of a susceptible wheat cultivar at the milk stage. Seeds were tested for infection by the freezing blotter method 1, 3, 5, 7, 14, and 21 d after inoculation. In addition, inoculation was targeted on leaves and peduncle only, glumes only, seeds only, and whole spikes with or without removal of anthers. Seeds were tested for infection by the freezing blotter method when ripe. *P. tritici-repentis* primarily invaded the pericarp of seeds via infected glumes, lemmas, or paleas. Infected seeds were first observed 3 d after inoculation, but the maximum proportion of infected seeds was reached at 21 d after inoculation. Targeted inoculation resulted in seed infection levels of 97% for seed inoculation, 89% for spike inoculation, 83% for spike inoculation after removal of anthers, 19% for glume inoculation, and 0% for leaf and peduncle inoculation. This indicates that seed infection predominantly occurs via the lemma and palea, rather than the glume. The presence of anthers adjacent to the seed apparently enhances seed infection.

A84

SEED QUALITY EFFECTS ON EMERGENCE AND YIELD OF HARD RED WINTER WHEAT. C. M. Rush and K. M. Vaughn, Texas Agricultural Experiment Station, Bushland, Texas 79012.

A three-year study was conducted to determine whether wheat seed quality affected seedling emergence and yield. Grain was collected from growers' fields and sorted into four categories (very good, good, bad, and very bad) based on seed weight. Seed were planted in randomized field plots, stand counts taken, and at harvest, total yield and test weight were determined. No field x quality interaction existed for stand, yield, or test weight any year of this study. Mean 100 seed weights for the four categories over three years, 3.60, 2.87, 2.27, and 1.44 g, were all significantly different. Despite large differences between categories, seed quality had minimal effect on any measured variable in 1989 or 1991. However, in 1990, seed from the very good and good categories produced better stands and higher yields than very bad seed. Planting date appeared to be associated with stand establishment and poor seed did better when planted in October than November.

A85

RELATIONSHIP BETWEEN SYMPTOMS ON TESTAE OF PEANUT (ARACHIS HYPOGAEA) AND ISOLATION OF CYLINDROCLADIUM CROTALARIAE. B.L. Randall-Schadel, NC Dept. Agri., Raleigh, NC 27611, J. E. Bailey, NC State Univ., Raleigh, NC 27695, and F. E. Dowell, USDA-ARS, Dawson, GA 31742.

Seeds from three peanut cultivars (NC 9, NC 10, NC 11) were harvested from plots treated or not treated with metam sodium in a *Cylindrocladium crotalariae* infested field. The number of colonies of *C. crotalariae* isolated on CBR selective medium was compared to nine categories of seed symptoms; presence/absence of metam sodium treatment; use of botran and clorox dips (1.74g botran/400 ml water for 2 min., 10% clorox for 1 min., distilled water for 1 min.); and disease incidence in the field. Metam sodium and botran, independently or in combination, did not affect the number of colonies isolated or symptoms observed. The 9 symptom categories were not equally associated with positive isolations. More colonies were isolated from seeds with cinnamon brown "speckles" than from seeds without "speckles" (P= 0.0001). Spectral analysis of reflected light (500-700nm) indicated curves of "speckled" seed tended to be flatter in slope and have lower relative reflectance than symptomless seed. Preliminary data indicate "speckled" seed can be separated by electric eye sorting.

A86

AN IMPROVED SEMI SELECTIVE MEDIUM AND METHOD OF EXTRACTION FOR DETECTING *CLAVIBACTER MICHIGANENSIS* SUBSP. *MICHIGANENSIS* IN TOMATO SEEDS. C. M. Waters and H. A. Bolkan. Campbell Research and Development 28605, County Road No. 104 Davis, CA 95616

A semi selective medium is described for detecting *C. m.* in tomato seeds. The new medium, designated mSCM, is based on the semi-selective medium of Fatmi and Schaad (Phytopathology 78: 121-126) and contains Pourite (0.013 g/L), $K_2HPO_4 \cdot 3H_20$ (2.62 g/L), KH_2PO_4 (0.5g/L), $MgSO_4 \cdot 7H_20$ (0.25 g/L), boric acid (1.5 g/L), mannose (10 g/L), yeast extract (0.1 g/L), nalidixic acid (Na salt) (0.03 g/L), nicotinic acid (0.1 g/L), cycloheximide (0.2 g/L), and agar (12 g/L). To extract *C. m.* from tomato seed, 24 g are soaked in 100 ml of PBT-2 buffer at 4 C for 15 minutes followed by stomaching for 15 minutes. Samples (.01ml) of nondiluted, 10^{-1} dilution, and 10^{-2} dilution are then each assayed on three plates of mSCM. At 7 to 9 days colonies are 3-5 mm in diameter, mucoid, clear and easily distinguishable from other mucoid colonies by internal flecks of yellow. *C. m.* colonies are easier to recognize and saprophytic contaminant colonies are reduced in size on the new medium.

A87

CARYOPSIS AND PLACENTAL INFECTION BY Aspergillus flavus TYPE ISOLATES IN FIELD INOCULATED MAIZE EARS. N. Zummo and G.E. Scott, USDA-ARS, P.O. Drawer PG, Miss. State, MS 39762.

Kernels from maize ears, inoculated in the field with individual Aspergillus flavus type isolates, were cut transversely into three sections and plated aseptically in petri plates. Placental tissue associated with each caryopsis was also assayed for infection by A. flavus. Although infection among individual kernel sections (top - 5.7%, middle - 6.1%, bottom - 5.4%) did not differ significantly, kernel infection was significantly higher than placenta infection (1.3%). This infection pattern was similar for all six isolates in this study. Recovery of A. flavus from 975 kernel segments and associated placental tissue was 123 in kernel segments only, five in placental tissue only, and 11 in both kernel and associated placenta. These data suggest that kernel infection in maize by A. flavus occurs through the pericarp rather than through the cob.

A88

ASSOCIATION OF *PSEUDOMONAS SYRINGAE* PV. *APII* WITH CELERY SEED. E. L. Little[1], S. T. Koike[2], and R. L. Gilbertson[1]. [1]Department of Plant Pathology, University of California, Davis 95616 and [2]Cooperative Extension, University of California, Salinas 93901.

Bacterial blight of celery, caused by *Pseudomonas syringae* pv. *apii* (*Psa*), is a new disease of celery in California. In 1991, *Psa* populations were not detected on leaf samples before March, when significant populations (10^5 to 10^7 cfu/gram tissue) were recovered from asymptomatic leaves in transplant houses. Outbreaks of disease occurred one week later. High temperatures, high water pressure from irrigation booms, and mowing of the transplants were associated with disease spread and severity. A seed assay was developed, and low populations of *Psa* (0.005 to 0.04 cfu/seed) were recovered from commercial seedlots. To determine if seed is a source of initial inoculum, celery plants at the vegetative stage of growth were inoculated with *Psa* and allowed to set seed. Few leaf symptoms were observed, but high populations of *Psa* were recovered from asymptomatic flowers (10^4 to 10^6 cfu/gram tissue) and seed (12.9 to 18.2 cfu/seed). This seed is being used to test for transmission of the bacteria to seedlings, and the efficacy of seed treatments for eradication of *Psa* from seed.

A89

Assessment of ergot among Kentucky Bluegrass fields grown for seed in Central Oregon. D.D. Coats[1], S.C. Alderman[2], and F.J. Crowe[1]. [1]Oregon State University-COARC, Madras, OR 97741 and [2]USDA-ARS NFSPRC, Corvallis, OR. 97331

During June, 1991, 400 seed heads were collected from each of 150 randomly selected Kentucky bluegrass (Poa pratensis) fields located in Jefferson County, Oregon. Ergot, caused by Claviceps purpurea, was detected in 23 of the fields, including eight out of 30 cultivars in the survey. Production of stroma among small (length equal to bluegrass seed), medium (one to two times longer than seed), and large (> twice the seed length) sclerotia was 23, 58,and 89%, respectively. Ergot among weed grasses was assessed at 127 $2m^2$ sites selected at random in the region. Infected weed grasses and seed production fields were located primarily in the southern area of the county. Weed grasses infected by C. purpurea were Bromus sp., Secale cereale, Festuca arundinaceae and Poa pratensis.

A90

ISOLATION OF FUSARIUM LATERITIUM FROM SWEETPOTATO TRUE SEED FROM DIFFERENT COUNTRIES. C. A. Clark, M. W. Hoy, and B. S. Stine, Dept. Plant Pathology & Crop Physiology, Louisiana State University Agricultural Center, Louisiana Agricultural Experiment Station, Baton Rouge, LA 70803-1720.

Fusarium lateritium, causal fungus of chlorotic leaf distortion (CLD) of sweetpotato, was isolated from true seed of sweetpotato that had been acid scarified and surface disinfested with NaOCl. The fungus was isolated from both floaters (nonviable seed) and sinkers (viable). It usually grew out of the seed when the seed coat cracked. The highest percentage isolation from a seed lot was 58%. The fungus was isolated from US seed as old as 13 yr. Frequency of isolation was greater from seed from the USA and Africa than from seed from Asia or South America. Isolates from US or African seed either reproduced CLD (59% of isolates), systemically colonized the surface of vines without inducing chlorosis (EMG-type = 34%), or had no effect (7%). All nine characterized isolates from Asia or South America were of the EMG type. Although *F. lateritium* does not invade the vines on which symptoms are produced, it was found inside true seed.

A91

BIOLOGY AND EPIDEMIOLOGY OF CARROT MOTLEY DWARF IN CALIFORNIA M. T. Watson AND B. W. Falk. Department of Plant Pathology, University of California, Davis CA 95616.

Carrot motley dwarf (CMD) is a severe disease of spring carrots in California's cool, coastal growing regions. CMD is caused by a co-infection of two viruses, carrot redleaf virus (CRLV) and carrot mottle virus (CMoV), which are transmitted by the willow-carrot aphid, *Cavariella aegopodii*. Field and greenhouse studies have identified factors affecting disease severity and spread. These include time of infection, temperature, location, and carrot cultivar. Forty-five carrot cultivars were screened for relative resistance/susceptibility to CMD. Several cultivars showed good resistance to CMD in both field and greenhouse environments. Virus and vector host range studies have shown carrots to be the only indigenous crop that is a host for both the viruses and the aphid vector. Mapping studies of disease occurrence have shown that CMD is greatest in spring carrots which were planted near or downwind of overwintered carrots. These results suggest that overwintered carrots are potentially the main inoculum source for spring carrots.

A92

COMMERCIALIZATION OF ZYMV CROSS PROTECTION FOR ZUCCHINI PRODUCTION IN HAWAII. J. J. Cho, D. E. Ullman, E. Wheatley, J. Holly, and D. Gonsalves. University of Hawaii, Honolulu and Cornell University, Geneva.

Zucchini yellow mosaic virus (ZYMV) severely affects stable zucchini production in Hawaii. Cross protection was shown effective in reducing zucchini crop losses from severe ZYMV infections. In two cross protection experiments fruit yield increased 3 and 16 times when natural ZYMV infections occurred 3 to 4 weeks and immediately after transplant, respectively. Cross protection effectively reduced disease incidence and allowed a 4 to 6 week harvest period. An attempt to transfer cross protection technology to farmers was initiated on August 14, 1991 through a cross protected zucchini seedlings distribution program. Fourteen farmers have participated and over 100,000 seedlings distributed to date. This program has been successful in increasing zucchini production on Maui, increasing grower confidence in cross protection, and attracting entrepreneurs to develop commercial nurseries to distribute cross protected zucchini seedlings.

A93

IDENTIFICATION AND DISTRIBUTION OF SOILBORNE WHEAT MOSAIC VIRUS IN ALABAMA. P. Jin[1], R. T. Gudauskas[1], D. J. Collins[1], K. B. Burch[1], A. K. Hagan[1], and P. L. Mask[2], [1]Dept. of Plant Pathology, and [2]Dept. of Agronomy and Soils, Auburn University, AL 36849.

Soilborne wheat mosaic virus (SBWMV) was originally found in Alabama in 1989, in wheat fields in Autauga County. Identification of the virus was based on particle size and morphology and cross reactivity in immunodiffusion and enzyme-linked immunosorbent (ELISA) tests with SBWMV isolates and/or antisera from FL, KS, NB, OK and the ATCC. During Feb-May, 1991, wheat leaves and roots were collected from randomly selected fields and tested by indirect ELISA using antiserum to the Alabama isolate of SBWMV. The virus was detected in at least one field in 28 counties located throughout the state and in all major wheat production areas. This widespread occurrence indicates that SBWMV likely was present in Alabama for some time prior to 1989, and that any effects on the wheat crop either went undetected or they were attributed to other causes.

A94

VECTOR SPECIFICITY OF MEXICAN BARLEY YELLOW DWARF VIRUS ISOLATES. B. van Os[1], R. Ranieri[2], R.M. Lister[3], P.A. Burnett[2]. [1] Plant Breeding Institute, University of Wageningen, Holland; [2] Wheat Program, International Maize and Wheat Improvement Center (CIMMYT), Apdo. Postal 6-641, 00600 Mexico D.F., Mexico; [3] Botany and Plant Pathology Department, Purdue University, W. Lafayette, IN 47907, USA.

Rhopalosiphum padi, *Metopolophium dirhodum*, *Sitobion avenae*, and *Rhopalosiphum maidis* were compared in greenhouse experiments as vectors of four Mexican isolates of barley yellow dwarf virus. The isolates - Mex-PAV, Mex-MAV, Mex-RPV and Mex-RMV - were PAV, MAV, RPV, and RMV serotypes, respectively. Aphids were allowed a 5-day acquisition feed on infected 'Centinella' barley plants, then transferred singly to each of about 250 plants for each aphid species, for a 5-day test feed. Infections among plants with surviving aphids (100-145 plants for each species) were assessed by ELISA. Mex-MAV was transmitted best by *M. dirhodum* (73% transmission) and *S. avenae* (44%); Mex-PAV by *R. padi* (60%), *M. dirhodum* (25%) and *S. avenae* (14%); Mex-RMV by *R. maidis* (52%) and *R. padi* (9%); and Mex-RPV by *R. padi* (8%). All other aphid/isolate combinations gave only 3% transmission or less.

A95

IMIDACLOPRID CONTROLS CEREAL APHIDS AND ALTERS BARLEY YELLOW DWARF EPIDEMIOLOGY. S. M. Gray[1] and G. C. Bergstrom[2]. [1] USDA, ARS, and [2] Dept. Plant Pathology, Cornell University, Ithaca, NY 14853

The effects of the seed applied insecticide, imidacloprid (NTN), on cereal aphid populations and on transmission and field incidence of barley yellow dwarf virus (BYDV) in winter wheat and spring oats were examined. In laboratory studies, NTN reduced adult aphid feeding time, fecundity, and survival. Aphid numbers in NTN-treated spring oat plots were reduced 93% relative to nontreated plots in 1991. Fewer BYDV symptoms were observed in treated than in untreated plots at stem elongation; but hot, dry weather precluded accurate assessment of late-season symptoms. The predominant aphid species and BYDV isolate were *Rhopalosiphum padi* and *Sitobion avenae* and PAV, respectively. Fall sampling of 1991 winter wheat plots revealed *R. maidis* and RMV to be the predominant aphid and BYDV isolate. Mean aphid counts/plot were 1.1 and 26.0 ($P < .001$) for NTN-treated and untreated plots, respectively, and 32% of field-collected alate *R. maidis* immigrants in treated or untreated plots transmitted RMV to indicator plants. Mean RMV incidence was 2% and 25% in treated and untreated plots, respectively.

A96

USE OF FLUORESCENT DYES TO TRACE ACTIVITIES OF APHIDS. P. E. Thomas and Shlomo Marco, USDA-Agricultural Research Service and Department of Plant Pathology, Washington State University, IAREC, Route 2 Box 2953A, Prosser, WA 99350-9687.

Labeling aphids with fluorescent dyes was a practical method to trace the dispersal patterns of both alatoid and apterous aphids. Aphid-infested source plants were dusted with powdered fluorescent dyes. The dyes adhered to the exoskeletons of the aphids. Dye-dusted aphids were then easily identified at a later date by their fluorescent glow under ultraviolet light. The dyes had no perceptible effect on aphid behavior. In flight chambers, about the same percentage of labeled and unlabeled alatae took flight, and their preference for white, yellow, or grey at landing was not affected as compared to unlabeled aphids from the same population. Labeling apterae did not affect aphid longevity, movement from plant-to-plant, or capacity to transmit potato leafroll virus (PLRV). This technology gives rise to new possibilities for elucidating the epidemiology and control of aphid-borne virus diseases.

A97

VIRUSES ISOLATED FROM COMMERCIAL OYSTER MUSHROOMS. R.R. Martin, A.M. Quail and F. Leggett, Agriculture Canada Research Station, Vancouver, B.C. V6T 1X2.

Pleurotus pulmonarius (oyster mushrooms) from a commercial grower in the Fraser Valley of British Columbia exhibited a severe deformation of basidiocarps in which virtually no bracts were produced and the stalks were swollen and greatly distorted. Attempts to purify virus from these diseased mushrooms yielded at least three different virus-like particles. There were spherical particles about 33 nm and 24 nm in diameter as well as bacilliform particles. There were five high molecular weight dsRNAs isolated from the diseased mushrooms exhibiting severe symptoms and none from healthy mushrooms. When purified on sucrose gradients the 33 nm sphere separated into two distinct fractions. The upper fraction contained three dsRNA bands and the lower fraction contained five dsRNA bands. Antisera prepared to particles from either fraction decorated particles from both the upper and lower fractions in immunosorbent electron microscopy. Antisera to the viruses from *Agaricus bisporus* (obtained from A.A. Brunt, Glasshouse Crops Research Institute) did not react with any with the particle types purified from *Pleurotus*. Also, antisera to the 33 nm spherical particle upper or lower sucrose gradient fractions did not decorate virus particles purified from *Agaricus bisporus*.

A98

ETIOLOGY OF CHERRY STEM PITTING DISEASE IN CALIFORNIA. Y-P. Zhang, *J. K. Uyemoto, and B. C. Kirkpatrick. *USDA/ARS and Department of Plant Pathology, University of California, Davis, CA 95616.

Cherry stem pitting (CSP) disease causes a rapid decline of sweet cherry (Prunus avium L.) trees in California. The disease can be graft transmitted but its causal agent is unknown. Extensive testing of CSP-infected trees using serological and nucleic acid hybridization assays that are specific for tomato ringspot (TmRSV) and tomato bushy stunt (TBSV) viruses were negative. A double stranded RNA (dsRNA), approximately 4.5 kb, was found in most CSP-infected, but not healthy, cherry trees. Complementary DNA was synthesized from CSP-associated dsRNA and cloned in E. coli. Twenty two unique clones were identified that hybridized with RNA from CSP-infected, but not healthy cherry or RNA from TmRSV- or TBSV-infected plants. Restriction enzyme mapping, hybridization and sequence analyses of the CSP-specific clones are underway.

A99

THE STRUCTURE AND BIOLOGICAL PROPERTIES OF THE SECONDARY METABOLITES OF THE APPLE SOOTY BLOTCH FUNGUS, *GLOEODES POMIGENA*. P. Venkatasubbiah, T. B. Sutton, and W. S. Chilton, North Carolina State University, Raleigh, NC 27695.

Gloeodes pomigena, the causal agent of sooty blotch of apple fruit, produced four metabolites in liquid cultures which were isolated and structurally identified as trichothecolone, trichothecolone acetate (TA), 6-methylsalicylic acid (MSA), and 2,5-dihydroxybenzoic acid. All four toxins caused necrosis at concentrations of 50 to 100 µg/ml when spotted on leaves of different apple varieties and eight weed species, thus the four toxins were not-specific. MSA and TA at 100 µg/ml were most phytotoxic to apple varieties and weed species, respectively. MSA and TA showed strong antifungal properties against species of *Botryosphaeria* and *Colletotrichum* in vitro. The zone of inhibition at 400 µg of TA was greater for both *B. obtusa* and *B. dothidea* than *C. gloeosporioides* or *C. acutatum*. The zone of inhibition increased with increasing rates of MSA for each fungus tested except *B. dothidea*. When detached fruit were treated with MSA and subsequently inoculated with *C. acutatum*, there was a significant decrease in the percent fruit pieces infected with increasing concentrations of MSA. The possible secretion of two mycotoxins, trichothecolone and TA on infected apple fruit and the role of MSA as a defense mechanism against other fungi on apple fruit will be discussed.

A101

ROSE ROSETTE DISEASE: SOME DISEASE-INDUCED CHANGES IN SYMPTOMATIC MULTIFLORA ROSE. A. H. Epstein, J. H. Hill, and W. A. Miller. Dept. Plant Pathology, Iowa State Univ., Ames, 50011.

Rosa multiflora Thumb. (multiflora rose), a thorny shrub introduced into North America from northeastern Asia, has naturalized and established itself on over 2 million acres of nontilled land in Iowa. Conventional methods of controlling this noxious plant have been used sparingly because of the high cost of cultural methods and negative environmental effects associated with chemicals. Rose rosette disease is lethal to multiflora rose and has been proposed as an agent for biological control of this plant. However, little is known about the nature of the causal agent or the manner in which the affected plant is killed. Infected shoots are characterized by rapid growth rates, degradation of chloroplasts, and rapid depletion of carbohydrate reserves. Other parts of the plant also undergo starch depletion and virtual cessation of growth. The disease was lethal to graft inoculated plants in the greenhouse in 18-23 months. In the field, symptomatic (naturally inoculated) plants are more subject to freezing injury than normal plants and rarely survived past the third year.

A102

Partition of soluble carbohydrates in plant organs of tolerant and non-tolerant wheat cultivars infected with Septoria tritici blotch. E. Zuckerman, A. Eshel, and Z. Eyal. Department of Botany, Tel Aviv University, Tel Aviv 69978, Israel.

Losses of 21.7% and 40.6% in kernel weight were recorded for similar AUDPCs under field conditions in the susceptible, spring bread wheat cvs Miriam (tolerant) and Barkai (non-tolerant), respectively infected with isolate ISR8036 of Septoria tritici. Quantitative analyses of carbohydrates extracted from roots, stems, leaves and spikes of infected and uninfected plants at 4 growth stages were performed by HPLC. Less than 5% of the total sugars in the plant were found in the roots. Significant differences in monosaccharides were recorded in the leaves between infected and uninfected for each cultivar and between the two cvs. The major part of the soluble carbohydrates in the stems were monosaccharides. All these makes the hypothesis that storage of oligosaccharides and their translocation to the spike at grain filling a less likely explanation of tolerance. The differences in carbohydrate content in the spikes of the two cultivars were of the same magnitude as the losses in kernel weight.

A103

ENHANCED DEPOSITION OF WOUND GUM IN RADISH ROOTS BY CHITIN, CALCIUM AND *PSEUDOMONAS SYRINGAE* PV TOMATO. R. R. Stange, Jr., J. W. Eckert. Department of Plant Pathology, University of California, Riverside, CA 92521.

Roots of *Raphanus sativa* var. 'Summer Cross Hybrid' were surface sterilized, sliced 2 mm thick, and incubated at 20 C in covered plastic trays lined with moist paper. Vascular lignin (VL) and pseudocicatrix were selectively stained with 1% Schiff's Reagent in aqueous 0.25% sodium meta-bisulfite for 2 h. Color of VL-free tissue was determined with a Minolta Chroma Meter R-100. Change in color (ΔE) was calculated using stained freshly-sliced tissue as a standard. Compared to water controls 48 h after treatment, 0.27 M $CaCl_2$, crab-shell chitin (4g/L), and ca. 10^7 cells/mL *Pseudomonas syringae* pv. tomato caused increases in ΔE of 1.42-, 1.55-, and 1.59-fold, respectively. ΔE began increasing 12 h after injury, and continued increasing for 4 d. Induction of phloroglucinol/HCl-positive compounds, extractable in 70% ethanol, followed a similar pattern.

A104

ANALYSIS OF PEANUT (*ARACHIS HYPOGAEA* L.) LEAFLETS FROM MATURE ZYGOTIC EMBRYOS AS A TARGET TISSUE FOR BIOLISTIC GENE TRANSFER. T. E. Clemente[1], J. A. Schnall[2], M. K. Beute[1] and A. K. Weissinger[2]. Departments of Plant Pathology[1] and Crop Science[2], North Carolina State University, Raleigh, NC 27695-7616.

Leaflets from mature peanut embryos are a useful recipient tissue for biolistic DNA transfer. Fertile plants were regenerated from leaflets representing all known botanical peanut types. A maximium of 25% of the cultured leaflets formed buds, while up to 12.1% of the leaflets formed plants. Estimates of integration frequencies of foreign DNA, based on the recovery of stably growing callus masses isolated from leaflet tissue on selection in which plasmid DNA (pRT99 GUS) was delivered by the biolistic process, ranged from 0.025 to 0.24 calli per leaflet. A subset of the callus lines generated (30) were characterized by PCR, ELISA and flourometery. PCR analysis revealed all 30 carried the selectable marker NPT II sequence of pRT99 GUS and 28 of the 30 carried the sequence for the assayable marker ß-GUS of pRT 99 GUS. ELISA and flourometry results demonstrated that all 30 lines expressed NPT II and 8 of the 30 co-expressed ß-GUS. Several plants have been regenerated under selection, but to date none appears to be stably transformed.

A105

RELATIONSHIPS BETWEEN POPULATIONS OF *Rhizoctonia solani* AG-2-2 FROM MAT RUSH, SUGAR BEET, CORN AND ST. AUGUSTINE GRASS. J. Stevens Johnk and R. K. Jones, Dept. of Plant Pathology, University of Minnesota, St. Paul, 55108.

Four populations of *Rhizoctonia solani* that cause sheath blight of mat rush (*Juncus effusus*) (AG-2-2 IIIB), crown and root rot of sugar beet (AG-2-2 IV), crown and brace root rot of corn (AG-2-2) and brown patch of St. Augustine grass (AG-2-2) were differentiated using cluster analysis and principal component analysis of fatty acids identified by gas chromatography. A dendrogram, based on cluster analysis of fatty acid compositions, shows that isolates from AG-2-2 IIIB and isolates from corn are closely related while isolates from turf are distinct from AG-2-2 IIIB and AG-2-2 IV. All isolates tested from these populations are auxotrophic for thiamine and each population has a unique but highly variable cultural appearance when grown on potato dextrose agar.

A106

ANTAGONISTIC EFFECT OF CHITINOLYTIC BACTERIA AGAINST TOXIN-PRODUCING FUNGI. P.A. Gay[1], K.V. Saikumar[1], T.E. Cleveland[2], and S. Tuzun[1], [1]Dept. of Plant Pathology, Auburn University, AL 36849, and [2]USDA Southern Regional Research Center, New Orleans, LA 70124.

Eight chitinolytic bacteria were tested *in vitro* for antagonistic effects against *Aspergillus flavus*, *A. parasiticus*, and *Fusarium oxysporum* f. sp. *moniliformae*. Bacteria were grown in the presence and absence of chitin for three days prior to the introduction of fungi. Antagonism was only found in the presence of chitin, not in the absence of chitin. Only *Bacillus cereus* (BP24a) inhibited growth of all three fungi, even though *Serratia marcescens* exhibited greater inhibition of *Aspergillus* sp. and *B. chitinosporus* exhibited greater inhibition of *Fusarium* than BP24a. Additionally, *S. marcescens* exhibited no inhibition of *Fusarium* and *B. chitinosporus* did not inhibit *Aspergillus* sp. Results may indicate a specificity between chitinases produced by the different bacteria and their enzymatic properties and antifungal activities against various fungi. Studies are underway to isolate the BP24a chitinase gene(s), express them constitutively in either secreted or nonsecreted forms in transgenic plant tissues utilizing engineered plant expression vectors, and determine the level of resistance of transgenic plant tissues to toxin-producing fungi.

A107

POPULATION DYNAMICS OF GRAPE EPIPHYTIC MYCOFLORA IN THE SAN JOAQUIN VALLEY AS INFLUENCED BY LEAF REMOVAL AND REDUCED PESTICIDE APPLICATIONS. R. A. Duncan[1], G. M. Leavitt[2], and J. J. Stapleton[1], University of California, Kearney Agricultural Center, Parlier, CA 93648[1] and Cooperative Extension, Madera, CA 93637[2].

Population dynamics of epiphytic fungi associated with the summer bunch rot complex of grapes (Vitis vinifera) in the San Joaquin Valley of California were monitored biweekly on berries of several wine grape varieties during 1989-92. Species of Cladosporium, Aspergillus, Penicillium, Geotrichum, and Alternaria predominated. Berries from vines sprayed with pesticides at 50 or 100% of recommended rates frequently had lower levels of epiphytic mycoflora than nonsprayed controls. Regardless of pesticide treatment, basal leaf removal resulted in more consistent reductions of epiphytic mycoflora. Incidence and severity of rot at harvest also tended to be lower in leaf removal treatments.

A108

EFFECT OF LEAF REMOVAL AND FUNGICIDES ON MANAGEMENT OF GRAPE POWDERY MILDEW. J. J. Stapleton, G. M. Leavitt, and P. S. Verdegaal. University of California, Cooperative Extension, Kearney Agricultural Center, Parlier, CA 93648.

Vines of wine grape varieties susceptible to powdery mildew (Uncinula necator) were subjected to basal leaf removal at several locations in the San Joaquin Valley of California, and compared to control vines. Fungicide spray treatments also were evaluated. Incidence and severity of mildew on clusters were effectively reduced by fungicides in all cases. Reduction of mildew by leaf removal occurred in two of the five experiments. Significant interactions between leaf removal and fungicides were sometimes found, and leaf removal improved spray coverage of fungicides. However, leaf removal alone should not be relied upon to provide adequate control of powdery mildew.

A109

EFFECT OF LEAF REMOVAL AND FUNGICIDES ON INCIDENCE AND SEVERITY OF BOTRYTIS BUNCH ROT IN THOMPSON SEEDLESS GRAPES IN CHILE. J.C. Broome, J.J. Marois, B.A. Latorre. Department of Plant Pathology, University of California, Davis, CA 95616 and Department of Pomology and Enology, Catholic University of Chile, Santiago, Chile.

Botrytis bunch rot of grape (cv. Thompson Seedless) was significantly reduced by leaf removal in 1991 and 1992 in the central valley of Chile. Leaf removal was used in a split plot design with or without fungicides (captan or vinclozolin). Leaf removal was performed 2 weeks after bloom. In Buin, leaf removal reduced disease incidence (percent diseased clusters) from 17% to 11.25% in 1991 and from 29.3% to 13.0% in 1992. Application of fungicides reduced disease incidence from 17.1% to 8.8% in 1991, and from 20.3% to 16.3% in 1992. Disease severity (percent diseased berries) was not reduced significantly by leaf removal in 1991, but was reduced significantly in 1992 from 0.42% to 0.13%. In San Fernando, leaf removal reduced disease incidence from 13% to 8.5% in 1991, and from 8% to 3.55% and in 1992. Disease severity was reduced by leaf removal from 0.05% to 0.02% in 1992. In Colina, leaf removal reduced disease incidence from 7.2% to 4.7% in 1991, and from 21.5% to 11.5% in 1992. Bunch rot severity was reduced by leaf removal in 1992 from 1.8% to 0.65%. Fungicides did not significantly reduce disease in San Fernando or Colina.

A110

USING SUMMER PRUNING TO REDUCE FLYSPECK AND SOOTY BLOTCH OF APPLE IN THE NORTHEAST. D. R. Cooley[1], C. Telgheder[2], W. A. Autio[3] and J. Gamble[1]. [1]Dept. of Plant Pathology, and [3]Dept. of Plant & Soil Sciences, Univ. of Massachusetts, Amherst, MA 01003, and [2]Cornell Cooperative Extension, Hudson, NY, 12534.

Flyspeck (FS) caused by *Schizothyrium pomi* (Mont. & Fr.) Arx and sooty blotch (SB) caused by *Gloedes pomigena* (Schwein.) Colby are summer fungal diseases of apples. Disease damage is primarily cosmetic, and disease pressure is generally low in the Northeast, but disease-incidence has increased significantly over the past 3 years and apple growers have been increasing fungicide applications to manage the problem. In response, we have examined summer pruning, a horticultural practice used to increase fruit color, as a technique which might also reduce incidence of FS and SB. Tests were run in in Massachusetts for two years, and in New York for 1 year. In two tests, fungicide treatments were also used in conjuction with pruining. In all tests, SB and FS were significantly reduced by summer pruning. In one test, the percentage of out-of-grade fruit were reduced 79% in non-pruned trees to 55% in pruned trees. Under lower pressure, infected fruit were reduced from 18% to 9%. Tests also indicated that factors such as fungicides and initial tree density are significant.

A111

MANAGING MELOIDOGYNE CHITWOODI (MC) ON POTATO WITH RAPESEED AND SUDANGRASS AS GREEN MANURE. H. Mojtahedi, G. S. Santo, R. E. Ingham, A. N. Hang, and J. H. Wilson. Washington State University, Prosser, WA 99350, and Oregon State University, Corvallis, OR 97331.

In greenhouse experiments, shoots and roots of rapeseed, and shoots but not roots of sudangrass reduced MC populations when incorporated into soil. The zone of incorporation was also protected from MC recolonization for 6 weeks. Second stage juveniles were more sensitive than egg masses to these treatments. In field experiments, incorporating rapeseed and sudangrass into soil as green manure reduced MC damage on potato tubers for two consecutive years. Augmenting green manure with nematicide ethoprop produced commercially acceptable potato crop, similar to fumigation with 1,3-dichloropropene.

A112

IRRIGATION MANAGEMENT OF POTATO EARLY DYING. M. R. Cappaert[1], M. L. Powelson[1], and N. W. Christensen[1], W. R. Stevenson[2], and D. I. Rouse[2]. [1]Dept. of Botany and Plant Pathology, Oregon State University, Corvallis, and [2]Dept. of Plant Pathology, University of Wisconsin, Madison.

Field trials were established in Oregon and Wisconsin to evaluate the effect of early season irrigation regimes on suppression of potato early dying in Russet Burbank potatoes. Treatments were three inoculum levels (0, 5 or 25 colony forming units of Verticillium dahliae/g of soil) and three irrigation regimes. The three irrigation regimes supplied 75, 100, or 125% of estimated consumptive use from planting to tuberization. After tuberization, plots were irrigated at 100% of estimated consumptive use. In Oregon, disease severity was suppressed two-fold in both the 75 and 100% irrigation treatments compared to the 125% irrigation treatment when disease was assessed in early and mid-August. Early season rains in Oregon negated any effect between the 75 and 100% irrigation regimes. In Wisconsin, disease was two to four-fold less severe in plants grown under 100 and 75% compared to the 125% water treatment. In Oregon, tuber yield was suppressed by 19% in the 125 compared to the 100% irrigation regime. In Wisconsin, yield was suppressed (8%) in the 100 compared to the 125% treatment.

A113

PRE-PLANTING MANAGEMENT OF COTTON SEEDLING DISEASE CAUSED BY RHIZOCTONIA SOLANI AG-4. S. M. Moustafa-Mahmoud[1], Mona M. Ragab[2], D. R. Sumner[1], and M. M. Ragab[2], University of Georgia, Coastal Plain Experiment Station, Tifton, GA 31793[1] and Department of Plant Pathology, Faculty of Agriculture, Cairo University, Giza, Egypt[2].

Lankart 57 was more susceptible to Rhizoctonia solani AG-4 than three multi-adversity resistant (MAR) cultivars, Tamcot CAB-CS, CAMD-E and SP-37. Variation in susceptibility within cultivars was associated with significant differences in germination percentage, seed vigor, seed weight, and fungi and bacteria isolated from interior or exterior seed tissues. Poor germination was eliminated by disinfesting seeds or seed treatment with fungicides except in Lankart 57. Linted seed increased percent stand in steamed soil compared with delinted seed. On the other hand, linted seed decreased plant numbers in infested soil. Clumping seedlings increased disease incidence in the field but not in pots. Pre-emergence herbicides increased disease severity in all cultivars in the greenhouse.

A114

INFLUENCE OF RYE RESIDUES ON DISEASES CAUSED BY RHIZOCTONIA SOLANI AG-4 AND AG2-2 IN A RYE-CORN-SNAP BEAN-RYE-PEANUT ROTATION. D. R. Sumner and D. K. Bell, University of Georgia, Coastal Plain Experiment Station, Tifton, GA 31793-0748.

Soil in field microplots was infested with Rhizoctonia solani AG-4, AG2-2, or noninfested, and planted in a 2-yr rotation of winter rye-corn-snap bean-winter rye-peanut in a factorial experiment that was continued for 2 cycles (4 yr). Residue treatments were incorporated separately into soil each spring before planting corn or peanut as follows: rye plants (roots + foliage), rye foliage (into fallow soil), rye roots, or no rye (fallow). In soil infested with AG-4, populations of AG-4 were high (avg 21 to 43 colony forming units (CFU)/100 g soil) for 16 mo, but declined to low levels after 2 yr. Populations of AG-4 were low (<2 CFU/100 g) in soil infested with AG2-2 or in noninfested soil, and AG2-2 was detected rarely in soil. Rye residue treatments did not influence populations of AG-4 or AG2-2 in soil, nor root disease severity in corn and snap bean or pod rot in peanut.

A115

Integration of cultural and biological methods for control of postharvest decay in pear. D. Sugar, R.G. Roberts, K.A. Powers, and S.A. Basile. Oregon State University, Medford, OR 97502.

Five cultural and biological factors influenced severity of postharvest decay of Bosc pears by Penicillium expansum (blue mold) and Phialophora malorum (side rot). Fruit nitrogen and calcium management, harvest maturity, postharvest yeast treatment, and storage atmosphere were integrated in a factorial experiment. Treatment with Cryptococcus laurentii resulted in the greatest reduction in decay. Nutrient management, harvest maturity, and storage atmosphere were more important factors in reducing decay severity in the absence of yeast. Early harvested, low nitrogen, high calcium fruit treated with yeast and stored in 2% oxygen had the least decay following 2-3 mo storage at 0 C. Optimum levels of cultural factors without yeast reduced severity of blue mold by 67% and of side rot by 97%.

A116

BACTERIAL BLIGHT OF CARNATION CAUSED BY PSEUDOMONAS WOODSII AND SUSCEPTIBILITY OF CARNATION VARIETIES. Nagata, N. and Trujillo, E. E. Department of Plant Pathology, University of Hawaii, 3190 Maile Way, Honolulu, HI 96822.

Pseudomonas woodsii blight of carnation was confirmed from 8 Maui farms by pathogenicity tests. Small, water-soaked, yellow specks were the initial symptoms on potted carnation plants, spray-inoculated with 2.9 x 10^6, and 4.5 x 10^8 cfu/ml, and they appeared 6-days after inoculation. Specks enlarged to 2-3 mm in dia forming discrete spots at the lower inoculum level, but at the higher inoculum level lesions coalesced, causing extensive blight at 10 days after inoculation. Plants inoculated with 10^6 to 10^8 cfu/ml, produced comparable number of lesions when incubated for 24-48 hr in a moist chamber and/or outdoor at ambient relative humidity. The lowest inoculum level to produce lesions, was 2.9 X 10^3 cfu/ml was. Aliette at 4,800 and 9,600 ppm ai significantly reduced leaf spot counts; however, it did not control the disease. In a field evaluation of 66 varieties, "Cal Red" and "Cal Improved White" showed high disease resistance.

A117

PATHOGENIC VARIATION AND DISTRIBUTION OF RACES OF *XANTHOMONAS CAMPESTRIS* PV *MALVACEARUM* (*XCM*) IN TEXAS COTTON. P. M. Thaxton and K. M. El-Zik, Dept. of Soil and Crop Sciences, Texas A&M University, College Station, Texas 77843.

Bacterial blight of cotton causes a 1% yield loss annually in the USA, and more than 50% loss during epidemics in Africa. Naturally infected plant tissues were collected from three regions in Southern and Central Texas, and *Xcm* was isolated and identified to race. Race identification of isolates was based on pathogenic reactions on a set of 10 upland (*Gossypium hirsutum*) host differential lines. Fourteen isolates were obtained and tested on cotyledons and true leaves. Race 18, the most virulent *Xcm* race, was the predominant race isolated from the 3 regions, and identified in 86% of the samples. Race 11 was identified from two isolates. A shift in races of *Xcm* has occurred in Texas, from races 1, 2 and 7 to the more virulent race 18. Growing cotton cultivars with horizontal resistance to all *Xcm* USA races will reduce disease incidence and severity, and control bacterial blight.

A118

INFECTION TYPES CAUSED BY *ZANTHOMONAS ORYZAE* PV. *ORYZAE* ON RICE NEAR ISOGENIC LINES. Bai, Jianfa and Mew, T.W. International Rice Research Institute, Los Baños, Laguna, Philippines.

Infection types were analyzed on eight near-isogenic lines for bacterial blight resistance in IR24 recurrent background. For compatible host-race combinations, expanding water-soaked lesions similar to those on the susceptible IR24 were observed after five days. For incompatible combinations, three infection types developed. Dark-necrotic lesions observed around the point of inoculation (pin-pricking) were on the flag and the three older leaves. As *Xa-3* conveys adult plant resistance, at tillering stage typical bacterial blight lesion was demonstrated. In lines carrying *Xa-4*, *xa-5* and *Xa-7*, chlorotic type was observed from tillering stage until flag leaf. However, as the plant grew older, restricted chlorotic lesion was developed on lines carrying *Xa-4*, while lines with *xa-5* and *Xa-7*, these were observed since tillering stage. Asymptomatic infection type was recorded on lines carrying *Xa-10* with races 2 and 5 from tillering to flag leaf stage, while those with *Xa-7* were asymptomatic on flag leaf only. Infection type was unaffected by temperature, inoculum concentrate and N fertilization.

A119

EPIDEMIOLOGY OF BACTERIAL STREAK AND ROT OF ONION, CAUSED BY PSEUDOMONAS VIRIDIFLAVA. R. D. Gitaitis, D. Sumner, D. Smittle, B. Maw, Coastal Plain Experiment Station, Tifton, Georgia, D. Gay, Cooperative Extension Service, Tifton, Georgia, B. Tollner, and Y. Hung, Georgia Experiment Station, Griffin, Georgia.

A medium (T-5) containing 5.0 g of NaCl, 1.0 g of K_2HPO_4, 1.0 g $(NH_4)H_2PO_4$, 0.2 g of $MgSO_4 \cdot 7H_2O$, 3.0 g of D-tartaric acid, 0.01 g of phenol red, and 20 g of agar per liter of medium and adjusted to pH 7.2 was used to recover Pseudomonas viridiflava. Selectivity was afforded with 10 mg of bacitracin, 6.0 mg of vancomycin, 75 mg of cycloheximide, 45 mg of novobiocin, 50 mg of penicillin G and incubation at 5 C. P. viridiflava was detected as an epiphyte but not in soil or irrigation water. Disease was more severe in plants receiving excess fertilizer through the winter. Furthermore, P. viridiflava was recovered consistently from atypical symptoms (tip burn) from plants receiving excess fertilizer but not from plants receiving recommended rates of fertilizer.

A120

A SIMPLE AND EFFICIENT METHOD TO ASSESS SUSCEPTIBILITY OF POTATO TO STEM ROT BY *ERWINIA* SPP. V. S. Bisht, P. S. Bains and J. R. Letal. Alberta Tree Nursery and Horticulure Center, R.R. # 6, Edmonton, Alberta, T5B 4K3 Canada.

A method using leaf petioles was developed for assessing susceptibility of potato to stem rot caused by *Erwinia* spp. Sterile autoclaved silica sand (50 g) in Majenta jars (GA-7 vessel, Majenta Corp., Chicago, Illinois) was drenched with bacterial suspension (16 ml) and freshly cut leaf petioles from 5 to 6-week-old plants were inserted to about 5 mm into the sand. The concentration of bacterial suspensions ranged from 2.6 x 10^1 to 2.6 x 10^8 cfu/ml. The Majenta jars with lids closed were kept on laboratory bench (20 ± 2 °C, and about 12 hours light). Length of rot was recorded 72 hours after inoculation. Linear regression of inoculum concentration on the lesion development had $R^2 > 0.80$. The method produced measurable stem rot lesions with a bacterial concentration as low as 2.6 x 10^4 cfu/ml and differentiated between susceptibilities of cultivars Russet Burbank and Sangre. Sangre was found to be more susceptible than Russet Burbank at all the inoculum concentrations used. The method is simple and sensitive, and could be used for large scale screening for stem rot resistance in potato.

A121

THE USE OF MONOCLONAL-ANTIBODY-BASED SERODIAGNOSTIC ASSAYS FOR DETECTION OF *CLAVIBACTER MICHIGANENSIS* SUBSP. *MICHIGANENSIS* IN SYMPTOMLESS TOMATO SEEDLINGS. A. G. Gharbi and S. T.Nameth. Dept Plant Pathology. Ohio State University. Columbus, OH 43210

A monoclonal antibody was used to detect *Clavibacter michiganensis* subsp. *michiganensis* (CMM), causal agent of bacterial canker of tomato. Symptomless seedlings were clorox-surface-disinfested, chopped in carbonate/bicarbonate buffer, and the extract used in two serological testing procedures. In greenhouse tests, CMM was detected in symptomless inoculated plants using Enzyme-Linked Immunosorbent Assay and Dot Immuno Blot methods. In a field survey, both methods detected CMM in 13 of 1,000 commercial transplants. CMM was isolated on mCNS, KBTS and TTC media from all positive greenhouse and field samples and all but one of the CMM suspected colonies elicited a hypersensitive-like reaction in *Mirabilis jalapa*. Identity of colonies was confirmed by fatty acid analysis. The use of serological methods for early, accurate and rapid detection of CMM is a promising technique for the control of this disease.

A122

CONTROL OF BACTERIAL CANKER OF TOMATO BY APPLICATION OF SOIL SOLARIZATION. Tjamos, E. C., Antoniou Polymnia, and Panagopoulos C. G. Agricultural University of Athens, Laboratory of Plant Pathology, Votanikos 118 55, Athens, Greece

Three year field trials in tomato plastic houses of west Greece have shown that application of soil solarization (1-2 months soil mulching with transparent polyethylene films) drastically reduces symptoms caused by *Clavibacter michiganensis* subsp. *michiganensis* throughout the cropping season. On the contrary soil fumigation with regular doses of methyl bromide (70 g/m^2) is ineffective in controlling the disease. Bacterial cultures of *C. michiganensis* subsp. *michiganensis* grown in NAG medium within covered bijoux bottles were embedded at various soil depths (5, 15, 25 cm) prior to the application of soil solarization. Weekly estimations of bacterial populations during mulching showed a sharp decrease or elimination of the pathogen in solarized compared to the untreated control plots. Bacterial isolates (belonging to the genera of *Pseudomonas*, and *Bacillus* and to the group of streptomycetes) obtained from the rhizosphere soil of tomato plants, were tested for their antagonistic activity against the pathogen. A large proportion of the tested isolates of streptomycetes grown in PDA and *Pseudomonas* grown in King's B medium were highly antagonistic, while very few *Bacillus* grown on PDA exercised a similar effect against the pathogen. Most antagonistic isolates obtained from solarized rhizosphere soil grew fairly well at relatively high growth temperatures (35 and 40 C). This suggests that soil solarization is not adversely affecting the survival of potential *C. michiganensis* subsp. *michiganensis* antagonists. The data constitute the first evidence for the control of the bacterial canker of tomato by application of soil solarization.

A123

MONITORING FLOWERS FOR PRESENCE OF EPIPHYTIC *ERWINIA AMYLOVORA* BY STIGMA STREAKING. S. V. Thomson. Department of Biology, Utah State University, Logan, UT 84322-5305.

The detection of epiphytic populations of *Erwinia amylovora* on flowers is a valuable technique in the prediction of fire blight and in the study of interactions between other microorganisms. A simple pistil-streaking technique was evaluated in Utah, New York and New Zealand as an alternative to the time-consuming and cumbersome washing techniques. The stigmas of flowers were gently touched or stroked onto the surface of a selective medium and the resulting colonies examined for *E. amylovora*. There was a high correlation between the percentage of colonized pistils and the actual population per flower as determined by washing. The technique is effective because the bacteria on flowers are invariably present on the pistils. Lower populations are present on other flower parts but usually only after precipitation. Flowers can either be taken into the lab for testing or evaluated *in situ* when nondestructive sampling is desired. This technique is currently used to predict the need for bactericide sprays and to evaluate potential control treatments.

A124

First report of Rhizomonas suberifaciens, Pathogen of Corky Root of Lettuce, from Australia. A. H. C. Van Bruggen and K. N. Jochimsen. Department of Plant Pathology, University of California, Davis, CA 95616.

Typical symptoms of corky root were observed on lettuce in Queensland, Australia. Slow-growing bacteria with similar colonies as strains of Rhizomonas spp. were isolated from soil adhering to lettuce roots with corky root symptoms using lettuce seedlings as bait. Crude lysate from 27 out of 119 strains had moderate to high homology to DNA from R. suberifaciens strain CA1, and lysate from three strains had slight DNA homology to Rhizomonas sp. strain WI4. All strains with homology to strain CA1 induced corky root on 1-wk-old lettuce seedlings, cv. Salinas, and tested positive with monoclonal antibody MAb-Rs1 specific for R. suberifaciens in an enzyme-linked immunosorbent assay. Strains with slight homology to strain WI4 were nonpathogenic, and tested negative with the antibody as did strain WI4. Sequences of 250 base pairs of 16S ribosomal DNA, amplified by polymerase chain reaction, confirmed that the 27 pathogenic strains belong to R. suberifaciens, while the other strains belong to different genera.

A125

ISOLATION OF GENETIC MARKERS SPECIFIC TO LINKAGE GROUPS IN *ASPERGILLUS FLAVUS*. K. R. Foutz, C. P. Woloshuk and G. A. Payne. Dept. of Plant Pathology, North Carolina State Univ., Raleigh, NC 27695-7616.

Through parasexual analyses, 30 genes in *Aspergillus flavus* have been mapped to 8 linkage groups. Mapped are loci for aflatoxin biosynthesis, spore color, and nutrient utilization. Our objective is to isolate marker genes for each linkage group and use these genes as probes to identify the corresponding chromosomes. To isolate linkage group marker genes, auxotrophic strains were transformed with a genomic library and complementing clones were recovered. To date, 6 marker gene clones have been recovered: *arg-2, lys-4, aro-4, pdx-6, leu-7* and *arg-7*. Cloned marker genes will allow us to establish an electrophoretic karyotype of *A. flavus* and to identify those chromosomes containing genes mapped for aflatoxin biosynthesis.

A126

THE ROLE OF THE *AFL*-2 GENE FROM *ASPERGILLUS FLAVUS* IN AFLATOXIN BIOSYNTHESIS. C. P. Woloshuk, D. Bhatnagar, T. E. Cleveland, G. J. Nystrom, and G. A. Payne. Plant Pathology, North Carolina State Univ., Raleigh, NC 27695-7616 and USDA/ARS/SRRC New Orleans, LA 70179.

We are isolating genes involved in aflatoxin biosynthesis in *Aspergillus flavus* by complementation of pathway mutants with a wild-type genomic cosmid library. Strains 650-33 and 656-2, blocked in aflatoxin biosynthesis by a mutation at allele *afl*-2, were complemented by a 32 kb cosmid (B9) to produce high levels of aflatoxin. Subcloning of the cosmid B9 revealed a 4.6 Kb fragment that complemented aflatoxin production in the mutant strains. A cDNA clone corresponding to a transcript from this DNA fragment was isolated. Genetic evidence indicates that the *afl*-2 gene is involved in aflatoxin biosynthesis prior to the formation of norsolorinic acid, the first stable intermediate in the pathway. Metabolite feeding studies suggest that *afl*-2 regulates gene expression or the activity of other aflatoxin pathway enzymes.

A127

EXTRACELLULAR CHITINOLYTIC ENZYMES PRODUCED BY *TRICHODERMA HARZIANUM*: PURIFICATION, CHARACTERIZATION AND MOLECULAR CLONING. Christopher Hayes, Matteo Lorito, Antonio Di Pietro and Gary Harman, Department of Horticultural Sciences, NYSAES/Cornell University. Geneva, NY 14456 USA.

Isolates of *Trichoderma harzianum* are effective biocontrol agents against a variety of phytopathogenic fungi. Many of these pathogens contain chitin as a structural component of their cell wall. Extracellular chitinolytic enzymes produced by *T. harzianum* may contribute to biocontrol. *T. harzianum* produces different types of chitinolytic enzymes, some with multiple forms. Several extracellular enzymes from strain P1 have been purified to homogeneity and partially characterized. *In vitro* assays demonstrated that these enzymes are strongly active against all chitin-containing pathogens tested. A cDNA library was prepared from mRNA of strain P1 and cloned into λgt11. A genetic sequence coding for one of the purified enzymes was detected with polyclonal antibodies and isolated.

A128

BIOLISTIC TRANSFORMATION OF *TRICHODERMA HARZIANUM* AND *GLIOCLADIUM VIRENS* WITH PLASMID AND GENOMIC DNA. Matteo Lorito, Christopher K. Hayes, Antonio Di Pietro, and Gary E. Harman. Department of Horticultural Sciences, Cornell University, NYSAES, Geneva, NY 14456 USA.

Three biocontrol strains belonging to the species *Trichoderma harzianum* and *Gliocladium virens* have been transformed for resistance to 300 µg/ml of Hygromycin B using particle gun bombardment. Different conditions and several plasmids have been tested in order to achieve an efficient transformation system. The highest number of transformants for all target strains was obtained with a plasmid containing a 2.4 kb homologous insert from *T. harzianum*. Moreover, a new approach was attempted to increase versatility of transformation systems for filamentous fungi. The genomic DNA from a previously transformed *T. harzianum* strain, containing integrated copies of the Hygromycin B resistance gene, (Eli Lilly and Co.) was used to bombard conidia of *T. harzianum* and *G. virens*. Putative Hygromycin B resistant transformants, possibly originating from integration of foreign genomic DNA, were recovered from both genera on selective media. These results suggest that both plasmids and fragments of genomic DNA can be successfully used for biolistic transformation of filamentous fungi.

A129

Molecular Analysis of the dsRNA Associated with Hypovirulence in a Michigan Strain of the Chestnut Blight Fungus *Cryphonectria parasitica*. C.M. Durbahn[1], D.L. Nuss[2] and D.W. Fulbright[1]. [1]Dept. Botany and Plant Pathology, Michigan State University, East Lansing, MI 48824. [2]Dept. Molecular Oncology and Virology, Roche Institute of Molecular Biology, Roche Research Center, Nutley, NJ 07110.

The presence of double-stranded RNA (dsRNA) molecules has been correlated with hypovirulence in the chestnut blight fungal pathogen *Cryphonectria parasitica*. We have made a cDNA library of a 9.0 kb dsRNA molecule from *C. parasitica* strain GH2, a strain isolated from a nonlethal canker on an American chestnut tree in Michigan. To date, sequence analysis of the cDNA clones has revealed an open reading frame of at least 6,540 nucleotides. The deduced amino acid sequence contains both helicase and polymerase motifs similar to those reported for a European dsRNA molecule isolated from *C. parasitica* strain EP713. Interestingly, except for these motifs, the Michigan dsRNA molecule thus far shows low nucleic acid and deduced amino acid identity to sequences derived from dsRNA molecules isolated from geographically distinct *C. parasitica* strains.

A130

ELECTROPHORETIC KARYOTYPE OF *CRYPHONECTRIA PARASITICA*. W.A. Powell, K.F. LoBuglio, and J.L. Beckerman. SUNY, College of Environmental Science and Forestry, Syracuse, NY, 13210-2788

To determine the electrophoretic karyotype of *Cryphonectria parasitica*, chromosomal-size DNA from lysed protoplasts was separated in an agarose gel using a CHEF-DRII (Bio-Rad Laboratories) electrophoresis system. Six bands were detected with estimated sizes of 8.4, 6.7, 5.2, 4.8, 3.8, and 3.2 megabase pairs (Mb). The top two bands were larger than the largest *S. pombe* chromosome size standard, therefore the estimated sizes for these bands are probably low. The 5.2 Mb band may be a doublet due to its higher staining intensity. Using a *Neurospora crassa* rDNA clone as a probe, the rDNA was detected on this 5.2 Mb band. There was no detectable variation in the banding pattern between five American and three European isolates of *C. parasitica* examined. The banding pattern was significantly different from the related pathogenic fungi, *C. gyrosa* and *C. cubensis*.

A132

SEQUENCE ANALYSIS OF TWO CLONED *FUSARIUM OXYSPORUM* TELOMERES. W.A. Powell and Z.H. Yan. SUNY, College of Environmental Science and Forestry, Syracuse, NY, 13210-2788

To determine the origins of the telomeres and origin of replication in the autonomously replicating plasmid, pFOLT4 (Powell and Kistler, 1990, J. Bact. 172:3163-3171), telomeres and adjacent sequences from *F. oxysporum* f.sp. *conglutinans* and *F. oxysporum* f.sp. *raphani* were cloned for comparison. One clone from each strain was obtained using anchored PCR techniques and then sequenced. The telomere repeats were identified with 28bps of adjacent sequences in each clone. The sequences from the two strains of *F. oxysporum* differed from each other by a single base pair. These 28bp sequences were 61% similar to sequences

adjacent to the telomere repeats in pFOLT4 and 57% similar to a sequence in the *Amp* gene of pUC. The similarities between the adjacent telomere sequences of the fungus and the pUC sequences may have caused the *in vivo* production of autonomously replicating plasmids from pUC derived fungal vectors. This 28bp sequence will tested for its ability to support autonomous replication in *F. oxysporum*.

A133

INVASION AND EXCLUSION AMONG INTRASPECIFIC BACTERIAL EPIPHYTES
Linda Kinkel and Steve Lindow, University of Minnesota, St. Paul, MN 55108 and University of California, Berkeley, CA 94720

We quantified invasion and exclusion abilities among Pseudomonas syringae (Ps) strains coexisting on leaves. 29 Ps isolates were inoculated onto plants in 107 pairwise combinations. All pairs were duplicated so that each strain in a combination was inoculated as both antagonist (day 0) and as challenge (day 3). Population size of each strain in a mixture was quantified on day 6 following incubation under moist conditions. Invasion and exclusion abilities, quantified by contrasting population sizes of challenge strains in the presence and in the absence of coexisting strains, varied significantly among Ps strains. Successful invaders were significantly less likely to exclude challenge populations than were non-successful invaders. Population sizes of successful excluders were negatively correlated with populations of coexisting challenge strains, while population sizes of successful invaders were positively correlated with those of coexisting antagonists. These data suggest mechanisms for invasion, exclusion and coexistence among intraspecific epiphytes.

A138

FREQUENCY, DISTRIBUTION AND VARIABILITY IN ISOLATES OF ENDOPHYTIC FUNGI FROM TOBACCO LEAVES.
Harvey W. Spurr, Jr., USDA-ARS, Crops Research Laboratory, P. O. Box 1168, Oxford, NC 27565 and North Carolina State University, Raleigh.

Leaf tissue samples (discs) were cut from field-grown tobacco in a randomized, replicated manner in 1991. Discs were surface sterilized and plated on Czapek's agar with 6% NaCl. Endophytic fungi were evenly distributed throughout mature, healthy leaves. Eleven spp.of endophytic fungi were frequently isolated. The two most frequently isolated fungi were Alternaria alternata in 86% of samples and Cladosporium cladosporoides in 37%. Frequency patterns were similar to previous observations in 1970. Endophytic A. alternata isolates from healthy tissue were generally nonpathogenic when compared to A. alternata isolates from brown spot lesions. C. cladosporoides isolates displayed a range in variability in culture. The impact of sampling technique on results will be discussed.

A141

T. R. Gottwald and J. H. Graham. A device for precise and non-disruptive stomatal inoculation of leaf tissue with bacterial pathogens. USDA/ARS, Orlando, Florida 32803, and University of Florida, CREC, Lake Alfred, Florida 33850.

A stomatal inoculation apparatus (SIA) was developed to produce water congestion of leaf tissues and provide a reproducible non-injurious means of introducing two Xanthomonas campestris pathovars of citrus into leaf tissues without wounding. The SIA consisted of a small inoculation chamber attached to an intact leaf. Water and/or inoculum were metered into an air stream and focused to impact on a one-mm diameter area of the leaf surface. Leaf tissues on the abaxial surface of 50-75 percent expanded Duncan grapefruit leaves were more susceptible to infection than other growth stages. Inoculum concentrations of 10^6 cfu/ml consistently induced infection and resulted in discrete individual lesions. Air stream impact pressures of 0.64 to 0.82 g/mm^2 against the leaf surface consistently produced tissue congestion and infection without wounding. These same pressures were the minimum threshold for increasing water volume in the leaf. From calculations of volume versus concentration of inoculum that enter a leaf via SIA, it was determined that as few as 2 cfu were necessary to cause a single lesion.

A142

STOMATAL INOCULATION AND GROWTH OF STRAINS OF *XANTHOMONAS CAMPESTRIS* IN CITRUS SPECIES VARYING IN SUSCEPTIBILITY TO CITRUS BACTERIAL DISEASES.
J. H. Graham, T. R. Gottwald, T. D. Riley, and D. S. Achor. Univ. of Florida, IFAS, CREC, Lake Alfred, FL 33850 and USDA-ARS, Orlando, FL 32803

A stomatal inoculation apparatus (SIA) was used to inoculate 2/3-expanded leaves of citrus species with *Xanthomonas campestris* pathovars. Penetration and growth of *X.c.* pv. *citri* and *X.c.* pv. *citrumelo* in leaves did not vary among species from 5-48 h. Populations continued to increase up to 168 h in citrus species susceptible to citrus canker, and in trifoliate orange and its hybrids susceptible to citrus bacterial spot (CBS). After 48-72 h, growth of *X.c. citri* was slower in species moderately resistant to canker and growth of *X.c. citrumelo* ceased in citrus species resistant to CBS. In susceptible species, lesion numbers were correlated with populations at 168 h but were not in species resistant to CBS. Resistance of citrus leaf tissue was expressed as a reduction in bacterial growth but not in the number of bacteria that penetrated through stomata and produced lesions.

A143

A SIMPLE MODEL TO DESCRIBE THE PECULIAR RESPONSE OF LATENT PERIOD TO TEMPERATURE FOR UROMYCES STRIATUS VAR. MEDICAGINIS IN ALFALFA.
R.D. Berger and D.A. Roberts, Plant Pathology Department, University of Florida, Gainesville 32611.

The latent period of Uromyces striatus var. medicaginis in alfalfa is strikingly responsive to small changes in temperature in the range of 21-29 C. The shortest latent period (7 days) occurred at a constant temperature of 24 C. Between 21 and 29 C, the latent period lengthened 1.3 days per each °C below the optimum and 1 day per each °C above the optimum. However, the latent period lengthened only 0.36 day per °C from 16 to 21 and from 29 to 33 C. A simple model, based on the relative latent periods at constant temperatures, was used to predict the length of the latent period at changing temperatures. The rates of latency for hourly intervals of recorded air temperatures were accumulated for appropriate time periods in natural epidemics of rust. The length of latent periods calculated with the model were in close agreement with those estimated from the breadth of waves of disease in the early stages of six epidemics in plots of alfalfa.

A144

THE EFFECT OF TWO TYPES OF INSECT INJURY ON THE SEVERITY OF SOYBEAN STEM CANKER.
G.B. Padgett[1], J.S. Russin[1], J.P. Snow[1], D.J. Boethel[2], and G.T. Berggren[1]. [1]Department of Plant Pathology and Crop Physiology and [2]Department of Entomology. Louisiana Agricultural Experiment Station, Louisiana State University Agricultural Center, Baton Rouge, LA 70803.

Field experiments were conducted during 1988-1990 to determine the effects of defoliation by Pseudoplusia includens (soybean looper) or basal stem girdles by Spissistilus festinus (threecornered alfalfa hopper), on soybean stem canker severity. To evaluate the effect of stem injury, soybean plants (R6) infected with Diaporthe phaseolorum var. caulivora were collected, 50 plants with basal stem girdles caused by S. festinus and 50 plants without girdles. The effect of defoliation was evaluated using cage studies with P. includens in a soybean field where stem canker epidemics were occurring. Defoliation was controlled by placing larvae in screened cages surrounding selected areas of soybean (V6). Larvae were allowed to feed for 14 days and then removed. Stem canker severity was greater and plant height, stem diameter, and yield were lower in plants with main stem girdles compared to plants without girdles. Stem canker severity, number of perithecia, yield, stem height, and stem diameter were lower in defoliated soybean, compared to soybean not defoliated. This suggests that the type of insect injury can affect stem canker severity either positively or negatively.

A145

EFFECT OF CULTIVAR MIXTURES ON POPULATIONS OF *PUCCINIA STRIIFORMIS* RACES.
J.A. DiLeone and C.C. Mundt, Oregon State University, Department Of Botany And Plant Pathology, Cordley Hall 2082, Corvallis, OR 97331-2902.

We investigated the potential for cultivar mixtures of wheat to increase the frequency of *Puccinia striiformis* races with complex virulences. All mixtures were inoculated with the same three races of the pathogen, but the specific cultivars included in the mixture determined the number of host genotypes each race could infect. Complexity does not seem to be the strongest determinant of a race's frequency in the pathogen populations.

A146

EFFECTS OF HOST CULTIVAR, TEMPERATURE, AND INOCULUM CONCENTRATION ON CARNATION RUST CAUSED BY *UROMYCES DIANTHI*.
M. Polek and D. M. Ferrin, Department of Plant Pathology, University of California, Riverside 92521.

Disease incidence (DI, proportion of leaves infected) and severity (DS, # pustules/plant) were determined for 8 carnation cultivars incubated at 10, 15 or 20 C. Main effects of cultivar and temperature were significant ($P < .05$) for both DI and DS, but the cultivar X temperature interaction was significant only for DS. DI and DS were also determined for 2 cultivars inoculated with 10^3, 10^4 or 10^5 urediospores/ml and incubated at 10, 15 or 20 C. Main effects of cultivar, inoculum concentration (IC), and the cultivar X IC interaction were significant for

both DI and DS. The interaction between temperature and inoculum concentration was significant for DI only. Slopes of the regressions of $\log_{10}$ (DS+1) on $\log_{10}$ (IC) were 0.67, 0.63 and 0.43 for 'Nora' and 0.84, 0.72 and 0.79 for 'Improved White Sim' at 10, 15 and 20 C, respectively.

A147

A STATISTICAL TECHNIQUE FOR THE IDENTIFICATION AND CHARACTERIZATION OF POTENTIAL INFECTION PERIODS OF FOLIAR FUNGAL PATHOGENS H. Scherm and A.H.C. van Bruggen, Department of Plant Pathology, University of California, Davis 95616.

We present a stepwise procedure for the identification and subsequent characterization of potential infection periods (PIPs) of foliar fungal pathogens based on epidemiological and meteorological field data. The method involves the following steps: (i) use a degree-day model for latent period to identify PIPs from disease progress data; (ii) perform stepwise and canonical discriminant analyses to identify the most important environmental variables for differentiating between PIPs and days without infection; (iii) perform a path coefficient analysis to unravel causal relationships among the environmental variables and with respect to their importance for infection. Using this approach, the duration of leaf surface wetness in the morning (LWDM) was identified as the most important single variable for infection of lettuce by *Bremia lactucae* in California in 1991. LWDM was $\geq$ 4 h (mean: 4.5 h) for 75% of the PIPs, and $\leq$ 3 h (mean: 1.6 h) for more than 90% of the days without infection.

A148

THE INFLUENCE OF POTASSIUM FERTILITY ON THE DEVELOPMENT OF ALFALFA FOLIAR DISEASES. K.M. Emery and J.T. English, Dept. of Plant Pathology, Univ. of Missouri, Columbia, 65211.

Little is known of the influence of soil fertility on foliar diseases of alfalfa. To determine if the form of K fertilizer influences the development of foliar disease, five fertilizer treatments were replicated in a two-year-old alfalfa stand. Treatments included K in combinations with Mg and S. Sample shoots were harvested weekly through three four-week cutting cycles in 1991. Foliar disease and plant growth information were assessed on harvested shoots. The 1991 growing season was relatively warm and dry, and disease levels were low. A maximum of 4.5% of leaf area was diseased in any of the fertility treatments. No consistent significant relationships of plant growth or disease development to fertility treatment were observed; however, in all treatments disease levels were significantly higher in the lower portion of the plant canopy than in the upper portion.

A149

Analysis of dispersal mechanisms and spatial distribution of *Colletotrichum gloeosporioides f. sp. aeschynomene* in rice. X.B. Yang and D.O. TeBeest. Dept. of Plant pathology, University of Arkansas, Fayetteville, Arkansas 72701.

Colletotrichum gloeosporioides f. sp. aeschynomene is a fungal pathogen used as a commercial mycoherbicide to control northern jointvetch (NJV) in rice. Four dispersal mechanisms have been identified, including splashing rain, flood irrigation, grasshoppers, and green treefrogs. Efficacy of each mechanism was examined under controlled conditions in a wind tunnel, an irrigation tunnel, and vector-weed-rice cages. Dispersal by rain is more efficient than irrigation water, but is limited to short distances in rice. About 20% of grasshoppers from diseased plants caused infections after feeding on healthy plants. Treefrogs were efficient vectors, and they preferred NJV to rice as shelters. Spatial studies showed that NJV plants were patchily distributed in rice. The four mechanisms were effective for dispersal within a patch but not for dispersal between patches. In fields with natural infestations, the incidence of diseased plants varied among patches from 0 to 90%. Application of inoculum by air overcomes dispersal barriers of patchy weed distribution.

A150

INFLUENCE OF DEW PERIOD ON CELERY LATE BLIGHT (*SEPTORIA APIICOLA*) LESION DEVELOPMENT. M. L. Lacy, Department of Botany and Plant Pathology, Michigan State University, East Lansing, MI 48824.

Duplicate ten-week-old potted celery plants (cv. Florida 683) were sprayed with an aqueous suspension of *Septoria apiicola* conidia (10^6/ml) at the rate of 8-9 ml/plant. Conidia (75-95% viable on water agar) were obtained from air-dried field-collected infected celery leaves stored at 4 C. Inoculated plants were immediately placed in an unlighted Percival model I-35DL dew chamber at 21 C ±1, removed after various time periods, and then placed in a lighted growth chamber (12 hr photoperiod) also at 21 C ±2. Lesions appeared after 8 days and new lesions could be found up to 21 days after inoculation. Mean lesion numbers/leaflet increased with increasing dew periods, and increased over time. Significant numbers of lesions occurred after 12 hr continuous dew. The highest numbers of lesions/leaflet occurred with a 24 hr wet-12 hr dry-24 hr wet regime (>8 lesions/leaflet), more than twice that attained with 36 hr continuous dew.

A151

CHARACTERIZATION OF ENDEMIC AND NON-ENDEMIC AREAS FOR TUNGRO IN THE PHILIPPINES. S. Savary, N. G. Fabellar, and P. S. Teng. Joint ORSTOM-IRRI Project, International Rice Research Institute, P.O. Box 933, 1099 Manila, Philippines.

Rice tungro historical data from two endemic and one non-endemic areas in the Philippines have been analyzed. Four categorized variables, the planting date, the cropping season, the total vector population, and the proportion of viruliferous vectors were considered to characterize the variation in tungro incidence, using correspondence analysis. Maximum tungro incidence was associated with intermediate planting dates, whereas absence of tungro was associated with very late or very early planting dates. High proportion of viruliferous vectors can compensate moderate vector population size in the endemic areas. Epidemic years in the non-endemic area are primarily associated with the occurrence of viruliferous vectors, the size of the vector population playing a secondary role only. The analyses suggest that the non-endemic area is more responsive to inoculum, when present, than the endemic areas in producing tungro outbreaks.

A152

AGGREGATION OF *COLLETOTRICHUM ACUTATUM* IN RESPONSE TO SIMULATED RAIN EPISODES. L. V. Madden, Dept. of Plant Pathology, Ohio State Univ., Wooster, 44691.

The influence of rain splash dispersal on aggregation and variability of spores of *Colletotrichum acutatum*, causal agent of strawberry anthracnose, was studied with simulated rain over a soil surface. Sampling plates were positioned under rainshields at both 20 and 60 cm from an inoculum point source for 1-min exposure periods. Number of colonies growing in plates were used to measure spore density. Lloyd's index of patchiness (LIP) exceeded 1, indicating the high variability and clustering of colonies and, hence spores, resulting from splash dispersal. LIP increased with distance from the source and with rain intensity at the greater distance. Taylor's power law described the linear relation between ln(variance) and ln(mean). The slope (b) was 1.67, a further indication of clustering. Results indicate that the physical process of splash dispersal produces aggregation similar to that generated by population dynamic and other ecological processes.

A153

SPATIAL AND TEMPORAL PATTERNS OF ANASTOMOSIS GROUPS OF *RHIZOCTONIA SOLANI* FROM DISEASE PATCHES OF NATURALLY INFECTED TALL FESCUE. E.R. Champaco and J.D. Mihail, Dept. of Plant Pathology, Univ. of Missouri, Columbia, 65211.

Spatio-temporal patterns of anastomosis groups (AGs) of *R. solani*, causal agent of brown patch of tall fescue (*Festuca arundinacea*), were examined in naturally infected plants. An area (25-m^2) was established in May 1991 on a four-year-old stand of cultivar 'Hound Dog'. Samples of infected leaves and soil from within and outside disease patches were taken twice weekly to identify the AGs present. Preliminary characterization of isolates indicated that although AG 1 and AG 2-2 were present in leaves expressing symptoms, AG 1 was predominant within patches at each sampling date. Both AGs were also present outside patches, but isolates of AG 2-2 were obtained at higher frequencies than were isolates of AG 1.

A154

Sampling Design for Monitoring Canopy Air Temperature in Vineyards. J.A. Duthie and J.J. Marois. Dept. of Plant Pathology, Univ. of California., Davis, CA 95616.

Precise estimates of air temperature (AT) within grape canopies are needed to forecast outbreaks of powdery mildew. The number and spatial arrangement of electronic sensors for continuous monitoring of AT were evaluated on a 2,000-ha ranch comprised of 38 contiguous vineyards. In each of 3 equal regions, AT was sampled using a multi-stage design with 1, 2, or 3 vineyards/region, 1 or 2 sites/vineyard, and 2 vines/site (20 vines total). A thermistor, which was housed within a radiation shield and suspended at the height of grape clusters, measured AT every 15 min during a 150-day period (May-Sept). Daily mean AT (DMAT) was computed for each day. Averaged over all vines on the ranch, DMAT ranged from 10.7 to 28.4C but there was no evidence that precision depended on DMAT. For any given number of vines/vineyard, precision was not improved by sampling more than 1 site/vineyard because variation due to sites was negligible after accounting for variation among vines. On most days, the greatest source of variation was due to vineyards. Average DMAT on the ranch usually would have been estimated within 0.5C ($p = 0.05$) by sampling 5 vineyards, 1 site/vineyard, and 2 vines/site.

A155

STATISTICAL MODELS FOR 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, OH 44691.

When disease incidence (proportion of individuals infected) is recorded, it is often appropriate to specify a logistic linear model with a binomial error distribution for the statistical analysis of the resulting data. Maximum likelihood estimates of parameters can then be obtained. However, if the data are aggregated (as is usually the case with epidemiological data), this analysis will be misleading. Standard errors will be too small, and tests of significance for experimental effects will therefore indicate lower probability levels than are warranted. Suggested methods of dealing with this problem using variance-mean relationships as descriptions of aggregation are illustrated with data from virus-infected tobacco crops.

A156

GROWTH OF FLUORESCENT PSEUDOMONADS IN THE RHIZOSPHERE UNDER CONTROLLED SOIL ATMOSPHERES. D.H. KIM, I.J. MISAGHI, and E.A. PIERSON, Department of Plant Pathology, University of Arizona, Tucson, AZ 85721.

The growth of four isolates of fluorescent pseudomonads in the rhizosphere of tomato and cucumber seedlings was determined under soil atmospheres containing O_2:CO_2 percentages of 21:0.03 (ambient atmosphere), 18:3, 15:6, and 12:9. Growth responses varied depending on the isolate, the plant, and the composition of the soil atmosphere. In general, growth was greater under atmospheres in which the O_2 concentration was below 21 % and the CO_2 concentration was above 0.03 %. As compared with growth under the ambient condition, the average growth of the isolate most responsive to changes in the soil atmosphere increased by 17, 52, and 117 % under soil atmospheres containing O_2:CO_2 percentages of 18:3, 15:6, and 12:9, respectively.

A157

GROWTH OF PSEUDOMONADS IN SINGLE AND DUAL STRAIN INOCULATIONS ON SUGAR BEET SEED IN SOIL AND ITS EFFECT ON CONTROLLING PERICARP INVASION BY *PYTHIUM* SPP.
R. Fukui, J. G. Hancock and M. N. Schroth. Dept. Plant Pathology, University of California, Berkeley, CA 94720

Two distinct growth patterns among pseudomonads were observed on sugar beet seed planted in soil. When inoculated singly on the seed at initial densities of 10^4 CFU/seed, some strains showed short (0-4 hr) lag phases (SL strain) whereas others had long (up to 12 hr) lag phases (LL strain). Populations of all strains plateaued at 10^6-10^7 CFU/seed within 24 hr. When inoculated at $>10^6$ CFU/seed, populations did not increase or slightly decreased over 48-hr. The incidence of pericarp invasion by *Pythium* spp. was lower in seeds treated with SL than LL strains, and lowest at the highest initial inoculum densities on the seed. When different combinations of two SL strains were inoculated on the seed at different initial densities, the population of the strain applied at lower density either increased up to 40-fold or did not increase over 48-hr, depending on the combination. The effect of dual strain inoculation on controlling pericarp invasion by *Pythium* spp. was no better than that of single strain inoculation.

A158

Suppression of take-all by *Trichoderma koningii* used individually and in combination with fluorescent *Pseudomonas* spp. Brion K. Duffy[1] and David M. Weller[2], [1]Dept. of Plant Pathology, Washington State University and [2]USDA-ARS Root Disease and Biological Control Research Unit, Pullman, WA 99164-6430.

Trichoderma koningii significantly reduced the severity of take-all, caused by *G. g.* var. *tritici*, in repeated growth chamber and field trials when applied as colonized rye-grass seeds and was consistently more suppressive than fluorescent *Pseudomonas* spp. *T. koningii* reduced the number of infected crown roots by 40% and increased yield by 70% as compared to the nontreated check at Pullman and Mt. Vernon, WA, respectively. Mixtures of *T. koningii* and fluorescent *Pseudomonas* spp. provided substantially better take-all control than the bacteria used alone, but only slightly better disease control than *T. koningii* used alone. In growth chamber experiments, *T. koningii* and *P. aureofaciens* 30-84 reduced take-all by 57 and 9%, respectively, when used individually and by 61% when used in combination. The performance of *T. koningii* was significantly influenced by the dosage of inoculum and by the method of application. At a ratio of 0.1:1 (w/w) of *T. koningii* rye-grass inoculum to *G. g. tritici* oat-kernel inoculum in the soil, significant take-all suppression occurred, but control was best at a ratio of 1:1. Disease suppression was greater when *T. koningii* was applied to the soil as compared to the seed.

A160

INDUCED RESISTANCE IN THE BIOLOGICAL CONTROL OF PYTHIUM APHANIDERMATUM BY PSEUDOMONAS SPP. ON EUROPEAN CUCUMBER. T. Zhou, L. Rankin, and T. C. Paulitz. McGill University, Macdonald Campus, Ste. Anne de Bellevue, Quebec, Canada H9X 1C0

Cucumis sativus L. cv. Corona were grown in a split root system using 10-cm pots filled with granular rockwool. Four weeks after seeding, one pot of each plant was treated with 75,000 zoospores of Pythium aphanidermatum (Pa) or water and the other pot was treated with 10^6 cells/cm^3 rockwool of Pseudomonas corrugata isolate 13 or P. fluorescens C isolate 15. Disease symptoms on the crowns of bacteria-treated plants were delayed by 5-10 days, compared to plants treated with Pa alone. Disease severity in bacterial treatments at 2, 3, and 4 wks after inoculation was also reduced. The simultaneous treatment of separate pots with Pa and Isolate 15 resulted in greater plant height, total leaf area, fruit number, root volume, and root dry weight. The population density of Pa was significantly reduced after one week in all bacterial treatments. This is the first report of systemic resistance to Pythium spp. induced by Pseudomonas spp., with both the pathogen and biocontrol agent applied to the root.

A161

HAINESIA LYTHRI, A POSSIBLE BIOCONTROL AGENT FOR THIMBLEBERRY IN BRITISH COLUMBIA FORESTS. S. F. Shamoun and B. E. Callan, Forestry Canada, Pacific Forestry Centre, 506 W. Burnside Road, Victoria, British Columbia V8Z 1M5, Canada.

Diseased leaves and stems of thimbleberry (Rubus parviflorus Nutt.) were collected north of Trail, British Columbia (B.C.) for fungi potentially useful as mycoherbicides. One such, Hainesia lythri (Desm.) Höhnel fungus was isolated from blotched leaves. This is a new host record, and the first report of this fungus in B.C. The synanamorph, Pilidium concavum formed on PDA, but only the Hainesia state was present in nature. To determine pathogenicity, unwounded and wounded (pressed on sand paper or rubbed with abrasive celite), thimbleberry leaves were inoculated with conidia ($1x10^6$/mL) and enclosed for 48 hr in plastic bags in a growth chamber at 20°C/16 hr day, 15°C/night. One week later leaves were rated on a 0-4 scale for disease severity, where 0=no symptoms and 4=75-80% of leaf surface with lesions. Wounded leaves had significantly ($P=0.05$) more lesions than unwounded leaves. The results, suggest the potential of H. lythri, combined with a wounding agent, as a biocontrol for thimbleberry.

A162

RESTRICTION OF DNA AMPLIFIED FROM AFLATOXIN PRODUCING AND NONPRODUCING STRAINS OF ASPERGILLUS. D. S. Egel and P. J. Cotty, USDA, ARS, Southern Regional Research Center, P.O. Box 19687, New Orleans, LA 70179.

The Aspergillus flavus group is a collection of fungal species and strains which vary widely in ability to produce aflatoxins. Relationships among members of this group are being characterized to test questions on phyletic evolution between atoxigenic and toxigenic members. Using a published set of primers (T.J. White et al., PCR protocols), a fragment of mitochondrial rDNA approximately 1260 bps in length was amplified from fungal genomic DNA of the above group and A. nidulans for comparison. The amplified DNA was restricted with the endonucleases Hae III, Msp I, Sau 3A and Taq I. It was not possible to discriminate among A. flavus group species, but A. nidulans could be differentiated.

A163

BIOCONTROL OF CITRUS ROOT ROT USING SOILBORNE BACTERIA. J. K. Turney, J. A. Menge, C. H. Yang, and D. A. Cooksey. Department of Plant Pathology, Univ. of Calif., Riverside 92521.

Bacterial isolates (1200) were collected from rhizosphere soil of 12 citrus groves throughout California by baiting with mycelial mats of *Phytophthora parasitica* (Pp). A common characteristic of these isolates is that they attach to the hyphae of *Phytophthora in vitro*. Growth of Pp was inhibited by 141 isolates *in vitro* on PDA and/or PAF media. Groves with high levels of Pp yielded an average of 20 Pp antagonists while groves with low levels of Pp yielded an average of 6 antagonists. Propagule densities of Pp in the rhizosphere of citrus seedlings were reduced 67-96% by each of 8 isolates compared to the controls. Four isolates reduced propagule densities of *P. citrophthora* 89-96% in the rhizosphere of citrus seedlings. Tn*5* mutants of *Pseudomonas fluorescens* (Pfl) deficient either for attachment to *Phytophthora* or siderophore production were made. Pfl mutants were similar to the wild type in their biocontrol ability.

A164

INTERACTION OF PHYLLOPLANE BACTERIA IN THE ENHANCEMENT OF DISEASE CAUSED BY THE BIOHERBICIDE, *COLLETOTRICHUM COCCODES* ON VELVETLEAF. W.G.D. Fernando, A.K. Watson, and T.C. Paulitz, Department of Plant Science, Macdonald Campus of McGill University, Ste. Anne-de-Bellevue, Quebec, Canada H9X 1CO.

Colletotrichum coccodes (Wallr.) Hughes. is a bioherbicide on velvetleaf (*Abutilon theophrasti* Medik.), causing foliar lesions and/or plant mortality. Nine bacterial isolates (mostly fluorescent *Pseudomonas* spp.), from a collection of 203 isolates from the phylloplane of velvetleaf, were tested for their effects on disease caused by *C. coccodes*. When leaves of velvetleaf were treated with bacterial suspensions (10^8 cfu ml^{-1}), five of nine isolates increased the number of lesions and disease severity on leaves inoculated 48 hrs later with C. coccodes (1 X 10^8 conidia ml^{-1}), compared to leaves not treated with bacteria. When true leaves were treated with bacteria, symptoms of C. coccodes infection were expressed 4-6 days earlier, compared to plants not pre-treated with the bacteria. Studies are under way to determine the mechanism(s) involved.

A165

J.J. Morrell and C.M. Sexton. Effect of environmental variables on performance of bioprotectants against wood staining fungi. Dept. of Forest Products, Oregon State University, Corvallis, OR.

Bioprotection against wood staining fungi represents an excellent method for reducing dependency on chemical biocides, however, most field trials of potential organisms have produced inconsistent results. In this study, the effects of media, pH, temperature and method of wood sterilization on bioprotectant performance were evaluated using Pseudomonas cepacia, Pseudomonas putida, Bacillus subtilis and Trichoderma harzianum. T. harzianum was the most effective bioprotectant under a variety of conditions. Media consistently altered performance as did pH. Staining on wood sterilized by steaming or irradiation was consistently lighter than that found on unsterile wood. The results suggest that a variety of subtle factors can affect bioprotectant performance. Considerable research will be required before successful bioprotection against fungal stain of wood becomes reality.

A166

OPTIMIZING NUTRITIONAL CONDITIONS TO MAXIMIZE CONIDIAL YIELD AND EFFICACY OF THE BIOHERBICIDE COLLETOTRICHUM TRUNCATUM. M. A. Jackson and D. A. Schisler. USDA-ARS, NCAUR, Peoria, IL 61604.

Colletotrichum truncatum NRRL 13737 is a fungal plant pathogen which shows promise as a bioherbicide against Sesbania exaltata. Our previous studies showed that the carbon-to-nitrogen (CN) ratio of the conidiation medium had a significant impact on conidial yield and efficacy (CN=30:1, high yield; CN=10:1, more efficacious). In this study, conidia produced in semi-defined media with CN ratios between 30:1 and 10:1 were compared. Conidial yields in media with CN ratios of 25:1, 20:1, and 15:1 were like those in "30:1" media ~1.8 X 10^7 conidia/mL). "20:1" and "15:1" conidia possessed attributes similar to "10:1" conidia (protein content, germination rate, frequency of appressoria formation) but were frequently less efficacious in inciting disease in S. exaltata. Yield and efficacy data suggest that a medium with a CN ratio of 15:1 may be optimal for cost-effective conidia production.

A167

VISUAL PARTITION OF P (GLIOVIRIN) AND Q (GLIOTOXIN) STRAINS OF GLIOCLADIUM VIRENS ON SELECTIVE MEDIA. C. R. Howell, USDA-ARS Southern Crops Research Lab, Rt.5, Box 805, College Station, Texas 77845.

Strains of Gliocladium virens can be separated into the P group (gliovirin producers) or the Q group (gliotoxin producers) on the basis of their growth and sporulation on differential agar media. On PDA containing 0.5 µg/ml benlate P strains grow well, whereas the growth of Q strains is restricted. On PDA containing 60 µg/ml of gliotoxin Q strains grow well and sporulate profusely, whereas the growth of P strains is reduced and sporulation is severely restricted. Strains within the P group can be further segregated on PDA containing 1 µg/ml of flusilazol, which restricts the growth of some strains much more than others. These phenotypic markers should facilitate the transfer of genes coding for antibiotic synthesis and other characters from strains in one group to those in another.

A168

EFFICACIES OF *Sclerotinia sclerotiorum* MUTANTS FOR BIOLOGICAL CONTROL OF WEEDS. R. V. Miller, K. A. Glass, M. K. McCarthy, and E. J. Ford, Montana State University, Bozeman, MT 59717.

Results continue to strongly support the development of *Sclerotinia sclerotiorum* as a biocontrol of weeds. Turf plots located in Montana, Mississippi, and New Zealand were treated with non-sclerotial and auxotrophic mutants, and the wild-type fungus to determine efficacies on; dandelion, buttonweed, mock strawberry, dicondra, white clover, plantain, spotted knapweed, and Canada thistle. In Mississippi, up to 90 percent control was achieved on six of the eight weed species with the wildtype fungus and up to 80 percent control with mutants. Additional mutants demonstrated efficacies comparable to and possibly superior to the wild-type in the greenhouse and will be tested during the 1992 field season.

A170

EVALUATION OF GRANULAR SODIUM ALGINATE FORMULATIONS OF *SCLEROTINIA MINOR* AS POTENTIAL BIOLOGICAL CONTROL AGENT OF TURF GRASS WEED SPECIES. **S. C. Brière, A. K. Watson, and T. C. Paulitz. McGill University, Ste-Anne-de-Bellevue, Québec, Canada, H9X 1C0.**

Sclerotinia minor Jagger, has been shown to be a potentially effective biological control agent against dandelion (*Taraxacum officinale* L.), two species of plantain (*Plantago major* L., *P. lanceolata.* L.) and other turf weed species. Research was undertaken to develop a granular sodium alginate based formulation with *S. minor*, which would retain viability when stored dry at room temperature for an extended period. Two different formulations were tested; the first consisting of a suspension of 1% sodium alginate, 6% kaolin clay, and 11% expended growth medium, and the second consisting of 1% sodium alginate, 4% kaolin clay, 2% ground wheat bran and 11% expended growth medium. Both mixtures were amended with 55 gL^{-1} homogenized mycelium of *S. minor*. The mycelial-alginate mixtures were pelletized by dropwise addition into solutions of either 0.25 M $CaCl_2$ or 0.1 M Ca gluconate and dried in a laminar flow cabinet for 48 hours. These formulations were tested for viability and rate of spread from each pellet at weekly intervals. The ability of each formulation to kill the host was also evaluated for *T. officinale* and *P. major* under controlled environmental conditions.

A171

POPULATION GENETICS OF *PHYTOPHTHORA INFESTANS* IN POLAND (1985-1991). L.S. Sujkowski, Linda J. Spielman and W.E. Fry. Department of Plant Pathology, 334 Plant Science, Cornell University, Ithaca, NY 14853.

Allozyme alleles and mating type were used to characterize 229 *Phytophthora infestans* isolates collected in Poland from 1985 through 1991. A2 isolates were first recorded in southern Poland in 1988 and have spread throughout eastern Poland, where A2 frequency in 1989-1991 was *ca.* 25%. Collections in 1985-1986 contained only isolates with 86/100 genotype for glucose-6-phosphate isomerase (*Gpi*) and 92/100 for peptidase (*Pep*). These genotypes declined to a frequency of < 2% in 1990-1991. A Chi-square analysis using *Gpi* alleles revealed significant differences between A1 and A2 isolates. A1 were mostly represented by 90/100 while A2 were represented mostly by 100/100 genotypes. However, an unusual genotype (90/90 for *Gpi*) was detected at a frequency of 0.06, and might represent a recombinant type. No regional substructuring was found within the Polish *P. infestans* population.

A172

NUCLEAR DNA CONTENT OF FEULGEN-STAINED NUCLEI OF PHYTOPHTHORA INFESTANS USING SCANNING-INTEGRATING MICRODENSITOMETRY. S. S. Daggett and E. Goetz[1]. Dept. of Biology. Pennsylvania State University, University Park, PA 16802 and [1]Ernst-Moritz-Arndt-Str. 20 W-6338 Rechtenbach.

Scanning-integrating microdensitometry was used to determine the DNA content of Feulgen-stained hyphal nuclei for 101 isolates of Phytophthora infestans from Germany. These isolates were collected between 1976 and 1990. Hyphal tips were removed from formalin-fixed cultures and stained using the Feulgen reaction. Fifty nuclei per isolate were analyzed and isolates with known nuclear DNA contents were used as standards. A range was obtained for both A1 and A2 isolates from 2C, designated as diploid, to 4C, designated as tetraploid. Sixty-five of the isolates were 3C (triploid), while only four isolates were 2C. The four diploid isolates were collected in 1989 and 1990. All isolates collected prior to 1980 were 4C. The results are consistent with previous studies of European isolates of P. infestans using other cytophotometric methods.

A173

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

Phytophthora sojae races 1, 3, 4, 7 and an undescribed race isolated from infested soil were selfed. After one month, the oospores were harvested and single-oospore colonies were isolated on an oospore-germination medium. At least 100 single-oospore cultures from each race were evaluated for their virulence using a hypocotyl inoculation method on 7-day-old soybean seedlings of Williams near-isogenic lines having *rps*, *Rps*1-a, *Rps*1-b, *Rps*1-c, *Rps*1-k, or *Rps*3 and Harosoy near-isogenic lines with *Rps*7 or *Rps*7+*Rps*6 genes. One race 3 isolate had races 3, 7, 8 and 21 among its sexual progeny. All field isolates had a small number of single-oospore lines with undescribed virulence phenotypes. Data indicated that field isolates of *P. sojae* were heterogenic in nature which could have been due to heterokaryosis, mixture of races and/or heterozygosity.

A174 Withdrawn

A177

GENETIC CORRELATIONS IN RESISTANCE TO STEROL BIOSYNTHESIS-INHIBITING FUNGICIDES IN *PYRENOPHORA TERES*. T.L. Peever and M.G. Milgroom. Department of Plant Pathology, Cornell University, Ithaca, NY, 14853.

Cross-resistance to sterol biosynthesis-inhibiting fungicides (SBIs) in *Pyrenophora teres* has been studied using the technique of genetic correlations to determine if resistance is controlled by the same genetic factors. One method used to estimate genetic correlations involves calculation of the covariance between and within families using progeny sampled from crosses. The progeny of 7 full-sib crosses of *P. teres* were used to determine genetic correlations in resistance among 5 different SBIs. Genetic correlations in resistance to these same SBIs were also estimated using a different approach which involved the correlation of isolate means using the 14 parental isolates. This analysis was much simpler to perform as it did not involve generating crosses of *P. teres* and produced similar results to those obtained with the crosses. This type of analysis may also be more relevant to evolutionary studies of *P. teres* and other haploid, asexually reproducing fungal pathogens.

A178

MATERNAL INHERITANCE AND DIVERSITY OF MITOCHONDRIAL DNA IN THE CHESTNUT BLIGHT FUNGUS, *CRYPHONECTRIA PARASITICA*. M. G. Milgroom and S. E. Lipari, Cornell University, Ithaca, NY 14853-5908

Inheritance of mitochondrial DNA (mtDNA) in *Cryphonectria parasitica* was investigated using ascospore progeny from contolled lab crosses and field-collected perithecia. Progeny from reciprocal crosses where maternal strains were controlled had mtDNA RFLP haplotypes identical to the maternal strains and not the conidial parents. Progeny from another lab cross showed uniparental inheritance for all progeny (n = 43). All ascospore isolates from eight field-collected perithecia had mtDNA haplotypes identical to the maternal canker isolate. In a sample of 39 canker isolates from a natural population, we found 24 different mtDNA haplotypes. Fifteen haplotypes each occurred only once, while only one haplotype occurred more than three times. The genotypic diversity of mtDNA in this population was 0.968.

A179

GENETIC DIVERSITY OF *Fusarium moniliforme* IN SEED FROM TWO MAIZE CULTIVARS. C.L. Campbell, J.F. Leslie, & R. Farrokhi-Nejad. Dept. of Plant Pathology, Kansas State University, Manhattan, KS, 66506.

Isolates of *Fusarium moniliforme* were recovered from seed of two corn cultivars grown at 12 locations in the North Central United States. *nit* mutants were generated in 402 isolates to determine distribution of vegetative compatibility groups (VCGs) within and between seed lots for corn cultivars 3377 and 3475. Of the 216 VCGs identified, 93 were represented by a single isolate and comprised 12-47% and 12-46% of the VCGs found in seed lots for cultivars 3377 and 3475, respectively. Fifteen heterokaryon self-incompatible isolates were also detected. At six locations, isolates in a common VCG were recovered from both cultivars. At one location, 67% of the isolates belonged to a single VCG, which contained more isolates than any other VCG identified, but no members of this group were found at any other location. At least 12 VCGs from cultivar 3377 and eight VCGs from cultivar 3475 were found at more than one site. Small VCGs represented in two or more widely separated locations and the genetic diversity observed within fields implies that some of the genetic variability may be due to migration of the fungus through seed, providing a mechanism for gene flow between fields. Hermaphroditic isolates constituted 13-74% of the population at 12 sites and 46% of the total population. The "A" mating type constituted 97% of the population evaluated.

A180

AN ULTRASTRUCTURAL PACHYTENE KARYOTYPE FOR *Melampsora lini*. E.W.A. Boehm and W.R. Bushnell, Department of Plant Pathology and Cereal Rust Laboratory, USDA-ARS, University of Minnesota, St. Paul, MN 55108.

An ultrastructural karyotype has been derived from five serially sectioned pachytene nuclei of two isolates of *Melampsora lini*. The pachytene nuclei were selected for sectioning based on degree of chromatin condensation, using epifluorescence microscopy of fixed, DAPI-stained teliospore protoplasts from which walls had been removed mechanically. Each of the five reconstructed nuclei contained 18 distinct bivalents which terminated at both ends on the nuclear envelope. The 18 bivalents formed a finely graded series of lengths, ranging from 3.2 to 9.0% of the total length of all bivalents. No centromeres were resolved. One bivalent, in terminal association with the nucleolus, could be identified in all nuclei. The karyotype of n=18 is significantly larger than the n=5-6 estimated previously by light microscopy.

A181

FIRST REPORT OF HETEROKARYON FORMATION BY *SCLEROTINIA SCLEROTIORUM*. E.J. Ford, H. Casquilho, R. V. Miller and D. C. Sands. Department of Plant Pathology, Montana State University, Bozeman, MT 59717.

Auxotrophic strains were used to demonstrate the ability of *S. sclerotiorum* to form viable heterokaryons. Heterokaryons were formed between auxotrophic strains derived from the same isolate and from distinct isolates and detected by prototrophic growth on minimal media. Heterokaryon formation was verified by growth of single hyphal tips on a minimal medium lacking the growth requirements of either parent and the presence of both nuclear types within this mycelium. The latter was demonstrated by the production and isolation of single protoplasts that showed the auxotrophic and growth patterns of each of the two parents. Because of the multi-nucleate nature of the protoplasts many still showed prototrophic growth. Heterokaryons were stable in culture through repeated transfer on minimal and complete media. Separation of parental types occurred in less than 3 percent of hyphal tips taken from growth on either minimal or complete media. Pathogenicities of all heterokaryons studied were comparable to wild-types.

A182

ISOLATION OF *GLOMERELLA MUSAE* [TELEOMORPH OF *COLLETOTRICHUM MUSAE* (BERK. & CURT.) ARX.] AND SEGREGATION ANALYSIS OF ASCOSPORE PROGENY. R. J. Rodriguez and J. L. Owen, Department of Plant Pathology, University of California, Riverside, CA 92521.

The sexual stage of *Colletotrichum musae* was obtained under controlled laboratory conditions. Meiosis was apparent by the formation of perithecia typical of ascomycetes classified as *Glomerella*. Perithecia contained as many as 50-80 asci, each of which contained up to 8 ascospores. To determine if this meiotic system was homothallic or heterothallic, chlorate resistant mutants were isolated and crossed with the wild type. Chlorate resistance and susceptibility segregated in a 1:1 ratio which indicated that this system was heterothallic. Sexual recombination was observed by analyzing the segregation of several DNA markers identified by amplification of polymorphic fragments of genomic DNA using single oligonucleotide primers and *Taq* DNA polymerase.

A183

IDENTIFICATION OF MOLECULAR MARKERS LINKED WITH THE *RPG1* GENE FOR STEM RUST RESISTANCE IN BARLEY. B. J. Steffenson[1], A. Kilian[2], and A. Kleinhofs[2], [1]Dept. of Plant Pathology, North Dakota State Univ., Fargo, ND 58105, and [2]Dept. of Crop and Soil Sciences, Washington State Univ., Pullman, WA 99164.

The resistance gene *Rpg1* is considered durable because it has protected barley from losses due to the stem rust pathogen, *Puccinia graminis* f. sp. *tritici*, for nearly 50 years. The transfer of this gene in breeding programs would be more efficient if closely linked markers were identified. Over 200 molecular markers have been mapped in a population of doubled haploid lines (DHLs) derived from Morex (possesses allele *Rpg1*) and Steptoe (possesses allele *rpg1*) as part of the North American Barley Genome Mapping Project. DHLs were evaluated for resistance to race Pgt-MCC of *P. g.* f. sp. *tritici* in the seedling stage. Integration of data for stem rust reaction with the molecular map revealed that *Rpg1* is located at the terminus of the short arm of chromosome 1 between the subtelomeric marker (*Tel1S*) and the precursor of plastocyanin (*Plc*). The DNA probe for *Plc* provides a valuable molecular marker for use in breeding programs because it is closely linked (ca. 3 cM) to *Rpg1* and gives a strong, unambiguous hybridization signal.

A184

GENETIC ANALYSIS OF AVIRULENCE/VIRULENCE TO RICE CULTIVAR KATY IN *MAGNAPORTHE GRISEA*. G. W. Lau and A. H. Ellingboe, Departments of Bacteriology and Plant Pathology, University of Wisconsin-Madison, Madison, WI, 53706.

Isolates of *Magnaporthe grisea* have been developed that are sexually competent and differ in their avirulence/virulence on only one rice cultivar. Isolates 70-6 and 70-14 are both virulent on 10 rice cultivars. Isolate 70-6 is virulent on Katy whereas isolate 70-14 is avirulent on Katy. Progenies from a cross between 70-6 and 70-14 segregated 26 avirulent:28 virulent. All progenies were virulent on the other 10 cultivars. The data suggested that two alleles at one locus controlled avirulence/virulence on Katy. However, backcrosses of progenies to 70-6 or 70-14 and intercrossing among the progenies have shown that at least 4 loci control interactions with Katy rather than a single locus. Isolate 70-14 contains two avirulence genes and isolate 70-6 contains two genes that each specifically suppress the expression of an avirulence allele. From the proposed genotypes, we would expect a 7 avirulent:9 virulent segregation ratio on Katy in the cross between 70-6 and 70-14. Therefore, transformation of isolate 70-6 with an avirulence genes from 70-14 would not be expected to give an avirulent phenotype due to the suppression of the avirulence gene by the suppressors carried by 70-6.

A185

PHYLOGENETIC RELATIONSHIPS OF 15 MLOs ESTABLISHED BY PCR SEQUENCING OF VARIABLE REGIONS WITHIN THE 16S RIBOSOMAL RNA GENE. B. C. Kirkpatrick, J. Gao, and *N. Harrison. Dept. of Plant Pathology, University of California, Davis, CA 95616 and *University of Florida-IFAS, Ft. Lauderdale, FL 33314.

DNA fragments containing the entire 16S ribosomal RNA (rRNA) gene or the 16S rRNA gene plus the 16S/23S intragenic region were amplified by the polymerase chain reaction (PCR) from 15 MLO isolates collected from North America, Africa and the Caribbean region. The PCR fragments were gel-purified and used as templates in a PCR-based sequencing system. Sequencing primers were selected that flanked 5 variable regions within the 16S rRNA gene. Approximately 500 bp of the 16S rRNA gene were sequenced in all MLO isolates. Another 250 bp of the 16S/23S spacer region, which contained a tRNA, were sequenced in 6 isolates. 16S rRNA variable region sequences were analyzed by PAUP to determine the phylogenetic relationships between these MLOs. MLOs that induce virescence and phyllody in herbaceous plants were distinctly separate from MLOs that cause decline diseases in woody plants.

A186

TRANSMISSION STUDIES OF WESTERN X-DISEASE IN CHERRY BY BUD INOCULATION AND PROPAGATION. C. F. Luhn and J. K. Uyemoto, USDA-ARS, Department of Plant Pathology, University of California, Davis, CA 95616.

Budwood was collected from X-diseased and healthy Bing sweet cherry/mazzard (Prunus avium) trees in June and August. The June collections consisted of different aged shoots, i.e., current season growth and 1, 2, and 3 year old shoots. Ten or 20 bud- or spur-chips of each age group were singly grafted by T-budding onto indicator trees of Bing/mahaleb. The August collection was of current season shoots only, taken from four diseased trees and one healthy tree. Twenty single buds from each source tree were T-budded onto mazzard seedlings. All grafts united and were forced to make yearling trees. Results of the June grafts showed that regardless of shoot age, all inoculum sources caused disease symptoms in 10% of the indicator trees. In contrast, the August grafts produced disease in 80% of the trees. All control trees were healthy. These results suggest that the X-disease mycoplasmalike organism (XMLO) is less systemically distributed in early than in late summer. Hence, when indexing candidate trees for the presence of XMLO, shoots collected in August are preferred to obtain high transmission efficiency.

A187

EVIDENCE AGAINST TREE-TO-TREE TRANSMISSION OF ILARVIRUSES DURING POLLINATION OF TWO OBLIGATELY CROSS-POLLINATED PRUNUS SPECIES. J. K. Uyemoto, USDA-ARS, Dept. Plant Pathology, and J. H. Connell, Coop. Extension, University of California, Davis, CA 95616.

A Bing cherry (P. avium) orchard was assayed by ELISA and Shirofugen for prune dwarf (PDV) and Prunus necrotic ringspot (NRSV) viruses in April and June 1988, respectively. Seven of 100 trees tested positive for PDV. Mature fruit were collected from several trees in May 1990 and leaf tissues from the same trees in April 1991. From healthy trees, the percentage of fruit (tested extracts of skin-flesh tissues) with PDV ranged from zero to 45%, while fruit from a diseased tree were 100% infected. Assays of leaf tissues from the healthy trees were negative by ELISA a year later. Likewise, assays were done with almond (P. dulcis) also. Extracts of pericarp-kernel tissues taken from trees of Nonpareil, Peerless, and Price cvs infected with NRSV indicated that 92 to 100% of the nuts were NRSV-positive by ELISA. Similarly prepared extracts from nuts collected from 2 healthy Price trees showed incidences of 0 or 14%. Yet, a year later both Price trees were negative by ELISA. Apparently, even though healthy trees of almond and cherry bore virus-infected fruits, they were not systemically infected. Our results indicate that if ilarviruses are transmitted by pollen to healthy mother trees, then that process is highly inefficient or that virus spread in orchards occurs in an as yet undetermined manner.

A188

RELATION BETWEEN SPREADER-STICKER APPLICATION, BLOSSOM CAP RETENTION, BERRY SCARRING, AND BOTRYTIS BUNCH ROT IN 'CHARDONNAY' GRAPES. N. Martinez[1], T. K. Wolf[2], A. Baudoin[1], and M. J. Weaver[1]. [1]Dept. of Plant Pathology, [2]Dept. of Horticulture, VPI & SU, Blacksburg, VA 24061-0331.

A field study was conducted in 1991 to determine whether high rates of flower cap retention in 'Chardonnay' grapes contribute to increased levels of Botrytis bunch rot (Botrytis cinerea, Pers.), and whether spreader-sticker application might enhance cap retention. At bloom, water (control) or the sticker, Nu-Film 17®, was sprayed onto single clusters at diluted (0.63 ml /l) and concentrated (1.26ml /l) label rates. After fruit set, some clusters were completely cleaned of caps and other organic debris with pressurized air. Cap retention, debris retention, berry scarring, and Botrytis rot were monitored throughout the season. Spreader-sticker increased overall debris retention only slightly. Removal of caps and organic debris had no significant effect on berry scarring, but did significantly reduce Botrytis bunch rot.

A189

CONIOPHORA SP. IMPLICATED IN RAPID DEVELOPMENT OF WOOD ROT ON LIVING BRANCHES OF LEMON TREES IN ARIZONA. M.E. Matheron[1], R.L. Gilbertson[2] and J.C. Matejka[1].
[1]Yuma Agricultural Center, Univ. of Arizona, Yuma, 85364;
[2]Dept. of Plant Path., Univ. of Arizona, Tucson, 85721.

A brown heartwood rot on living branches of lemon trees has been observed in several orchards in Yuma, AZ. A Coniophora sp. was consistently isolated from decayed wood tissue. To determine the ability of the fungus to cause wood decay, mature lemon trees were inoculated by placing wooden dowels (8-mm-diam. x 12-mm-long) colonized by Coniophora into holes drilled into healthy branches. After 9 months, brown wood rot decay columns averaging 32.5 cm in length were recorded on lemon branches inoculated with the Coniophora sp. The ability to colonize and spread rapidly in lemon wood tissue suggests that this Coniophora sp. could be a major threat to lemon tree health in Arizona. This is the first known report of Coniophora as a pathogen on living citrus tissue.

A190

THE OCCURRENCE OF ENDOPHYTIC FUNGI AND LATENT PATHOGENS IN CRANBERRY AS INFLUENCED BY FUNGICIDE TREATMENT. S. E. Keates, L. M. Carris and P. R. Bristow, Dept. of Plant Pathology, Washington State University, Pullman, WA 99164-6430.

Fungi were isolated from surface-sterilized cranberry leaf, stem, flower, fruit and root tissues collected on four dates between bud break (March) and harvest (October) in 1991. Plant material came from untreated and fungicide treated plots. Commonly isolated fungi included cranberry pathogens such as *Phyllosticta elongata* and *Phomopsis vaccinii*. The predominant fungus isolated from all tissues was *Colletotrichum gloeosporioides*, the putative anamorph of *Glomerella cingulata*. Additionally, *C. gloeosporioides* was isolated from 63% of fruit rotted at harvest and 56% of rotted fruit after 2 months of refrigerated storage. Fungicide applications were effective in reducing colonization of tissues by *C. gloeosporioides*.

A191

TRUNK AND BRANCH CANKER OF PISTACHIO CAUSED BY *PHYTOPHTHORA* SPP. J.D. MacDonald, Z. Banihashemi, S.M. Mircetich, G. Browne and L. Bolkan. Department of Plant Pathology, University of California, Davis, CA, 95616

Phytophthora root and crown rot of pistachio is a common, serious disease problem in Greece and Iran, where there are long histories of pistachio cultivation. Recently, trees in California have been found infected with *Phytophthora* spp., but primarily as trunk and scaffold branch cankers. Cankers on affected trees appear as darkened, sunken areas of bark which typically exude profuse amounts of resin. Cankers expand and often girdle major limbs or trunks and cause tree death. Cankers are usually associated with pruning wounds or bark cracks on limbs. Isolations from cankers have consistently revealed the presence of *P. capsici* and a high-temperature *P. cryptogea*. Pistachio isolates are pathogenic to pistachio and they are morphologically similar to known isolates of the same species. Spread of these species among pistachio orchards is believed to be aided by surface irrigation water. Artifical inoculations and field observations indicate the pistachio cultivars 'Joly' and 'Ruehly' are more susceptible than the more widely-grown cultivar 'Kerman'.

A192

PATTERNS OF SPORE RELEASE FROM BLACK ROT (*GUIGNARDIA BIDWELLII*) INFECTED GRAPE MUMMIES THAT OVERWINTERED ON THE GROUND OR IN THE CANOPY. C.M. Becker, R.C. Pearson, Cornell Univ., NYSAES, Geneva, NY 14456.

As a result of machine pruning of vines and harvesting of grapes in New York, many black rot affected berries (= mummies) are not removed from the vine and may remain within the canopy area for more than one year. To determine the availability of inoculum from mummies: mummies from the canopy and the ground were collected from three cultivars at each of four sites during the 1991 growing season. They were soaked in water for two hours and the resulting discharged ascospores and conidia were counted. Ascospores were released from mummies (1×10^3 to 6×10^4 ascospores per mummy) overwintering on the ground beginning from one to three weeks after budbreak until approximately one month after bloom. Ascospore release from mummies that overwintered within the canopy began at least one week later and continued until harvest, with $>1 \times 10^5$ ascospores and $>1 \times 10^5$ conidia per mummy detected between veraison and harvest. From May until October, the daily mean temperature on the ground and in the canopy was similar. However, the duration of wetness was generally less within the canopy region than that on the ground, which may account for the delay in ascospore maturity.

A193

FACTORS AFFECTING THE INFECTION OF AVOCADO TREES BY THE TRUNK CANKER PATHOGEN (*PHYTOPHTHORA CITRICOLA*). J. A. Menge and Z. El-Hamalawi, Dept. of Plant Pathology, Univ. of California, Riverside, CA 92521.

Avocado trunk canker, caused by *Phytophthora citricola,* has recently emerged as a major threat to the avocado industry in California. Root and crown lenticels, adventitious roots and bark wounds were investigated as possible infection courts for *P. citricola*. Neither lenticels nor lenticel-free areas of the bark were infected by the pathogen. Infection of adventitious roots remained in the root base area but did not advance. Inoculum placed over cut wounds or injected under the bark on the crown or stem resulted in cankers in 100% of the cases. Time of inoculation, moisture, wounds above or below the inoculation site, and stress on the host were investigated. Little infection occurred during winter months. Infection was increased by plant wounding and moisture applied to the inoculation site. Stress applied by clipping roots increased canker development compared to nonstressed plants.

A194

EFFECT OF IRRIGATION CUT-OFF DATE ON HULL ROT OF ALMOND. B.L. Teviotdale, Dept. Plant Pathology, University of California, Berkeley/Kearney Agricultural Center, Parlier.

Hull rot, caused by Rhizopus stolonifer, results in leaf and shoot death (strikes) without damaging the edible nutmeat. The influence of deficit irrigation practices on hull rot was studied using groups of trees whose seasonal irrigation was terminated at eight weekly intervals before harvest. The incidence of natural infection increased as irrigation cessation was delayed. Fruit in the latest five irrigation cut-off treatments were inoculated at four weekly intervals during hull split with 0.1 ml of 10^3, 10^4, and 10^5 conidia/ml suspension of R. stolonifer. Fruit having small, medium, and large hull splits or loosely or firmly attached hulls were inoculated with 0.1 ml of the 10^4 conidia/ml suspension. Percent infected hulls increased with increased numbers of irrigations but was not affected by inoculum concentration or inoculation date. Percent strikes was positively correlated with deferral of irrigation cut-off date, unaffected by inoculum concentration and least at the latest inoculation date. Fruit with small- and medium- split hulls had greater percent infected hulls and strikes than hulls with large splits. Percent strikes, but not percent infected hulls, was greater when fruit with firmly than loosely attached hulls were inoculated.

A195 Withdrawn

A196

POPULATION DYNAMICS AND SURVIVAL OF COLLETOTRICHUM GLOEOSPORIOIDES, THE CAUSE OF CITRUS POSTBLOOM FRUIT DROP. J. P. Agostini and L. W. Timmer, Univ. of Florida, IFAS, CREC, 700 Expt. Sta. Rd., Lake Alfred, FL 33850

The slow-growing, orange strain (SGO) of Colletotrichum gloeosporioides (C.g.) infects blossoms producing lesions and inducing fruit drop and formation of persistent calices. Populations of C.g. in affected orchards were monitored during two seasons using selective isolation procedures. Abundant conidia formed on infected petals. Populations on vegetative tissues were high after bloom but later declined gradually. In greenhouse studies, some conidia applied to the leaf surface germinated to form appressoria. The remaining ungerminated conidia survived up to one month. Conidia, but not appressoria, germinated in the presence of free moisture alone. When a blossom extract was applied to leaves, many appressoria germinated and produced conidia on new hyphae. The SGO strain of C.g. did not compete well with saprophytic organisms in colonization of senescent or necrotic tissue.

A197

ERGOSTEROL AS AN INDICATOR OF GRAPE ROOT COLONIZATION BY PHYMATOTRICHUM OMNIVORUM. H. M. Escamilla and S. D. Lyda. Tecnologico de Monterrey-C. Queretaro, Departamento de Fitotecnia, Queretaro, Qro. 76000, Mexico and Department of Plant Pathology and Microbiology, Texas A&M University, College Station, TX 77843.

Grapes (Vitus vinifera) grown in soil (28 C) infested with sclerotia of Phymatotrichum omnivorum showed symptoms of Phymatotrichum root rot but few plants died over a period of 5 months. Lesions formed on roots of plants grown in infested soils; however, plants were sustained by new roots that formed above the lesions. The extent of root colonization by P. omnivorum was measured by quantitating the most prevalent sterol in fungal mycelium (ergosterol) by HPLC. Grape roots from infested and noninfested soil were dried (45 C), ground (60 mesh), and extracted twice with methanol. The lipid fraction was saponified with KOH (20 g) in ethanol (50 ml), and the extract was resuspended in methylene chloride-isopropanol (99:l) for analysis. The ergosterol content of Chenin blanc roots from noninfested, unsterilized soil was 2.3 to 4.1 ug/g, compared with16 to 24.9 ug/g from roots in infested soil. Mycelium of P. omnivorum contained 1.59 mg ergosterol/ g dry wt.

A198

RELATIONSHIP OF EUTYPA DIEBACK SEVERITY TO GROWTH AND YIELD OF GRAPEVINES. G.P. Munkvold, J.A. Duthie, and J.J. Marois. Dept. of Plant Pathology, University of California, Davis, CA 95616.

In each of 5 vineyards representing 2 cultivars, 50 grapevines with a wide range of severity ratings for Eutypa dieback (*Eutypa lata*) were selected for growth and yield evaluation. Severity was assessed during the spring as the proportion of spurs on each vine that were symptomatic or missing. At harvest, the number of grape clusters and the yield (kg) per vine were measured. Cluster count and yield decreased linearly with disease severity, but mean cluster weight was not affected. A small effect on pruning weight, measured during the dormant season, was detected in 2 of the 5 vineyards. Predicted values of cluster count and yield on symptomless vines varied among vineyards. However, when intercepts were standardized to a value of 1.00, the slopes of the linear yield loss models were not significantly different among vineyards or between cultivars for either response variable. Slopes ranged from -0.79 (R^2=.53) to -0.88 (R^2=.66) for cluster count, and from -0.80 (R^2=.67) to -0.94 (R^2=.67) for yield. This corresponded to a yield loss of 1.2 to 3.0 kg per affected spur. The models estimated total yield losses of 39% to 62% for the 5 vineyards. Thus, Eutypa dieback caused a substantial, linear reduction in yield by reducing the number of clusters, while causing little loss in vegetative growth.

A199

EFFECT OF INOCULUM DISTRIBUTION AND CONCENTRATION ON DEVELOPMENT OF ROOT AND CROWN ROT OF STRAWBERRY CAUSED BY *COLLETOTRICHUM ACUTATUM*. G.T. Browne,* C.Q. Winterbottom, S.M. Mircetich*, R.P. Buchner, R.W. Hoenisch, R.J. Wakeman*, and W.D. Gubler. *USDA-ARS, Department of Plant Pathology, University of California Davis 95616.

In greenhouse experiments, distribution and concentration of conidial inoculum in soil was varied to study effects on development of root and crown rot caused by *Colletotrichum acutatum* (*Cac*) in strawberry cv. Pajaro. Strawberry roots were exposed to different concentrations of *Cac* in two ways: in one set of treatments, roots were infested by dipping them into conidial suspensions (10^2-10^6 conidia/ml) before planting into noninfested soil; in the other treatments, noninfested roots were planted into infested soil (125-2000 conidia/g soil). In the roots dipped in conidial suspensions before planting, no significant disease developed from concentrations of 10^2-10^4

conidia/ml, but severe root and crown rot developed from concentrations of 10^5-10^6 conidia/ml. The strawberries with noninfested roots planted in artificially infested soil developed no disease, regardless of inoculum concentration. These results suggest that inoculum associated with infested transplants may be more important in causing root and crown rot phases of the disease in commercial strawberry fields than inoculum present in naturally infested soil.

A200

PERIOD OF TRANSMISSION OF BLUEBERRY SCORCH CARLAVIRUS AND BLUEBERRY SHOCK ILARVIRUS IN THE FIELD. P.R. Bristow and G.E. Windom, Washington State University, Puyallup 98371 and R.R. Martin, Agriculture Canada, Vancouver, British Columbia V6T 1X2

Two newly described viruses of highbush blueberry in the Pacific Northwest cause scorch-like symptoms and spread rapidly in the field. To determine when transmission occurs potted trap plants (cv. Berkeley) were set out in infected bushes in commercial fields. Trap plants were replaced with a fresh set every two weeks for blueberry scorch carlavirus (BBScV) and weekly for blueberry shock ilarvirus (BSIV). Upon removal from the fields, trap plants were examined for aphids, sprayed with an insecticide and overwintered in a screenhouse. The following spring, young leaves were assayed by ELISA procedures for the two viruses. BBScV was transmitted from early-May to mid-August and was associated with the colonization of the trap plants by aphids (mostly *Fimbriaphis* spp.). Transmission of pollen-borne BSIV took place only during bloom.

A201

DIFFERENTIAL INDUCTION AND SUPPRESSION OF POTATO HMG-CoA REDUCTASE GENES CORRESPOND TO ALTERED STEROID AND SESQUITERPENE PHYTOALEXIN BIOSYNTHESIS. Choi, D.-I., Ward, B. L., Bostock, R. M. Department of Plant Pathology, University of California , Davis, CA 95616

Induction of HMG-CoA reductase(HMGR) is essential for the synthesis of sesquiterpenoid phytoalexins and steroid derivatives in *Solanaceous* plants following stresses imposed by mechanical injury and pathogen infection. To better understand this complex step we cloned three cDNAs for HMGR(*hmg*1,2,&3) from a potato tuber library and gene specific probes were constructed on the basis of the determined sequences and used for expression studies in potato plant. Northern blot analysis revealed that *hmg*1 is strongly induced by wounding but the wound induction is suppressed by treatment of the tissue with the fungal elicitor arachidonic acid or by inoculation with an incompatible race of *Phythophthora infestans*. *hmg*2 and *hmg*3 mRNA also accumulated in response to wounding, but in contrast to *hmg*1 these mRNAs were strongly enhanced by arachidonic acid or inoculation. The suppression of *hmg*1 and induction of *hmg*2 and *hmg*3 transcript levels following elicitor treatment parallel the suppression in steroid and stimulation of sesquiterpenoid accumulations. The results are consistent with the hypothesis that HMGR isoforms are components of discrete organizational channels for sterol and sesquiterpene biosynthesis.

A202

EXPRESSION OF HMG CoA REDUCTASE PROMOTER-GUS REPORTER GENE FUSIONS IN TRANSGENIC PLANTS. B.A. Stermer, M.K. Bhattacharyya and R.A. Dixon, Plant Biology Division, The Samuel Roberts Noble Foundation, P.O. Box 2180, Ardmore, OK 73402.

Induction of HMG CoA reductase (HMGR) activity is an important step in the biosynthesis of sesquiterpenoid phytoalexins. Transcriptional fusions were made between the potato HMGR13 promoter and the GUS reporter gene in the binary vector pBI101. Transgenic tobacco plants carrying the fusions were analyzed for GUS activity. The HMGR13 promoter produced constitutive GUS expression in all plant organs examined. The highest level of expression was in the pollen where it also was developmentally regulated. Promoter fusions were not further activated in tobacco leaves by inoculation with *Phytophthora infestans* or TMV but were responsive to arachidonic acid. Studies of the HMGR13 promoter-GUS fusion in transgenic potato are underway.

A203

PHYTOALEXIN PRODUCTION IN BEAN ROOTS GROWN ON STERILE MEDIA AND IN NATURAL SOIL. L. Liu, Z. K. Punja, and J. E. Rahe. Department of Biological Sciences, Simon Fraser University, Burnaby, British Columbia, Canada V5A 1S6

Application of the herbicide glyphosate at sublethal doses has been previously shown to enhance infection by *Pythium* and *Fusarium* spp. on bean roots, and reduced the levels of phaseollin, phaseollinisoflavan, phaseollidin and kievitone in bean leaves. The purpose of this research was to determine if glyphosate influences phytoalexin production in roots, which in turn could influence root susceptibility to fungal infection. Production of phytoalexins in healthy roots grown in sterile growth media including 0.65% water agar, silica sand and metro-mix was compared to that in natural soil. Seedlings were grown for 7-10 days, and the phytoalexins were extracted in 95% boiling ETOH, and quantitative analysis was performed by HPLC. Only a trace amount of phaseollin was found in roots grown on water agar. Large amounts of phaseollinisoflavan and phaseollin were produced in roots grown in metro-mix and natural soil. Both phytoalexins were also found in roots grown on silica sand, but at a lower concentration. The effects of glyphosate on altering the concentration or ratios of phytoalexins produced in bean roots are being investigated.

A204

PURIFICATION AND CHARACTERIZATION OF TWO CUTINASES FROM ALTERNARIA BRASSICICOLA. [1]Frances Trail, and [2]Wolfram Koeller. [1]Dept. of Food Science and Human Nutrition, Michigan State University, E. Lansing, MI 48824, [2]Dept. of Plant Pathology, Cornell University, Geneva, NY 14456.

Previously, a relationship between tissue specificity and cutinase properties was shown for several plant pathogens. A cutinolytic pH optimum of 6.5 was associated with leaf pathogens, whereas cutinolytic pH optima of 8.5 to 10.0 were associated with stem pathogens. Alternaria brassicicola, a pathogen which causes disease on all above-ground plant parts, secreted cutinases with optima at pH 7.0 and at 9.0 (Trail and Koeller, 1990, Physiol. Mol. Plant Pathol. 36:495). These two cutinases, Ac and Ba, were extracted from the culture filtrate of the pathogen grown on cutin as the sole carbon source and were purified to homogeneity by SDS-PAGE followed by electro-elution. As previously reported for other cutinases, both enzymes were induced by cutin monomers and partially repressed by glucose. This is the first report of two enzymatically distinct cutinases produced by any organism.

A205

INDUCTION AND CHARACTERIZATION OF CHITINASES AMONG CUCUMBER (CUCUMIS SATIVUS L.) CULTIVARS Y. Y. Zhang and Z. K. Punja, Department of Biological Sciences, Simon Fraser University, Burnaby, British Columbia, Canada V5A 1S6

Plant chitinases(E.C. 3.2.1.14) have been reported to have antifungal activity in a number of pathogen-host systems. Different cultivars of cucumber (Cucumis sativus L.) (Calypso, Fidelio F1, Alaska and PSX 144388) were compared for their chitinase banding patterns before and after powdery mildew (Sphaerotheca fuliginea) inoculation. Cotyledonary tissues from 2-wk-old seedlings were extracted with buffer, and subjected to Native-PAGE gel electrophoresis. The gel was transferred onto an overlay gel containing glycol chitin and the banding patterns on the overlay gel were visulized under UV light after staining with fluorescent brightner 28. Three bands were observed in uninoculated Fidelio, Alaska, and PSX 144388, while four bands were seen in Calyspo. Chitinase activity was increased following infection by powdery mildew, and three new isozymes were induced. In inoculated cotyledons, the banding patterns were similar whether the cultivar was susceptible or resistant to powdery mildew. The molecular weight of chitinase from cucumber was estimated to be 25.6 kD on SDS-PAGE. The importance of constitutive and induced chitinases in plant defenses against disease will be discussed.

A206

EXPRESSION OF TRICHODIENE SYNTHASE FROM *FUSARIUM SPOROTRICHIOIDES* IN TRANSGENIC TOBACCO. M. Zook[1], T. Hohn[2], A. Bonnen[1] and R. Hammerschmidt [1]. [1]Department of Botany and Plant Pathology, Michigan State University, East Lansing, MI 48824. [2]Mycotoxin Research Unit, USDA/ARS, Peoria, IL 61604.

The gene encoding trichodiene synthase, a sesquiterpene cyclase from the fungus *Fusarium sporotrichioides*, was used to transform tobacco (*Nicotiana tabaccum*). Treatment of wild-type cell suspension cultures with cellulase induced a 20-fold increase in sesquiterpene cyclase activity. Five different unelicited transformant cell suspension cultures had cyclase activities equal to or greater than that of cellulase-treated wild-type cell suspension cultures. The cyclase product of cellulase-treated wild-type cells (5-epi-aristolochene) was separated and thus differentiated from the cyclase product of untreated transformant cells using argentation chromatography. The *in vivo* production of foreign sesquiterpene cyclase products may provide insights into tobacco isoprenoid metabolism and lead to the production of novel natural products.

A207

The use of DNA fingerprinting in detecting genetical variation among isolates of Septoria tritici differing in virulence. S. Pnini-Cohen[1], A. Zilberstein[1], S. Shuster[1], U. Lavi[2], J. Hillel[3], and Z. Eyal[1]. [1]Tel Aviv Univ., [2]Volcani Center, Bet Dagan, [3]Faculty of Agric., Hebrew Univ., Rehovot, Israel.

Genetic relatedness of 8 S. tritici isolates from 6 countries having different virulences was assessed using the VNTR probes 33.6 (human) and 22.3 (cattle). Five of the isolates exhibited completely different DNA hybridization patterns. Isolates from Israel, Montana and Uruguay showed small band variation. Lower banding and band sharing was revealed among the 8 isolates for PstI and EcoRI digests probed with 33.6 rather than with 22.3. Sub-culturing of isolates ISR398 and ISR8036 for 17 times at 10-15 days intervals decreased virulence while DNA patterns remained highly stable. Reisolation from pycnidia on leaves inoculated with attenuated cultures regained full expression of virulence while maintaining distinct DNA fingerprinting for each isolate. Cultures obtained from pycnidia of leaves inoc- ulated with a 1:1 conidial mixture of the 2 isolates revealed fingerprints identical to that of the composing isolates.

A208

PHYTOTOXICITY OF ALTERNARIA ALTERNATA SWSL#1 AND AAL-TOXIN TO WEEDS. H. K. Abbas,* USDA-ARS, SWSL, Stoneville, MS 38776.

An extract of an isolate of A. alternata grown on rice contained AAL-toxin (100 μg/g), tenuazonic acid (10 μg/g), and alternariol monomethyl ether (580 μg/g). Only AAL-toxin and crude and cell-free filtrates of fungus-infested rice caused injury on excised leaves or intact jimsonweed (Datura stramonium) plants. A dose-response study of AAL-toxin on excised jimsonweed and black nightshade (Solanum nigrum) leaves showed equal effects at concentrations of 1.56 μg/ml and 0.01 μg/ml, respectively. A dose-response study of AAL-toxin on duckweed (Lemna pausicostata) showed phytotoxic effects at concentrations as low as 0.01 μg/ml. These effects included an increase in electrolyte leakage, visual bleaching, reduction of chlorophyll synthesis (50%) and inhibition of duckweed growth (27%) 72 h after application. Preliminary study indicates that SWSL#1 and AAL-toxin have a broad-spectrum of phytotoxicity including grasses and broad-leaf plants and may have potential as weed control agents.

A209

COMPARISON OF THE PHYTOTOXICITIES OF FUMONISINS A & B WITH AAL-TOXIN USING A DUCKWEED (LEMNA PAUSICOSTATA L.) ASSAY. H. K. Abbas,* T. Tanaka, S. O. Duke, W. C. A. Gelderblom and M. E. Cawood. SWSL, ARS, USDA, Stoneville, MS, USA and MNR/MRC, South African Medical Research Council, Tygerberg, South Africa.

Fumonisins (produced by cultures of Fusarium moniliforme) and their aminopentol hydrolysis products (AP_1 and AP_2) as well as AAL-toxin (produced by Alternaria alternata) were assayed for phytotoxicity on duckweed. FB_1, (0.33 μM) increased cellular leakage, reduced chlorophyll synthesis 66% and inhibited growth by 42% after 72 h. Phytotoxicity of FB_1 was stable or increased with increase in concentration. FB_1, FB_2, FB_3 and AAL-toxin were more active than AP_1, AP_2, FA_1, and FA_2 (all 1 μM). AP_1 was more phytotoxic to duckweed than AP_2, FA_1, and FA_2 with an increase in cellular leakage and growth inhibition of 25%. Fumonisin phytotoxicity can be decreased by hydrolysis or acetylation and this has implications for the structure activity relationships of fumonisin.

A212

EFFECT OF PEROXIDASE ACTIVITY IN LEAF LEACHATES ON THE SUSCEPTIBILITY OF MAIZE LEAVES TO THE HOST-SPECIFIC TOXIN PRODUCED BY BIPOLARIS MAYDIS RACE T. M. O. Garraway, Dept. of Plant Pathology, The Ohio State University, Columbus, OH 43210.

Exposure of leaves of normal (N) and Texas male sterile (T) cytoplasm isolines of maize to high temperature stress (HTS) i.e. 42 C in the dark for at least 15 minutes caused an increase in peroxidase activity in the leaf leachates and increased leaf susceptibility to Bipolaris maydis race T toxin (BMT-toxin). This response was significantly greater for the T isolines which are susceptible to BMT-toxin. The role of peroxidase activity in the altered sensitivity to BMT-toxin was studied by measuring its effect on toxin-induced electrolyte leakage from T cytoplasm maize leaves. When detached leaves were infiltrated with a BMT-toxin solution amended with either horseradish peroxidase or with peroxidase-containing preparations from maize leaf leachates, there was an increase in the susceptibility of maize leaves to BMT-toxin. The response was greater in T than in N cytoplasm leaves. Also, the peroxidase-induced increase in the sensitivity of T cytoplasm leaves to toxin was comparable to that caused by HTS. Peroxidase appears to be one of the constituents in leaf leachates that mediates stress-induced alterations in the susceptibility of maize leaves to BMT-toxin.

A213

PURIFICATION, CHARACTERIZATION, AND SUBSTRATE RELATIONSHIPS OF THE TANNASE FROM ENDOTHIA PARASITICA. G. M. Farias, C. Gorbea, J. R. Elkins, and G. J. Griffin. Virginia Polytechnic Institute & State University. Blacksburg, Virginia 24061.

The tannase of E. parasitica was isolated from the mycelium and purified 142-fold with a 10 % yield by anion exchange chromatography and gel filtration. The estimated molecular weight was 240 kD and the molecule may be a tetramer composed of four subunits with a molecular weight of 58 kD. The pH optimum of the purified tannase was 5.5 and the temperature optimum for activity was 30 C. The enzyme was separated into six bands in the pH range of 4.6 to 5.1. Based on the Michaelis-Menten constant (K_m) of the tannase for three substrates tested, aleppo tannic acid was the best substrate (K_m = 0.95 mM). The V_{max}/K_m ratio was almost five times higher than that for hamamelitannin and seven times higher than that for methyl gallate. Hamamelitannin was a good substrate for the enzyme (K_m = 5.07 mM). The ellagitannins, vescalagin, and castalagin, were not good substrates for the enzyme and no inhibition of E. parasitica tannase by these compounds was found. Gallic acid was an effective competitive inhibitor of the tannase with all substrates and concentrations tested (K_i = 11.0-13.1 mM).

A214

RACES OF *COCHLIOBOLUS CARBONUM* DISTINGUISHED BY PCR-AMPLIFICATION. M. J. Jones and L. D. Dunkle. USDA-ARS, Dept. of Botany and Plant Pathology, Purdue University, W. Lafayette, IN 47907.

Four pathogenic races of *Cochliobolus* (*Helminthosporium*) *carbonum* are differentiated on the basis of the size and shape of necrotic lesions on susceptible maize leaves. Isolates of the races were analyzed by PCR amplification of DNA with arbitrary primers or specific primers within the *Tox 2* locus that is essential for production of a host-specific pathotoxin. The DNA fingerprints of the pathogenic races were substantially different from those of a nonpathogenic race 0 and from most other species. But fingerprints of species thought to be closely related to *C. carbonum* (e.g., *C. victoriae* and *C. sativum*) were very similar. With one of the arbitrary primers tested, race 3 isolates of *C. carbonum* consistently lacked two major amplification products that were present in the other pathogenic races. With the *Tox 2* gene primer, a single amplification product was detected only in race 1 isolates. DNA fingerprints of race 2 and race 4 were indistinguishable, suggesting that the recently discovered race 4 is a variant of race 2. The results indicate that the population of *C. carbonum* is variable and dynamic and confirm that the *Tox 2* gene is uniquely and universally present in the toxin-producing race 1.

A215

ANALYSIS OF COLLETOTRICHUM SPECIES USING RFLPS. C.L. Trout and D.O. TeBeest, Department of Plant Pathology, University of Arkansas, Fayetteville, Arkansas 72701

Species within the genus Colletotrichum are usually identified on the basis of morphological characteristics while various formae speciales are recognized on the basis of host specificity. However, morphological characters are uncertain taxonomic criteria in this genus due to the wide variability often exhibited, particularly within C. gloeosporioides. A ribosomal DNA (rDNA) probe and a glutamate dehydrogenase (GDH) probe both from Neurospora crassa were tested as molecular markers for examining relatedness and diversity in Colletotrichum. Ten isolates representing six species of Colletotrichum (C. gloeosporioides, C. graminicola, C. dematium, C. malvarum, C. orbiculare and C. coccodes) and four formae speciales of C. gloeosporioides were analyzed. Preliminary results indicate that while rDNA RFLP analysis may be a useful tool for distinguishing Colletotrichum at both the species and formae speciales levels, GDH RFLP analysis was able to distinguish only groups composed of several species or formae speciales.

A216

MONOSPECIFIC AND CROSSREACTIVE MONOCLONAL ANTIBODIES TO *TOSPOVIRUSES*. J. M. Hall and J. W. Moyer, Department Plant Pathology, North Carolina State University, Raleigh, 27695-7616.

The *Tospovirus* genus is the only genus in the bunyaviridae virus family which infects plants. Viruses and subgroups of viruses within each genus are designated in part by the serological relatedness of the structural proteins. The nucleocapsid (N) protein is the predominate determinant in the Tospovirus genus. A panel of 63 monoclonal antibodies (MAb's) were synthesized to the N proteins of tomato spotted wilt (TSWV) and Impatiens necrotic spot *Tospoviruses*. Three different MAb's with different specificities were identified for each virus. In addition, two MAb's were identified which reacted equally well with both viruses. Competition assays were performed to demonstrate that these cross-reacting MAb's were not mixtures. Western blot analysis was used to confirm specificity to the N protein. Other crossreacting MAb's were identified which detected a strain of TSWV-like virus which causes peanut bud necrosis.

A217

DETECTION OF GRAPEVINE CLOSTEROVIRUS A BY POLYMERASE CHAIN REACTION AMPLIFICATION. A. Minafra, A. Hadidi, and G.P. Martelli*. USDA-ARS, NGRL, Beltsville, MD 20705 and *University of Bari, Bari, Italy.

The nucleotide sequence of a 1529 bp clone of grapevine virus A (GVA) has been recently determined (Minafra et al, 1992, Vitis, in press). We have used this sequence to design oligonucleotide primer pair that specifically primes the cDNA synthesis and polymerase chain reaction amplification of a 430 bp DNA fragment from purified RNA and from nucleic acid extracts of GVA-infected grapevine tissue. The viral origin of this DNA fragment was confirmed by Southern blot hybridization with ^{32}P-labeled GVA cRNA probe. This DNA was absent from nucleic acid extracts of uninfected tissue. As little as 0.1 pg of purified viral RNA or 100 pg of nucleic acid extracts of grapevine-infected tissue were sufficient to synthesize and amplify the 430 bp DNA fragment. This accurate and sensitive assay will be useful for epidemiological studies and identification of GVA-related viruses in fruit crops.

A218

DETECTION AND CHARACTERIZATION OF WHITEFLY-TRANSMITTED GEMINIVIRUSES BY USE OF POLYMERASE CHAIN REACTION. M. R. Rojas[1], R. L. Gilbertson[2], D. R. Russell[3], and D. P. Maxwell[1]. [1]Dept. of Plant Pathology, Univ. of Wisconsin, Madison, WI 53706, [2]Dept. of Plant Pathology, Univ. of California, Davis, CA 95616, and [3]Agracetus, Inc., Middleton, WI 53717.

Whitefly-transmitted geminiviruses are serious threats to many crops in the Americas and the Caribbean Basin. Polymerase chain reaction (PCR) methods were developed that allowed the amplification of viral DNA fragments from the two components, DNA-A or DNA-B, for previously undescribed geminiviruses. PCR-amplified fragments of DNA-A (1.1 to 1.4 kb) were cloned and "signature sequences" obtained for three different regions (the Common Region, and partial sequences for AL1 and AR1) for 10 new geminiviruses from tomatoes from Mexico, Costa Rica, and the Dominican Republic; from Sida sp. and Calopogonium sp. from Costa Rica; from soybeans from Puerto Rico; from Macroptilium lathyroides from the Dominican Republic; and from cassava and a legume weed from Malawi. Names are proposed for four geminiviruses: Sida golden mosaic geminivirus (SGMV); Macroptilium golden mosaic geminivirus (MacGMV), Calopogonium golden mosaic geminivirus (CalGMV), and soybean golden mosaic geminivirus (SoyGMV). Phylogenetic analysis of DNA sequence data placed 21 whitefly-transmitted geminiviruses from the Western Hemisphere into four major branches.

A219

USE OF PCR TECHNOLOGY FOR THE DETECTION AND ANALYSIS OF BADNAVIRUSES. N.E. Olszewski and B.E.L. Lockhart, Departments of Plant Biology and Plant Pathology, University of Minnesota, St. Paul, MN 55108.

We have designed and tested oligonucleotides that can prime PCR amplification of many badnaviruses (bacilliform DNA viruses). These primers have successfully detected eleven of twelve different badnaviruses tested. The viruses detected include: banana streak, *Commelina* yellow mottle, canna yellow mottle, *Kalanchoe* top spotting (one of two isolates tested was detected), *Piper* yellow mottle, rice tungro bacilliform, shefflera ringspot, and sugarcane bacilliform (four of four isolates were detected). We have also used these primers in conjunction with nucleic acid hybridization and DNA sequencing to assess the amount of variability among badnaviruses. Our results indicate that the group is highly polymorphic and that even some badnaviruses infecting the same host do not cross-hybridize. These results have implications on the utility of the various badnavirus detection strategies.

A220

IDENTIFICATION AND INCORPORATION OF TSWV RESISTANCE INTO COMMERCIAL LETTUCE. Wang, M., J. J. Cho,, and J. S. Hu, Dept. of Plant Pathology, University of Hawaii, Honolulu, Hawaii 96822

Tomato spotted wilt virus (TSWV) has been devastating to Hawaii vegetable industries and is the limiting factor for lettuce production. Progenies of an interspecific cross between *Lactuca sativa* L. and *L. saligna* L. have been screened for resistance to the virus. TSWV-L strain isolated from Maui, Hawaii was mechanically inoculated to plants from 130 lines of this cross. ELISA was used to confirm virus infections of survivors. Among 4489 plants inoculated, 192 from 42 lines survived virus infection and grew to maturity. TSWV was detected in the inoculated leaves but not in new developing growth. An early flowering (Ef) lettuce variety is being used in our program to accelerate the incorporation of TSWV resistance into commercial lettuce. The Ef lettuce (obtained from Dr. E. Ryder) which flowers in one-half the time required by normal varieties, was crossed with our resistant survivors, and their progenies backcrossed to commercial inbred lettuce lines. Identification of TSWV resistant lettuce will not only give both scientists and growers a means for combating this virus problem, but it may also save the lettuce industry in Hawaii.

A221

EXPRESSION OF RESISTANCE TO WHEAT SOILBORNE MOSAIC VIRUS (WSBMV) IN HARD RED WINTER WHEAT (*TRITICUM AESTIVUM* L.) IS TEMPERATURE DEPENDENT. J. L. Sherwood, L. D. Myers, and R. M. Hunger. Department of Plant Pathology , Oklahoma State University, Stillwater, OK 74078-9947.

The resistance to WSBMV in cultivars (cvs) of *T. aestivum* is not well characterized. After inoculation of susceptible (Sage and Vona) or resistant (Hawk and Newton) cvs using root washings from WSBMV-infected wheat at 15 C, a temperature conducive to virus transmission by *Polymyxa graminis*, WSBMV is detected in the roots of all cvs and in the foliage of susceptible cvs. When foliage of resistant and susceptible cvs is mechanically inoculated, WSBMV accumulates in the foliage and roots of all cvs. When susceptible and resistant cvs were inoculated via root washings, maintained at 15 C for at least 6 days to allow for WSBMV infection, and then placed at 22 C; WSBMV was found in the foliage of all cvs although symptoms were not evident in Hawk and Newton. Therefore, expression of resistance, as measured by virus accumulation in foliage, is temperature dependent.

A222

RESISTANCE TO SOIL BORNE WHEAT MOSAIC VIRUS (SBWMV) IS DUE TO INHIBITION OF AN UPWARD MOVEMENT OF THE VIRUS IN RESISTANT SOFT WINTER WHEAT CULTIVARS TESTED AND COULD BE INDEPENDANT OF ITS VECTOR (Polymyxa graminis).
RUMJAUN A., LAPIERRE H., Institut National de la Recherche Agronomique, Versailles, France.

The presence of SBWMV in three resistant(Pernel,Fandango,Fidel) and one susceptible(Festival)wheat(Triticum aestivum)cultivars (cvs.) was investigated in field trials during 1990-1991.Foliar symptoms were observed on susceptible cv. during the growing season and unfrequently observed on secondary tillers of resistant cvs. late in the season.After concentration by high speed centrifugation SBWMV was detected in roots and shoots by ELISA. Resistant cvs. were infected in some of the roots and secondary tillers tested lately in the season rise. Soil transmission in growth chamber after 35 days shows that resistant cvs.were only root infected whereas susceptible one was both shoot and root infected.To understand the mechanism of resistance,the four cvs. were sap inoculated.Mechanical inoculation of leaves of resistant and susceptible cvs.gave mosaic symptoms and virus was detected in roots of both types of cvs.Newly experimented mechanical inoculation of roots gave foliar symptoms on susceptible cv. only and virus was detected in the roots of both types of cvs. However no virus was detected in the shoots of resistant ones. These results suggest that the resistance to the upward migration of the virus is independant of Polymyxa graminis infection.

A223

SEROLOGICAL DETECTION OF TOMATO SPOTTED WILT VIRUS (TSWV) NON-STRUCTURAL PROTEIN. Frank A. Cantone[1], Thomas L. German[1], Diane Ullman[2], Delta Wescot[2], and John L. Sherwood[3] [1]Department of Plant Pathology, University of Wisconsin, Madison 53706 [2]Department of Entomology, University of Hawaii, Honolulu 96822 [3]Department of Plant Pathology, Oklahoma State University, Stillwater 74078

The genus *Tospovirus* has been created in the family Bunyaviridae to include those viruses formerly assigned to the monotypic TSWV group of plant viruses. The Bunyaviridae have tripartite genomes with RNA segments designated as S, M and L. Typically, the S RNA segments of these viruses encode a non-structural protein (NSs) found in infected cells but not in purified intact virions. The TSWV S RNA segment codes for a 52.4 kD protein also designated NSs. We used PCR to clone the NSs gene from TSWV into the pET-11 vector (Novagen) and expressed the protein in *E. coli* cells. The 54 kD fusion product was purified from preparative polyacrylamide gels and used to produce a polyclonal antiserum in a rabbit. The antiserum specifically reacted with TSWV infected plant tissues in ELISA. Western blot analysis indicates the presence of a 52.4 kD protein in infected plants that is absent in healthy plants. The antiserum was also used to label fibrous paracrystalline structures in sectioned plant material.

A224

IMMUNOCYTOCHEMICAL EVIDENCE FOR TOMATO SPOTTED WILT VIRUS (TSWV) REPLICATION IN CELLS OF THE WESTERN FLOWER THRIPS, Frankliniella occidentalis (Pergande). Ullman, D.E.[1], Westcot, D.M.[1], Cantone, F.[2], Sherwood, J.L.[3] and T.L. German[2]. [1]Department of Entomology, University of Hawaii, Honolulu, HI. 96822, [2]Department of Plant Pathology, Madison, WI. 53706, [3]Department of Plant Pathology, Oklahoma State University, Stillwater, OK. 74078.

Immunocytochemical analyses for TSWV encoded proteins in thrips cells provide the first direct evidence that *Tospoviruses* (Bunyaviridae) replicate in insect vectors. In cells of larval thrips fed on plants infected with TSWV; virions, viroplasm, dense masses, and inclusions of fibrous paracrystalline material were observed with transmission electron microscopy. Only viroplasms, dense masses and intact virions were labelled with polyclonal antibody specific to TSWV. Inclusions of fibrous, paracrystalline material, but no other TSWV related structure, were labelled with polyclonal antibody specific to the 52.4K TSWV encoded nonstructural protein (NsS). No labelling was observed with normal serum or in noninfected thrips cells. These results show TSWV NsS, an indicator of replication,is present in thrips.

A225

EFFECT OF YARD WASTE COMPOST ON PHYTOPHTHORA ROOT ROT OF PAPAYA. A.W. Barkdoll, D.J. Mitchell, P.A. Rayside and R.A. Nordstedt. University of Florida, Gainesville, FL 32611.

Many states are composting yard waste in an effort to conserve landfill space. Various types of compost, other than yard waste compost, have been shown previously to control several soilborne diseases. Yard waste compost from Alachua County, Florida was used for disease suppression studies. The pathosystem used was papaya and Phytophthora palmivora. Compost was sieved and mixed with soil at various levels (0, 20, and 50%) on a volume basis. Inoculum was mixed with the soil compost mixtures at planting at 0, 0.1, 1, and 10 chlamydospores/g soil. In repeated experiments, survival of papaya was greater with 20 and 50% compost than in soil alone. At 10 csp/ g soil, 100% of the plants in soil alone died after 8 wks; 60-80% and 50% of the plants survived in 50% and 20% compost, respectively. In spite of increased survival, papaya roots were infected at the end of 8 wks. In later experiments

with and without fertilizer additions, plants were harvested at 2, 4, 6, and 8 wks. Root infection was delayed, root and shoot weight were greater, and soil counts of P. palmivora were greater in compost mixtures than in soil alone. Fertilizer applications increased the rate of plant mortality in all treatments.

A226

Control of Phytophthora damping off of vinca *(Catharanthus roseus)* with drenches of aluminum sulfate. D. M. Benson, Dept. of Plant Pathology, N. C. State University, Raleigh.

A peat: vermiculite mix (1:1) amended with 1.8 or 3.0 g lime/L was added to plug trays, seeded with vinca, and covered with mix infested with *P. parasitica.* Aluminum at 133 meq/100 cm³ mix was drenched as $Al_2(SO_4)_3$ onto the surface of plug trays. Trays were placed under mist for 14 days as soil pH, exchangeable aluminum, populations of *Phytophthora* and stand counts were determined. Pre-emergence damping off was severe (>96%) in trays of seedlings not treated with aluminum, but only 29% and 0% in mix drenched with $Al_2(SO_4)_3$ at 1.8 and 3.0 g lime/L, respectively. Exchangeable aluminum was above 6 meq/100 cm³ for 14 days after drenching with $Al_2(SO_4)_3$ but less than 0.6 meq in undrenched mix. Populations of *P. parasitica* were suppressed 10-fold in $Al_2(SO_4)_3$ drenched mix. Exchangeable aluminum at 1.0 meq or higher in potting mixes may suppress populations of *P. parasitica* and control pre-emergence damping off of vinca.

A227

ALUMINUM AND A CALMODULIN ANTAGONIST INHIBIT GERMINATION AND REPRODUCTION OF *PHYTOPHTHORA PARASITICA* VAR. *NICOTIANAE.* A. M. Weaver and H. D. Shew, North Carolina State University, Raleigh, NC 27695-7616.

Soils high in exchangeable aluminum are suppressive to development of tobacco black shank. Aluminum is toxic to many fungi and is a potent inhibitor of the calcium binding protein, calmodulin. To examine the effects of aluminum and a calmodulin antagonist, W-7, on reproduction of *P. p. nicotianae*, hyphal mats were grown in 5% V-8 juice broth for 48 hours, incubated in test solutions for 48 hours, then examined for presence of sporangia. Mean number of sporangia per square millimeter was 13 for 3 ppm Al, 37, 20, and 8 for 10, 50 and 100 ppm W-7, and 41 for deionized water. To determine if spore germination was affected similarly, encysted zoospores were placed onto 0.5, 1.0 or 2.0% carrot agar amended with 10, 50, or 100 ppm W-7 or 2.5, 5.0 or 10 ppm Al. Increasing concentrations of W-7 and Al reduced germination. A nutritional effect also was observed; increased levels of nutrients decreased the toxicity of W-7 and Al. Disease suppression may be the result of Al effects on primary and secondary inoculum of the fungus.

A228

DISPERSAL OF PHYTOPHTHORA CAPSICI AND P. PARASITICA IN FURROW IRRIGATED ROWS OF TOMATO, PEPPER AND SQUASH. A.C.Café-Filho, D.A.Neher & J.M.Duniway. Plant Path. Dept., UC-Davis, CA 95616.

Dispersal of Phytophthora capsici (PC) and P. parasitica (PP) from point sources buried in soil near the upper end of 72m-long irrigation furrows was studied with 3 hosts. Furrows next to tomato were inoculated with PC or PP, while furrows next to pepper and squash were inoculated with just PC. Furrows were irrigated 5 times after inoculation, on a 14-day schedule. Dispersal of each fungus in the water was monitored by leaf disk baits, transplants in the furrows, incidence of root rot on all hosts, and incidence of buckeye rot on tomato fruit. For both fungi, spread upstream from the source was negligible while both were detected 2-32m downstream. PC and PP caused gradients of disease on roots that peaked at the source and decreased to low levels of root rot that extended 32m down the rows. Conversely, numbers of infected tomato fruit increased with distance downstream from the points of inoculation, implying accumulation of secondary inoculum in the furrows. PC was more virulent on tomato roots and caused more buckeye rot on fruit than did PP.

A229

Effects of inoculum density, nitrogen fertilizer, and soil management on corky root of tomato. F. Workneh and A. H. C. van Bruggen, Plant Pathology Department, University of California, Davis, CA 95616.

Incidence and severity of corky root of tomato, caused by Pyrenochaeta lycopersici, were significantly lower in organic farms than in conventional farms in the Central Valley of California. To determine if the difference in disease severity was due to suppressiveness, a greenhouse experiment was conducted with 4 inoculum levels of P. lycopersici (0, 10^3, 10^4, 10^5 viable microsclerotia/ml of soil) added to soils from two organic and two conventional farms. Tomatoes were planted in pots of these soils and corky root severity was assessed two months later. Corky root severity was significantly higher in conventionally than in organically managed soils. Organically managed soils also had higher levels of microbial activity. Addition of NH_4NO_3 at 100 kg/ha increased the disease in organically managed soils at all levels of inoculum but decreased disease in conventionally managed soils which were higher in available nitrogen (significant interaction P=0.0001). In a separate experiment with 6 levels of NH_4NO_3 (0, 50, 100, 150, 200 and 250 Kg/ha) added to pasteurized Yolo loam soil initially low in available nitrogen, severity of corky root increased linearly with increasing fertilizer rate (R^2 = 0.89).

A230

INCUBATION TIME AND SOIL TEMPERATURE EFFECTS ON SUGAR BEET AND APHANOMYCES DAMPING-OFF AFTER SOIL-INCORPORATION OF A GREEN OAT CROP. Carol E. Windels and Jeffrey Nielsen, Northwest Experiment Station, University of Minnesota, Crookston, 56716.

Soils collected from two fields naturally infested with *Aphanomyces cochlioides* were planted to oat or left fallow in the greenhouse (18 C). After 4 wk, plants were removed at soil level, cut into pieces, and incorporated into the soil in which they had grown. Sugar beets planted in soil immediately after oat residues were incorporated averaged 19% emergence compared to emergence of seedlings in fallow soil 83%. Seedlings planted in soil incubated for 3 wk at 15, 25, or 35 C after oat residues were added averaged 99% emergence. Incubation of soil for 3 wk at 15, 25, or 35 C after oat residues were incorporated resulted in sugar beet root rot indices (0-100 scale) of 24, 25, and 88%, respectively compared to indices in fallow soil of 57, 74, and 81%, respectively. Thus, incubation time and temperature of soil after incorporation of a green oat crop affect reduction of Aphanomyces damping-off.

A231

SUGAR BEET RESPONSE TO MULTIPLE SOILBORNE PATHOGENS. R. M. Harveson and C. M. Rush, Texas Agricultural Experiment Station, Bushland, Texas 79012.

The test was conducted on a clay loam naturally infested with four soilborne pathogens: beet necrotic yellow vein virus (BNYVV), *Rhizoctonia solani* (AG2-2), *Aphanomyces cochlioides*, and *Fusarium oxysporum f. sp. betae.* Twenty sugar beet entries (15 hybrids, 5 parental lines) were planted in a randomized, split plot design with six replications. Half the test was fumigated and half left untreated. Each plot was evaluated twice during the season by ELISA for BNYVV infection. A rating was made at midseason based on stand and foliar symptoms of root rot. At harvest, a rhizomania rating and root rot index were taken and analyzed with yield data. Fumigation had no effect on virus incidence or sugar percentage, but some entries showed improvement in root yield and lower disease ratings. Inverse correlations were observed between disease ratings at harvest and percent sugar and between midseason disease ratings and root yield in tons/acre.

A232

PATHOGENICITY OF PYTHIUM SPECIES ON SUGAR BEET USING A THERMOGRADIENT PLATE. R.A. Kuznia, C.E. Windels, and M.E. Stanghellini*, NW Expt. Stat., Univ. Minnesota, Crookston, 56716 and *Dept. Plant Pathology, Univ. Arizona, Tucson, 85721.

Pathogenicity of *Pythium* spp. isolated from diseased sugar beet seedlings was evaluated in a thermogradient plate composed of 96 acrylic cells (16 x 6); each cell measured 4 x 11 x 4.5 cm. Soil temperatures at seed depth ranged from 13 to 32 C across six cells. *P. acanthicum*, *P. aphanidermatum*, *P. ultimum* var. *sporangiiferum* and an unidentified species (*P. irregulare*-, *P. paroecandrum*-like) were grown on oat grains for 2 wk at 24 C. Two oat grains were placed next to 10 sugar beet seeds per cell. At 10 days after inoculation, >75% seed rot and damping-off incidence occurred at 20-32 C for *P. aphanidermatum* and at 13-29 C for *P. ultimum* var. *sporangiiferum.* Neither *P. acanthicum* nor the unidentified species caused seed rot, damping-off, or root necrosis; *P. acanthicum* was isolated from healthy roots. The thermogradient plate allows concurrent pathogenicity evaluations of *Pythium* spp. over a wide range of soil temperatures.

A233

TRANSMISSION OF SOILBORNE YIELD DECLINE OF SUGARCANE IN NORTHERN QUEENSLAND, AUSTRALIA. T. Isakeit[1], R.C. Magarey[2], and B.J. Croft[2]. [1]Dept. of Plant Pathology, University of Arizona, Tucson AZ 85721. [2]Bureau of Sugar Experiment Stations, P.O. Box 566, Tully, Qld. 4854, Australia.

In northern Queensland, unidentified soil microorganisms reduce sugarcane yield by 20-40%. Associated symptoms consist of root browning and reduced growth of fine roots. The transmission of yield decline (YD) to soil fumigated with methyl bromide (i.e. non-YD soil) was studied with greenhouse pot experiments. The addition of root tissue from symptomatic plants to soil had no effect on root growth. Only large proportions of YD soil (>50%) added to fumigated soil reduced growth (g root weight = 5.54-0.023[%YD soil], p=0.001). The addition of sand, silt or clay components from YD soil did not adversely affect growth, while 125-1000 μ sieve size fractions of organic matter from YD soil caused root browning and significantly (P=0.05) reduced root weights to 52-57% of the control. These results indicate that YD is not root-infecting and may be attributable to microorganisms associated with the organic matter fraction of soil.

A234

SOILBORNE SEEDLING DISEASES OF TALL FESCUE: INFLUENCE OF THE ENDOPHYTE, ACREMONIUM COENOPHIALUM. C.A. Blank and K.D. Gwinn, Dept. Entomology and Plant Pathology, University of Tennessee, Knoxville, TN 37996.

A negative correlation exists between endophyte infestation level of tall fescue seed lots and seedling loss due to Rhizoctonia zeae and R. solani under certain environmental conditions. The objective of this research was to determine if endophyte infestation level influences seedling losses due to Pythium aphanidermatum and Magnaporthe poae. Two seed lots with endophyte infestation levels of < 1% (E-) or 65% (E+) were planted (10 g/flat) in Fafard® soilless medium or Fafard® amended with M. poae or P. aphanidermatum. The number of seedlings per 5 cm core was counted; 3 cores were sampled from each flat. Five flats per treatment were used and each experiment was repeated 3 times. Endophyte infestation level did not influence seedling losses to either pathogen.

A235

INFECTION OF POTATO ROOT TIPS BY *VERTICILLIUM DAHLIAE* AS AFFECTED BY *PRATYLENCHUS PENETRANS* AND *P. CRENATUS*. J. H. Bowers, R. M. Riedel, and R. C. Rowe. Dept. of Plant Pathology, The Ohio State University, OARDC, Wooster 44691.

Factorial experiments were conducted in the greenhouse with soil infested with known densities of *Verticillium dahliae* (*Vd*) and/or *Pratylenchus penetrans* (*Pp*) and *P. crenatus* (*Pc*), and destructively sampled after 3, 5, and 7 wk. Root samples were excised and stained by using an immunoenzymatic procedure. Percent colonization was estimated and healthy and infected root tips counted. Five weeks after planting, roots grown in soil infested with *Vd* alone had a very low percentage of infected root tips (1.2%) which was significantly less ($P<.05$) than roots growing in soil infested with *Vd*+*Pp* or *Vd*+*Pc* (2.3 and 2.5%, respectively). However, roots and basal stem segments were colonized by *Vd* to a significantly greater extent ($P<.05$) when grown in soil infested with *Vd*+*Pp* than in soil infested with *Vd* alone or with *Vd*+*Pc*. Low levels of initial infection resulted in high incidences of stem colonization in treatments with *Vd*+*Pp*. Infection was not observed to be associated with nematode feeding and the effect of nematodes on initial infection may be non-specific. The interaction between *Vd* and *Pp* in potato early dying may occur within the root as an altered or delayed host response to colonization by *Vd*.

A236

EFFECT OF CROPPING SEQUENCES ON THE EFFICIENCY OF COLONIZATION OF POTATO ROOTS BY VERTICILLIUM DAHLIAE AND FUSARIUM SPECIES. O.C. Huisman and J.R. Davis. Univ. of California, Dept of Plant Pathology, UC Berkeley, CA 94720 and Univ. of Idaho Research and Extension Center, Aberdeen, ID 83210

Colonization of potato roots by selected fungi was monitored for two seasons following a previous two to three year cropping sequence with green manures. Verticillium dahliae, Fusarium oxysporum and F. equiseti differed in their root colonization efficiency (number of cortical colonies per cm root and per unit soil inoculum density). Per unit inoculum, V. dahliae colonized potato roots about ten fold higher than did F. oxysporum or F. solani, while for F. equiseti, the efficiency was less than half that of the other Fusaria. Cropping sequences also affected colonization efficiency. Colonization efficiency was reduced for V. dahliae in plots previously cropped to green manures as compared to previously fallow plots. For V. dahliae, colonization efficiency was equivalent for both seasons, whereas colonization efficiency for the Fusaria was only one fourth as effective in the second potato crop as compared to the first crop.

A237

USE OF F(ab')$_2$-ELISA IN DETECTING VIRUSES AFFECTING FRUIT AND NUT TREES. Adib Rowhani and J. K. Uyemoto, Specialist, Department of Plant Pathology and Research Plant Pathologist, USDA-ARS, University of California, Davis, CA 95616.

An enzyme-linked immunosorbent assay using F(ab')$_2$ fragments prepared from polyclonal rabbit antibodies specific for Prunus necrotic ringspot virus (PNRSV), a prune isolate of tomato ringspot virus (TmRSV), and a walnut isolate of cherry leafroll virus (CLRV) were evaluated in the detection of homologous antigens. The plates were precoated with the F(ab')$_2$ preparations, and homologous IgG as probes and alkaline phosphatase labelled protein A as the anti-probe. F(ab')$_2$-ELISA was compared with indirect ELISA (I-ELISA) whereby the probe antibody sources were from different animal species than the trapping antibody. In our laboratory, the F(ab')$_2$-ELISA is used in preference to the I-ELISA due in part to the lower nonspecific reaction and the convenience of using antibody produced in a single animal species and a standard commercial source of enzyme-labelled anti-probe.

A238

GENETIC DIVERSITY OF SATELLITE TOBACCO MOSAIC VIRUS FIELD ISOLATES. G. Kurath, J. A. Heick and J. A. Dodds, Department of Plant Pathology, University of California, Riverside, CA 92521.

Fifteen field isolates of satellite tobacco mosaic virus (STMV) were collected from wild *Nicotiana glauca* plants within fifteen miles of Riverside, California. RNase protection assays showed that there were ten different genome types within the fifteen samples, including some with extreme divergence from the STMV type strain. Isolates from adjacent locations were not always identical but were more similar than those from more distant locations. Mapping of the sites of heterogeneity revealed that the 3' untranslated region of the genome was more conserved than the 5'coding regions. This high level of genetic diversity between populations will be discussed with regards to STMV transmission, epidemiology and evolution.

A239

CONTROL OF SOYBEAN SEVERE STUNT THROUGH THE USE OF CROP ROTATION AND REISTANT CULTIVARS AND FURTHER CHARACTERIZATION OF ITS CAUSAL AGENT. T. A. Evans and D. A. Schaff, Department of Plant and Soil Sciences, University of Delaware, Newark, DE 19717.

Soybean severe stunt (SSS), caused by the soybean severe stunt virus (SSSV), is a new virus disease affecting Delaware soybeans and has not been detected elsewhere. The disease is transmitted via soil and has been observed in many of the same fields year after year, particularly those continuously cropped with the susceptible soybean cultivar Essex. Evaluation of soybean cultivars in naturally infested fields and by mechanical inoculation in the greenhouse have identified a number of apparently resistant cultivars including Asgrow A-5149, HyTest-5203 and Sparks. Results of a crop rotation study indicate that both SSS and its probable vector, the dagger nematode (Xiphinema americanum Cobb), were greatly reduced after a two year rotation to corn, grain sorghum and wheat. Rotation to Essex and HyTest 5203 resulted in moderate and high levels of SSS, respectively. Characterization of the causal agent continues. A partial cDNA library for SSSV has been constructing using oligo dT and random primers. Insert size of postive clones of SSSV and SSSV-RNA size(s) will be reported.

A240

SYMPTOM MODIFICATION BY SATELLITE TOBACCO MOSAIC VIRUS IN PEPPER CULTIVARS INFECTED WITH HELPER VIRUSES. G. Rodriguez-Alvarado, G. Kurath, and J. A. Dodds, Department of Plant Pathology, University of California, Riverside, CA 92521.

The presence of satellite tobacco mosaic virus (STMV) in co-infection with 8 different helper viruses and/or 23 different host plants, causes no change in the symptoms induced by the helper viruses alone. This is not the case for systemic infection of certain cultivars of pepper (*Capsicum annuum*). On Jalapeno and Pimiento pepper plants, the presence of STMV in co-infections with TMV-U2 caused increased chlorosis in the form of yellow patches relative to the mosaic caused by TMV-U2 alone. On Jalapeno the presence of STMV ameliorated the blister leaf distortion caused transiently by TMV-U2 at 6 wk after inoculation. Symptom modifications were also observed when tobacco mild green mosaic virus and pepper mild mottle virus were used as helper viruses.

A241

FIELD STUDIES OF SATELLITE TOBACCO MOSAIC VIRUS. D.M. Mathews, J.A. Heick, J.A. Dodds. Department of Plant Pathology, University of California, Riverside, CA 92521.

Approximately 250 *Nicotiana glauca* trees, primarily in So. California, were tested for the presence of satellite tobacco mosaic virus (STMV). The virus was not uniformly distributed, but overall 28% of the plants tested were infected with STMV. Four controlled field plots consisting of 60 *N. glauca* trees each were planted near UCR. Trees were tested by dsRNA analysis over a 2 1/2 year period. Viruses detected included CMV, CARNA 5, TEV, TMV, and STMV. The STMV was limited to 2 of the 4 plots although each plot was within 1/2 mile of the others. Transmission experiments using insects, seed, soil, and mechanical means are in progress at this time.

A242

VARIATION IN LONG TERM STABILITY OF APHID TRANSMISSION PHENOTYPE OF RMV ISOLATES OF BARLEY YELLOW DWARF VIRUS. D. Hazelwood, S.M. Gray, USDA-ARS, Cornell University, Ithaca, NY 14853, and T.W. Carroll, Montana State University, Bozeman, MT 59717.

Serologically similar isolates that differ in aphid transmission phenotype were previously separated in our lab from plants infected with an RMV serotype of barley yellow dwarf virus (BYDV). *Rhopalosiphum maidis* transmits each isolate efficiently. Following six serial passages through *Schizaphis graminum*, normally an inefficient vector, transmission of some isolates was increased to 65-90. The stability of aphid transmission phenotype was examined from oat plants infected with RMV isolates efficiently transmitted by both aphid species and maintained for two years. Transmission efficiency from these two year old plants remained high (70-80%) for *R. maidis*, but was reduced to 5-20% for *S. graminum*. Serial passage of the isolates through *S. graminum* resulted in increasing transmission efficiency (up to 55%). It appears that isolates varying in aphid transmission phenotype can be selected from a common serotype virus population; however, in the absence of selection by aphids (in this case *S. graminum*), the majority of virus particles in the population will have a transmission phenotype reflective of the serotype. This may be important with regard to perennial grasses that can serve as long term reservoirs of populations of BYDV.

A243

TRANSMISSION, MOVEMENT, AND INACTIVATION OF CYMBIDIUM MOSAIC VIRUS AND OTHER ORCHID VIRUSES. J. S. Hu, S. Ferreira, M. Wang, M. Q. Xu, and J. Uchida, Department of Plant Pathology, D. Ullman, Department of Entomology, Univ. of Hawaii, Honolulu, HI, 96822.

Cymbidium mosaic virus (CyMV), odontoglossum ringspot virus (ORSV), and tobaco mosaic virus common strain (TMV) were mechanically transmitted to Dendrobium orchid leaves. CyMV was detected from inoculated leaves within 6 days, from roots within 15 days, and other leaves within 40 days post-inoculation. ORSV and TMV were detected only in the inoculated leaves even three months after inoculation. Inoculation of Dendrobium orchids by slashing leaves with razor blades dipped in a suspension of CyMV failed to infect the orchids, but was successful on flower stems (sprays). Tomato spotted wilt virus was purified from infected Oncidium orchids (with low yield), but was not transmitted to Oncidium orchids or herbaceous plants by mechanical or thrips inoculation. Skim milk was an effective, safe, inexpensive reagent for the inactivation of CyMV. When CyMV was mixed with skim milk, the virus lost infectivity within 15 seconds. After skim milk treatment, CyMV was not observed in electron microscopy, but was positive in ELISA. Heat-treated skim milk (90 C, 10 min) was similarly effective.

A244

APHID/PLANT/VIRUS INTERACTIONS: A CLOSER LOOK AT FACTORS DETERMINING VECTOR SPECIES INTENSITY. C. Y. Yuan, Department of Plant Pathology, and D. E. Ullman, Department of Entomology, University of Hawaii, Honolulu, HI 96822.

At least 8 species of aphids, some colonizing and some landing on but not colonizing cucurbitaceous plants, are able to transmit zucchini yellow mosaic virus (ZYMV) in Hawaii. The purpose of this investigation was to accurately measure vector species importance under laboratory conditions. A method in which aphid access to plants and movement between plants was controlled experimentally was compared to an "arena" method in which insects were given free access to all plants and could move freely. Results from these comparative transmission assays indicate the arena method is accurate, more cost and labor effective than the assay in which aphids are experimentally manipulated. In addition, the noncolonizing aphid species was more efficient as a vector than the colonizing species for transmission of ZYMV. It is likely that the increased efficiency observed in noncolonizing species is due to feeding behavior and increased movement between plants. These findings are significant for future projects involving simulation of ZYMV epidemics, as well as providing a method for testing aphid propensity.

A245

TRANSMISSION OF TOMATO SPOTTED WILT VIRUS BY FRANKLINIELLA FUSCA FROM TWO LOUISIANA WILD COMPOSITE HOSTS. H.A. Hobbs, L.L. Black, R.N. Story, R.A. Valverde, W.P. Bond, J.M. Gatti, Jr., D.O. Schaeffer and R.R. Johnson. Dept. of Plant Pathology and Crop Physiology, Louisiana State University, Baton Rouge, 70803.

Laboratory experiments were carried out to determine the ability of Frankliniella fusca to transmit tomato spotted wilt virus (TSWV) from two Louisiana wild hosts of the virus, spiny-leaved sowthistle, Sonchus asper, and wild lettuce, Lactuca floridana, both members of the Compositae. Using five thrips per test plant, F. fusca was able to transmit TSWV from both S. asper and L. floridana virus acquisition host plants to S. asper test plants and to bell pepper (Capsicum annuum) test plants. F. fusca was also able to transmit TSWV from bell pepper virus acquisition host plants to bell pepper test plants and to S. asper test plants. Highest levels of transmission occurred with S. asper as virus acquisition host, followed by L. floridana, then bell pepper. Higher levels of transmission were obtained with S. asper as test plant than with bell pepper as test plant.

A246

DIFFERENTIAL RESISTANCE OF A PINK SHADDOCK TO SELECTED ISOLATES OF CITRUS TRISTEZA VIRUS (CTV). S. M. Garnsey, USDA, ARS, USHRL, 2120 Camden Rd., Orlando, FL 32803.

A large pink shaddock (Citrus maxima) tree at the Waimea Experiment Station, island of Hawaii, remained free of CTV symptoms although other cultivars nearby were severely affected. CTV infection was not detected in this tree by ELISA, and glasshouse-grown propagations were not infected by graft inoculation with four CTV isolates. Most seedlings of this tree were resistant to infection when inoculated by a Florida isolate of CTV. Propagations of the pink shaddock and of one of its seedlings were readily infected by a hassaku dwarf (HD) isolate of CTV from Japan. HD-infected plants showed stem-pitting symptoms and tested positive for CTV infection by ELISA. The HD isolate did not infect the hybrid citrus breeding line US 119, which has the trifoliate orange (Poncirus trifoliata) gene for CTV resistance. The CTV resistance in pink shaddock is isolate specific and distinct from the CTV resistance in trifoliate orange.

A247

POTATO LEAFROLL VIRUS RESISTANCE AS DETERMINED BY INOCULATION ACCESS PERIOD IN A GREENHOUSE MICROPLOT SYSTEM. G. E. Sánchez and S. A. Slack. Department of Plant Pathology, Cornell University, Ithaca, NY 14853.

A greenhouse microplot system was used to compare the relative resistance of potato cultivars (CV) to potato leafroll virus (PLRV). *In vitro* plantlets of Russet Burbank (susceptible), Katahdin (moderately resistant) and Abnaki (resistant) were transplanted into styrofoam plug trays, established for 1 week, and then caged individually with 3 aphids, previously allowed a 3-day PLRV acquisition period. Four replicates of 15 plants/cv were exposed to inoculation access periods (IAP) of 0, 1, 3, 9, 27, 81 and 168 hours. Plantlets were grown for 30 days and then scored for PLRV by visual inspection and ELISA. Logistic regression analysis indicated that CV and IAP significantly affected transmission but the concentration of initial inoculum (CI) and the interactions IAP x CV and CI x CV were not significant. The probability that PLRV transmission would occur in Katahdin or Abnaki compared to Russet Burbank was 51% and 8%, respectively. ELISA titers in PLRV-infected Russet Burbank, Katahdin and Abnaki were 1.02, 1.13 and 0.16 and qualitative agreement between visual and ELISA scores was 90%. This system is being expanded to establish quantitative PLRV relationships among potato CVs.

A248

VARIABILITY OF CAPSID PROTEIN SIZE AMONG DASHEEN MOSAIC VIRUS (DMV) ISOLATES. Li, R.H., F.W. Zettler, C.A. Baker, D.E. Purcifull, E. Hiebert, and G.C. Wisler. Dept. Plant Pathology, Univ. Florida, Gainesville 32611-0680

Western blot tests, performed as described by Li *et al.* (1991, Plant Dis. 75:130) with DMV antiserum, were used to compare molecular weight (MW) values of the capsid proteins (CPs) of 7 DMV isolates which induced symptoms of varying severity in *Philodendron selloum*. In their original hosts, the MWs were 47 (*Xanthosoma*), 45 (*Colocasia, Zantedeschia*) and 38-46 kD (*Caladium* sp.). Virus propagation in *P. selloum* did not affect the CP MW values of the individual isolates. The high (relative to other potyviruses) MW values for each of the respective 7 DMV isolates were confirmed when polyclonal antisera to the following potyviruses were tested: passionfruit crinkle, peanut stripe, tobacco etch, tobacco vein mottling, watermelon mosaic 2, zucchini yellow mosaic. Similar MWs of DMV CPs were also noted when tested against the following monoclonal antisera: Agdia potygroup, papaya ringspot type W, and watermelon mosaic 2. Lower MW products representing degraded DMV CPs (45-15 kD) reacted with polyclonal antisera of DMV and the other 6 potyviruses tested. However, few or no such immunoreactive components were noted when DMV isolates were tested against the monoclonal antisera. The high MWs noted for the major CP components of DMV isolates in this study are consistent with the report of Abo El-Nil *et al.* (1977, Phytopathology 67:1445), who also noted high MWs for the CPs of 2 DMV isolates from *Colocasia*.

A250

INTERACTIONS AMONG VAM MYCORRHIZAE, MOISTURE STRESS AND MACROPHOMINA PHASEOLINA ON MELON PLANTS. Maria Pilar Castro and R. M. Davis, Department of Plant Pathology, University of California, Davis, CA 95616.

Eight days after sowing melon seedlings were inoculated with two VAM mycorrhizae fungi, Glomus intrarradices and/or G. deserticola, and Macrophomina phaseolina. Half of the plants were subjected to water stress; the water potential of the leaves was maintained slightly above the permanent wilting point. The water status in all plants was measured in a pressure chamber. The effect of VAM mycorrhizae on the visual reduction of charcoal disease symptoms and plant biomass production was evaluated. The incidence of disease was greater in water stressed plants. Mycorrhizal fungi significantly increased plant growth only in non-water stressed plants. A simple selective medium for recovering M. phaseolina sclerotia in infected soils is proposed.

A259

ACREMONIUM COENOPHIALUM ENDOPHYTE OF TALL FESCUE SUPPRESSES GLOMALES MYCORRHIZAL FUNGI. B. Z. Guo, Z. -Q. An, M. Chu- Chou, and J. W. Hendrix. Dept of Plant Pathology, Univ of Kentucky, Lexington 40546.

Mycorrhizal stunt, caused by Glomus macrocarpum, is the reason tobacco must be rotated to maintain soil productivity for tobacco. Tall fescue is the best rotation crop. Acremonium coenophialum, the endophyte of tall fescue, produces toxic chemicals. As assay hosts, seedlings infected with Acremonium resulted in detection of lower populations of most of the eleven mycorrhizal species detected in a field soil than seedlings free of Acremonium. In single-spore inoculations, Acremonium did not reduce the number of seedlings infected by G. macrocarpum and G. mosseae, but the extent of colonization by both fungi was reduced. Sporulation by both fungi also was reduced, perhaps as a result of the effect on colonization. The inhibitory effect of Acremonium on mycorrhizal fungi probably is due to the transport of toxic metabolites to the roots, for Acremonium resides only in shoots, and mycorrhizal fungi only in roots.

A260

LONG- AND SHORT-TERM CROPPING HISTORY IN RELATION TO PATHOGENICITY OF GLOMUS MACROCARPUM TO TOBACCO. J. W. Hendrix, B. Z. Guo, and Z. -Q. An. Dept of Plant Pathology, Univ of Kentucky, Lexington 40546.

Tobacco stunt, caused by G. macrocarpum, was more severe on land with a long-term history of sorghum-sudangrass hybrid than on land in tall fescue. Of 17 species of mycorrhizal fungi found, populations of only G. macrocarpum were related to stunt. Fescue with a low incidence (6.5%) of infection with Acremonium coenophialum, and grown for two years, suppressed G. macrocarpum and stunt to only a limited extent. Acremonium present in fescue probably is necessary for maximum maintenance of soil productivity for tobacco. Cropping history and crop rotation have extensive effects on the composition of the mycorrhizal community of soils and may be used to promote or suppress individual mycorrhizal species if desirable.

A273

YIELD OF MUSKMELON (CUCUMIS MELO) AS AFFECTED BY FUMIGANTS IN FIELDS INFESTED WITH MONOSPORASCUS CANNONBALLUS. M. E. Miller, R. D. Martyn, and B. D. Bruton. Texas A&M University, Weslaco 78596 and College Station 77843, and USDA, ARS, Lane, OK 74555.

In 1989 and 1990, fumigants at different rates were injected into the center of muskmelon (cantaloupe cv. 'Magnum 45') beds in 0.3 m treatment zones to determine their effects on fruit yield in fields naturally infested with Monosporascus cannonballus. Busan 1020 (metham-sodium) at 70.1, 140.3, and 210.4 l/ha was injected into the soil using winged-shanks with orifices at 30.0, 23.0, 15.0 cm depths. Telone C17 (1,3-Dichloropropene 77.9% plus chloropicrin 16.5%) at 84.2, 168.2, 252.4 l/ha and Telone II (1,3-Dichloropropene 94%) at 65.5, 130.9, and 196.4 l/ha were also injected with the winged-shank, but only at the 30.0 cm depth. All plots treated with fumigants had significantly increased yield over the nonfumigated controls. Yield was significantly increased by increasing application rate in plots treated with Telone C17 and Telone II, however, a rate response was not observed in plots treated with Busan 1020. In 1991 fumigants were applied in the center of 2.0 m beds in 0.3, 0.6, and 0.9 m bands to determine if extending the treatment zone would increase efficacy of the fumigants, however, no significant yield increase was observed.

A274

POTENTIAL CONTRIBUTION OF WEATHER FORECASTS TO IMPROVED FUNGICIDE SPRAY SCHEDULING AND EFFICACY OF POTATO EARLY BLIGHT CONTROL. S.N. Bergeron and W.E. Fry, Plant Pathology Department, Cornell University, Ithaca, NY.

Flexible, weather-based disease forecasters are designed to help reduce the amount and frequency of fungicide application while maintaining adequate disease control. Such forecasters currently rely on previous weather and might benefit from the incorporation of weather forecasts in scheduling fungicide sprays. A computer simulation analysis of the potato early blight system was conducted to assess the maximum potential contribution of a 24-hour weather forecast to a flexible spray scheduling scheme. Simulations were run using 10 years of actual weather data, for 3 potato cultivars with different levels of resistance to early blight. Empirically derived rules for the initiation and termination of spraying during the season were also included in the analysis. Cumulative disease severities resulting from flexible spray schedules were compared with those resulting from fixed spray intervals (from every 5 to 12 days). The incorporation of a perfect 24-hour weather forecast in a flexible weather-based disease forecaster resulted in a marginal reduction in the number of sprays applied while maintaining comparable disease control.

A275

EFFECT OF WOUNDING AND WETTING DURATION ON INFECTION OF POTATO FOLIAGE BY COLLETOTRICHUM COCCODES. D. A. Johnson, and E. R. Miliczky, Washington State University, Prosser WA 99350.

Dark brown to black lesions developed on leaves, petioles and stems of Russet Burbank potato after wounding by sand blasting and inoculating with Colletotrichum coccodes in the greenhouse and field. Significantly more lesions developed on sand blasted foliage than on non-sand blasted foliage ($P = 0.01$). Leaves of infected plants became chlorotic, wilted, and blighted. C. coccodes was reisolated. Symptoms did not develop on plants that were wounded but not inoculated. Successful infections decreased as the interval between wounding and inoculation increased from 0 to 2 days. After 3 days, wounded foliage was no more susceptible than non-wounded foliage ($P = 0.01$). Number of lesions on foliage of wounded and inoculated plants increased ($P = 0.001$) as the duration of the post-inoculation wet period increased from 2 to 48 hr. Conidia from foliage and applied to the soil surface washed through the soil and infected below-ground stems and roots.

A276

INFLUENCE OF DEW PERIOD AND TEMPERATURE ON INFECTION OF TOMATO FOLIAGE BY *COLLETOTRICHUM COCCODES*. M.K. Hausbeck and S.D. Linderman. Dept. of Botany and Plant Pathology, Michigan State University, East Lansing 48824.

A selective medium was developed for the isolation of *Colletotricum coccodes* and quantification of latent infections on inoculated greenhouse-grown tomato (cv. Bonnie Best) foliage. Plants were inoculated with conidia of *C. coccodes* and exposed in a dew chamber to 0, 4, 8, 12, 16, 20, or 24 h of wetting at 15, 20, or 25 C. One wk after inoculation, the severity of infection was quantified by placing explants from leaves on the surface of a medium consisting of the following ingredients/L: 300 ml filtered V-8 juice; 700 ml double-distilled water; 20 g Difco agar; and the following antimicrobial agents added after autoclaving: 0.13 g pentachloronitrobenzene; 0.14 g benomyl; 0.10 g streptomycin sulfate; 0.10 g tetracycline HCl, and 0.1 g chloramphenicol. On this medium colonies of *C. coccodes* were compact and easily identified and quantified after 4 days. At 15 C, a minimum of 16 h of leaf wetness were necessary for infection, whereas only 12 h were necessary at 20 and 25 C. Numbers of colonies of *C. coccodes* were higher on explants removed from bottom than from mid to top leaves of plants that received 24 h leaf wetness at 20 or 25 C.

A277

ROLE OF *PYTHIUM* SPP. IN THE DEVELOPMENT OF CAVITY SPOT ON CARROTS IN BRITISH COLUMBIA. D. Benard and Z.K. Punja, Department of Biological Sciences, Simon Fraser University, Burnaby, British Columbia, Canada V5A 1S6.

Isolates of *Pythium* spp. were recovered from cavity spot lesions on carrots in 1989, 1990, and 1991, by plating infected tissues onto PVPP medium. Among the 110 isolates, 15 distinct groups, possibly representing different species, were identified. The isolates were compared for pathogenicity to in-vitro grown carrot seedlings, mature carrot roots, and carrots grown in infested soil. All isolates were pathogenic to carrot seedlings, but varied in their ability to induce cavity spot on mature carrot roots and on soil grown carrots, with infection ranging from none to severe disease. The largest group of isolates, which was also the most pathogenic to carrots, was identified as *P. violae*. Enzyme assays demonstrated the secretion of pectolytic enzymes by various *Pythium* isolates. Histopathological studies were conducted to determine the pathogen-host interaction during cavity spot development. Fungal hyphae and oospores were observed among diseased host cells. Representative isolates of *P. violae* were also used to inoculate 36 carrot varieties. Differences in varietal susceptibility to cavity spot were detected in laboratory inoculations, which correlated well with results from field evaluations.

A278

HOST RANGE AND NUTRITIONAL REQUIREMENTS OF PYTHIUM VIOLAE, THE CAUSE OF CAVITY SPOT OF CARROT. J. K. Schrandt, J. J. Nunez and R. M. Davis. Department of Plant Pathology, University of California, Davis 95616.

Cavity spot, caused by Pythium violae, is a disease of carrots in California. The host range of the fungus was determined in greenhouse pathogenicity tests or in naturally infested field soils. Five new symptomless hosts (celery, broccoli, cowpea, sugar beets and cucumber) were identified in the greenhouse tests. Other known hosts confirmed in the test were alfalfa and wheat, both which showed root lesions. The utilization of C and N compounds by P. violae was examined in a chemically defined medium under controlled conditions. P. violae metabolized glucose, fructose and sucrose as carbon sources and glutamine, ammonium nitrate, serine and proline as favored nitrogen sources.

A279

CONIDIAL PRODUCTION BY VIRULENT AND AVIRULENT FUSARIA ON ASPARAGUS PLANTLET ROOT AND STEM SEGMENTS. Youn Su Lee and W. J. Manning, Dept. of Plant Pathology, University of Massachusetts, Amherst, MA 01003

Three-cm root and stem segments of asparagus (Asparagus officinalis L.) plantlets (female clone NJ362M) were placed on LSR medium on glass slides within sterilized glass petri dishes. Washed spores of fusaria, $3x10^3$/ml, from 10-day-old PCA plates, were placed in droplets on root and stem segments. Conidial populations were determined 7 days after inoculation. On root segments, conidial counts increased for avirulent Fusarium oxysporum (AVFO1) by 76% and for virulent F. oxysporum (FO) by 533%, and decreased for F. moniliforme (FM) by 42.3% and for F. solani (FS) by 25%. On stem segments, conidial counts increased for FO by 410%, for FS by 17.7% and for FM by 28%, and decreased for AVFO1 by 7.3%. The experiment was repeated twice.

A280

HINOKITIOL, A NATURAL SUBSTANCE, TO CONTROL POSTHARVEST DECAY ON'GALIA' MELONS. Yair Aharoni, Azica Copel, and Elazar Fallik. Department of Fruit and Vegetable Storage, Agricultural Research Organization, Bet-Dagan 50250, Israel.

Hinokitiol, a volatile oil component present in Taiwanese Hinoki tree oil, Japanese Hiba tree oil and in Western Red Cedar tree oil, was found to reduce decay on'Galia' melons Cucumis melo cv reticulatus, caused by Alternaria alternata and Fusarium spp. Melons coated with wax containing 750 ppm Hinokitiol had much less decay than the control fruit during 14 storage days at 6°C and additional 6 shelf-life days at 20°C. 'Galia' melons designed for export from Israel to Europe are coated with wax containing 2000 ppm Imazalil for decay control. This treatment leaves on the fruit a residue of 4-5 ppm Imazalil, an amount that exceeds the tolerance of some European countries that demand an Imazalil residue below 0.5 ppm. Hinokitiol, which is a natural substance, has a potential of replacing the Imazalil for'Galia' melon decay control.

A281

RESPONSE OF CAPSICUM SPP. TO FUSARIUM OXYSPORUM F. SP. CAPSICI AND VCG CHARACTERIZATION OF PATHOGENIC ISOLATES.
M. M. Jones and L. L. Black. Dept Plant Path. & Crop Phys., La. Agric. Expt. Sta., LSU Agric. Ctr., Baton Rouge, Louisiana 70803

Fusarium wilt of pepper, caused by Fusarium oxysporum f. sp. capsici (FOC), has been observed in 'Tabasco' pepper production fields at Avery Island, Louisiana since 1978. Using root-dip inoculation, high levels of resistance were found among accessions of Capsicum baccatum, C. annuum, and C. chacoense. Sixteen of 27 C. baccatum accessions were highly resistant showing no wilt and little or no root damage. Single accessions of C. annuum and C. chacoense were found to be highly resistant. All accessions of C. frutescens tested were highly suceptible. Vegetative compatibility groupings (VCG) of 27 pathogenic isolates were determined. All pathogenic isolates were of the same VCG, but were distinct from 32 nonpathogenic isolates from pepper tissue and field soil. Limited studies using VCG testers from f. spp. conglutinans, matthioli, melongenae, and raphani showed incompatibility with FOC testers.

A282

CHARACTERIZATION AND VIRULENCE OF RHIZOCTONIA ISOLATES ASSOCIATED WITH BOTTOM ROT OF LETTUCE. L. J. Herr, Dept of Plant Pathology, Ohio State Univ., Wooster, OH 44691.

Rhizoctonia isolates from diseased field lettuce collected at Celeryville and Hartville, OH were grouped by anastomosis (AG) and, when feasible, intraspecific groups (ISG). Thirty-three of 51 Celeryville isolates and 28 of 59 Hartville isolates were AG-1-1B. Ten AG-1-1C isolates were obtained from Celeryville and four from Hartville. AG-2-1 isolates were found at Hartville only, as was an AG-5 isolate, whereas AG-2-2 isolates were found only at Celeryville. Other less frequently obtained isolates were AG-4, binucleate Rhizoctonia spp. and Laetisaria arvalis at both sites. AG-1-1B isolates were the most virulent AG in greenhouse tests, ranging from avirulent to highly virulent on a disease rating scale (DR) of 1=healthy, 2=diseased, 3=dead plants, with the av. DR of isolates=2.6. AG-1-1C and AG-2-1 isolates were of lesser virulence, AG-1-1C (av DR=2.1), >AG-2-1 (av DR=1.4).

A283

DEVELOPMENT OF A SEMI-SELECTIVE MEDIUM FOR THE RECOVERY OF ALTERNARIA RADICINA. B. Pryor, R. M. Davis, and R. L. Gilbertson, Department of Plant Pathology, University of California, Davis, CA 95616.

A semi-selective medium was developed to aid in the isolation and identification of Alternaria radicina, the causal agent of black rot disease of carrots, from soil and carrot seed. The medium utilizes sodium polypectate as a substrate and incorporates benomyl, dichloran, and several antibiotics to suppress the growth of other organisms. A. radicina was only recovered from carrot fields with a history of the disease. Population densities ranged from 30-200 CFU/g soil. The fungus was recovered from soil collected from fallow fields at least 2 yrs out of carrots. With the incorporation of 2,4-D, the medium was successfully used to index seed lots contaminated with A. radicina. Fifteen seed lots were screened; levels of infestation ranged from 0-6.0%.

A284

SOIL SOLARIZATION FOR CONTROL OF PYTHIUM SPP. AND HOME GARDEN PESTS IN WEST TENNESSEE. C. H. Canaday, J. E. Wyatt, and R. M. Hayes, University of Tennessee, West Tennessee Experiment Station, Jackson, TN 38301, and J. Logan, University of Tennessee, Knoxville Experiment Station, Knoxville, TN 37901.

Methods for solarizing soil were evaluated under west Tennessee growing conditions in replicated field plots in 1989-91. Highest soil temperatures were achieved with two layers of clear polyethylene plastic, separated by a 2.5 cm air space (CsC). This combination was more effective for solarizing soil than one layer of black plastic, one layer of clear plastic, two layers of clear plastic without an air space, clear over black plastic without an air space, or clear over black plastic separated by a 2.5 cm air space. Doubling the height of the air space reduced solarization efficiency. Significant decreases in soil populations of Pythium spp. and weed infestations and significant increases in plant stand, growth, and yield were achieved using the CsC combination to solarize vegetable garden plots.

A285

PERICARP AND ALEURONE TISSUE THICKNESS ASSOCIATED WITH THE RELATIVE RESISTANCE OF 15 CORN CULTIVARS TO FUSARIUM EAR ROT. R.W. Hoenisch, R.M. Davis, Dept. of Plant Pathology, University of California, Davis 95616

Sections of kernels from 15 corn hybrids were examined microscopically to determine the relationship between thickness of pericarp and aleurone layers and relative resistance to ear rot caused by *Fusarium moniliforme*. In 1989 the thickness of the layers during the dough and early dent stages were not significantly different between susceptible, intermediate, and resistant cultivars. In 1990, the thickness of the pericarp in resistant and intermediate hybrids was significantly greater than that in susceptible hybrids ($P \leq 0.01$). In contrast the thickness of the aleurone layer in susceptible hybrids was significantly greater than that in resistant and intermediate hybrids ($P \leq 0.01$). These results suggest that the relative resistance of corn hybrids to Fusarium ear rot may be positively correlated with the thickness of the pericarp layer and negatively correlated with the thickness of the aleurone layer.

A286

NEWBONNET SOMACLONES WITH VARIATION FOR INCREASED PARTIAL RESISTANCE TO RICE BLAST. J. Narvaez, M.C. Rush, and D.E. Groth. Louisiana State University Agricultural Center, LA Agricultural Experiment Station, Baton Rouge.

Newbonnet R2 somaclonal lines and the Newbonnet cultivar were evaluated in field nurseries for leaf blast and rotten-neck blast in 1990 at the Louisiana Rice Research Station at Crowley, LA. The somaclones were also evaluated for panicle blast and yield during the 1991 season at the same location. The same lines and an additional set of 266 R2 Newbonnet somaclones were evaluated for leaf blast in nursery beds during 1991. The majority of the somaclonal lines were more resistant than Newbonnet to leaf, rotten-neck, and panicle blast. Most of the somaclones headed earlier and were taller than Newbonnet. Rotten-neck blast had more impact on yield reduction than leaf and panicle blast. Forty seven of the somaclonal lines yielded more than 30 percent higher than Newbonnet.

A287

RELATIVE RESISTANCE OF TWELVE STRAWBERRY CULTIVARS TO *PHYTOPHTHORA CACTORUM* AND *P. CITRICOLA*. S.M. Mircetich*, C.Q. Winterbottom, G.T. Browne*, R.W. Hoenisch, R.J. Wakeman*, and W.D. Gubler. *USDA-ARS, Department of Plant Pathology, University of California Davis 95616

Strawberry cultivars (Capitola, Chandler, Commander, Douglas, Fern, Irvine, Muir, Pajaro, Parker, Sheehy, Tioga, and Yolo) were evaluated in greenhouse studies for their relative resistance to *Phytophthora cactorum* (*Pcac*) and *P. citricola* (*Pcit*), which cause decline and death in commercial strawberry plants in California. In spring/summer evaluations with soil infested with *Pcit*, Fern, Irvine, Muir, Pajaro, Sheehy, and Yolo were highly susceptible (mean root rot (MRR) 52-85%); Parker, Tioga, Capitola, and Commander were highly resistant (MRR 1-8%); and Chandler and Douglas were intermediate (MRR 21-30%). During the same period, with soil infested with *Pcac*, most cultivars were relatively resistant; only Irvine, Sheehy, and Yolo developed significant levels of disease (MRR 28-31%). In soil infested with *Pcit* or *Pcac*, the 12 cultivars generally developed less disease in fall/winter tests than in spring/summer tests. Our research revealed marked differences in resistance to *Pcit* and *Pcac* among the twelve commercial strawberry cultivars and suggests seasonal variations in development of the disease.

A288

TOXINS AS A TOOL TO SCREEN FOR RESISTANCE TO ASCOCHYTA BLIGHT OF CHICKPEA. H. Morjan, M. Harrabi, M.Halila and R.N. Strange. INAT 43 Ave. Charles Nicolle 1082 Tunis, TUNISIA.

Cultural filtrates of Ascochyta rabiei, the causal agent of chickpea blight containing the solanopyrones A,B and C were used to develop two bioassays to screen chickpea cultivars for tolerance. When cells, isolated from chickpea leaves, were exposed to culture filtrates, those from the cultivars ILC482 and ILC3279 were less sensitive than those from the very susceptible cultivar, local Amdoun. This result was corroborated by an electrolyte leakage assay. Greater leakage occured from leaflets of cultivar local Amdoun after exposure to culture filtrates for 3 hr than from leaflets of cultivars ILC482 and ILC3279. These results are compatible with the hypothesis that receptors on the plasma membrane are responsible for susceptibility and that these may be more plentiful in susceptible cultivars. Both assays appear to have potential as tools for rapid screening of numerous lines of chickpea such as F_2 populations.

A289

ROLE OF THE CUTICLE AND PHENOLIC CONTENT IN RESISTANT AND SUSCEPTIBLE PEACH GENOTYPES TO BROWN ROT CAUSED BY *MONILINIA FRUCTICOLA*. J.E. Adaskaveg, J.M. Ogawa, A.J. Feliciano, and M. Dunlap. University of California, Davis, CA 95616

In previous studies, a thicker cuticle (greater amounts of cutin and cuticular waxes) found in resistant (R) but not in susceptible (S) peach genotypes was implicated in host resistance to *M. fructicola*. To elucidate the role of the cuticle, pectinase of *Aspergillus niger* (Sigma Chemical Co.) or cutinase of *Venturia inaequalis* (Dr. W. Köller, Cornell University) were used in pre-inoculation or during inoculation treatments, respectively. Fruit of R- (Bolinha) and S- (Corona) genotypes were harvested, treated and washed (pectinase treatments only), non-wound inoculated with a 20 μl conidial suspension (25,000/ml), exposed to a wetness period (0, 4, 8, or 12 hr), and incubated for 5 days at 20C, 90% RH. Regressions of lesion diameter on wetness periods showed that pectinase or cutinase treatments significantly increased susceptibility of fruit when compared to buffer (no enzyme) treatments. Using wavelength dispersive X-ray analyses of histological sections stained with Mn or Br, or UV-spectrophotometry of acidified methanol extracts, R-genotype showed a concentrated cuticular and epidermal distribution and an overall greater amount of phenolic materials than S-genotype. These comparisons between R- and S-genotypes suggest that the cuticle and epidermal cells function as a barrier delaying pathogen penetration of resistant fruit.

A290

QUANTIFICATION OF APHANOMYCES RESISTANCE IN PEAS. J. M. Kraft and W. L. Boge, USDA-Agricultural Research Service, WSU-IAREC, Route 2 Box 2953A, Prosser, WA 99350-9687.

Pea (Pisum sativum L.) germplasm, resistant to Aphanomyces euteiches f. sp. pisi, has been identified. Resistant and susceptible pea lines, were compared by measurement of: 1) oospore production in roots of zoospore-inoculated plants, and 2) pathogen development in inoculated pea roots using a polyclonal antiserum developed against A. euteiches. Oospore production was greatest in the root tip of the susceptible (DS Perf) as compared to the resistant (PI 189693, 86-2236) lines. The antiserum was determined to be specific to mycelium and oospores of Aphanomyces spp. ELISA readings (A_{405} nm) were significantly less for the resistant lines at a low (100) and high (1000 zoospores/ml) inoculum level after 5-9 days of incubation, respectively. The data indicate that resistant pea roots inhibit oospore production and fungal growth of A. euteiches within inoculated root tissues.

A291

Influence of *Pratylenchus penetrans* and *Verticillium dahliae* on Verticillium-resistant potato cultivars. T. A. Wheeler, R. C. Rowe, R. M. Riedel, and L. V. Madden. O.A.R.D.C., The Ohio State University, Dept. of Plant Pathology, Wooster, OH 44691.

Microplots were infested with *Verticillium dahliae* (VD) and/or *Pratylenchus penetrans* (PP) during 1988, 1989, and 1991. Two potato cultivars susceptible to *Verticillium* (Superior and Kennebec) and two resistant cultivars (Reddale and Russette) were tested. In 1988 and 1989, VD alone lowered yields of the susceptible, but not the resistant cultivars. PP alone did not affect yield in any of the cultivars in all 3 years. An interaction ($P < 0.10$) between VD and PP for yield losses occurred in 1989 with Kennebec, Reddale and Russette. When all 3 years data were combined for each cultivar, a yield-loss model for Superior and Kennebec included the terms VD and VD x PP, while for Russette and Reddale, only VD x PP was significant. The decrease in yield caused by the interaction of PP and VD was greater for the susceptible cultivars than for the resistant ones, though $\leq$ 5% of the yield was explained by the interaction term for any cultivar.

A292

EARLY DYING-RESISTANT MUTANTS THAT OUT YIELD STANDARD POTATO CV. RUSSET BURBANK. G. D. Easton. Washington State University, Rt. 2, Box 2953-A, Prosser, WA 99350-9687.

Over 1000 "giant hill" mutants were hand harvested from commercial fields of potato cv. Russet Burbank and field tested the following two years for resistance to early dying (primarily caused by Verticillium dahliae), root knot nematode (Meloidogyne chitwoodi), leaf roll virus, and Virus Y. In 1991, 18 of the better selections from the 1989 disease trials were further evaluated for yield as well as disease response. Seven of these 18 mutants (RBM) had significantly less Verticillium wilt (7 to 40% wilt) than standard Russet Burbank (SRB), which had 92% wilt. Four of the mutants produced yields ranging from 1320 to 1490 q/ha, significantly more than the 950 q/ha yield of SRB. These same four yielded 800 to 910 q/ha of U.S. No. 1 tubers compared to 650 q/ha by SRB. None of the RBM selections showed important improvements in resistance to damage by root knot nematode, leaf roll or Virus Y.

A293

RESISTANCE TO POTATO STORAGE ROTS CAUSED BY ERWINIA AND FUSARIUM SPECIES. D.L. Corsini and J.J. Pavek. USDA-ARS, University of Idaho, Aberdeen, Idaho 83210.

The degree of suberization as measured by relative conductance of cut tuber surfaces was highly correlated with potato tuber soft rot resistance but not with dry rot. Ten cultivars and breeding selections were tested over a three year period. Ability to suberize was estimated with a LICOR porometer after several months storage. The reaction to Erwinia soft rot (E. atroseptica) and Fusarium dry rot (F. roseum var sambucinum and F. solani var coeruleum) was measured in both bruise and stab inoculated tubers. Resistance was expressed as both the number of inoculated tubers that developed active rot, and the relative severity of rot. Several clones highly resistant to soft rot were highly susceptible to one or both Fusarium species. Two clones, Frontier Russet and ATD63-7 (a Solanum microdontum hybrid), appeared to have a degree of resistance to all rot species.

A294

GENETIC MULTIPLE VIRUS RESISTANT PAPRIKA PEPPERS, Benigno Villalon, Texas Agricultural Experiment Station, 2415 E. Hwy 83, Weslaco, TX 78596

Paprika pepper (high extractable red), one of about 20 different cultivated Capsicum annuum L. types, has for many years been associated with the dehydrated red chile and processing industry. Increased market demand for high red color chile powder, high vitamin C, and low caloric pepper product has stimulated production in Texas and other areas throughout the world. All known commercial paprika type peppers are susceptible to virus diseases, a limiting factor in most pepper production areas throughout the world. The Texas Agricultural Experiment Station at Weslaco has developed several hundred high red color inbred lines with resistance to tobacco etch virus, pepper mottle virus, potato virus Y, tobacco mosaic virus, tobacco ringspot virus and cucumber mosaic virus. Improved lines of pungent and non-pungent long fruited, thin walled paprika peppers are in the process of being released.

A295

FIELD INOCULATION STUDIES TO QUANTIFY RESISTANCE IN SPINACH TO RACES 3 AND 4 OF THE DOWNY MILDEW PATHOGEN. L. P. Brandenberger, J. C. Correll, T. E. Morelock[1], and R. W. McNew[2]. Dept. of Plant Pathology, [1]Dept. of Horticulture and Forestry, and [2]Dept. of Agricultural Statistics, University of Arkansas, Fayetteville, AR 72701.

Resistance to races 3 and 4 of the spinach downy mildew pathogen (Peronospora farinosa f. sp. spinaciae) was examined in separate field inoculation experiments. Resistance was quantified in three Arkansas spinach cultivars (Fall Green, Ozarka II, and 88-354) and three or four other commercial spinach culitvars (St. Helens, Grandstand, Hybrid 424, and/or RS1242) by periodically scoring individual leaves for disease severity 7 to 28 days after inoculation. Leaves were scored on a 0 to 6 scale with 0 = 0% and 6 = 90-100% of the leaf surface exhibiting evidence of sporulation. Resistance was evaluated statistically by using the mean disease severities for a given sample date and the areas under disease progress curves for each cultivar. The three Arkansas cultivars exhibited significantly lower disease severity ratings in field inoculation tests for all sample dates for both races 3 and 4 when compared to the known susceptible cultivars. Similar results were obtained when areas under the disease progress curve were compared. The data indicate that the Arkansas cultivars tested have race non-specific resistance to races 3 and 4.

A296

REACTION OF 1RS.1BL AND 1B NEAR-ISOLINES OF WHEAT TO LEAF RUST (LR), WHEAT SOILBORNE MOSAIC VIRUS (WSBMV), TAN SPOT (TS), AND WHEAT STREAK MOSAIC VIRUS (WSMV). R.M. Hunger[1], B.F. Carver[2], J.L. Sherwood[1], C.K. Evans[1], and J.R. Montana[1]. Departments of Plant Pathology[1] and Agronomy[2], Oklahoma State University, Stillwater, OK, 74078.

The reactions to LR, WSBMV, TS and WSMV were determined for 27 pairs of hard red winter wheat genetic stocks developed from crosses between the breeder line OK83398 (TAM W-101*2/Aurora) and Chisholm or Arkan. Members of each pair are genetically similar except for the presence or absence of the 1RS.1BL translocation, which contains the gene for LR resistance designated *Lr26*. Results from inoculation of the genetic stocks with the appropriate races of Puccinia recondita f. sp. tritici was consistent with the cytological determination for the presence of 1RS.1BL. Resistance to WSBMV was found in pairs obtained from crosses involving Chisholm, but was not related to the translocation. Resistance to TS appears to have been derived from the parent line OK83398. No resistance to WSMV was found.

A297

PHYLOGENETIC RELATIONSHIPS AMONG PLANT PATHOGENIC MYCOPLASMALIKE ORGANISMS (MLOs) BASED ON 16S rRNA SEQUENCE ANALYSIS. I.-M. Lee, R.W. Hammond, R.E. Davis, and D.E. Gundersen. USDA/ARS, MPPL, BARC, Beltsville, MD 20705.

Partial (about 86%) 16S rRNA sequences of various MLO strains were amplified by polymerase chain reaction (PCR) using a primer pair chosen from an MLO 16S rRNA gene (Lim, P.-O. and Sears, B.B. 1989. J. Bacteriol. 171:5901-5906). This primer pair broadly detected essentially every MLO in infected periwinkle and some other plants. No PCR products were generated in samples containing DNA extracts from healthy plants. The partial 16S rRNA sequences generated from various MLOs were compared through restriction fragment length polymorphism (RFLP) analyses. Phylogenetic relationships based on coefficients of similarity in these RFLP patterns delineated several distinct Strain Clusters which coincided with MLO Genomic Clusters previously delineated on the basis of dot hybridization analyses using cloned chromosomal DNA probes.

A298

PROTEASE PRODUCTION BY GRAPE STRAINS OF *XYLELLA FASTIDIOSA*. S. M. Fry, J.-S. Huang, and R. D. Milholland, Department of Plant Pathology, North Carolina State University, Raleigh, NC, 27695-7616.

Grape strains of *X. fastidiosa* grew on milk agar and nutrient agar plus 1% skim milk but did not produce zones of hydrolysis. Strains grew and produced zones of hydrolysis on nutrient agar and PD3 each amended with 0.4% gelatin. Less virulent strains produced slightly less protease on PD3 amended with 1% gelatin compared with virulent strains. A less virulent strain (79) and a virulent strain (P) were grown in PD3 broth. Growth curves for these strains were similar, however, strain 79 exhibited about half as much protease activity as strain P over time. Protease samples from strains P and 79 yielded two bands of activity on native-PAGE activity gels and three bands of activity on SDS-PAGE activity gels. The protease was most abundant in the 51-70% ammonium sulfate fraction. The protease did not degrade azocoll, but did degrade azocasein and gelatin.

A299 Withdrawn

A300

DODONAEA YELLOWS DISEASE IN HAWAII IS ASSOCIATED WTH VIRUSLIKE PARTICLES AND MYCOPLASMALIKE ORGANISMS. W. B. Borth, J. S. Hu, Dept. of Plant Pathology, D. E. Gardner, Cooperative Parks Study Unit, Dept. of Botany, Univ. of Hawaii, Honolulu, HI 96822.

The yellows disease of Dodonaea viscosa in the Hawaiian islands is characterized by the production of pendulous, chlorotic witches' brooms on afflicted plants. The disorder is not seed transmitted. Flexuous, rod-shaped viruslike particles of 16 nm diameter and 700 nm length and dsRNA of 3 x 10^6 daltons have been detected in symptomatic but not symptomless plants. Oligomeric probes capable of detecting highly conserved nucleic acid sequences from the 16S ribosomal gene of a wide variety of MLOs were used to screen symptomatic plants and symptomless plants. 80% of symptomatic and 30% of symptomless plants sampled from sites on all the major Hawaiian islands reacted positively with this probe. Pleiomorphic bodies bound by a single unit membrane were also observed in necrotic phloem elements in roots of symptomatic plants using TEM. Partial alleviation of symptoms on diseased plants was achieved following application of oxytetracycline by direct injection. The results suggest a complex etiology for this disease which involves both viruslike particles and MLOs.

A302

16S rRNA PRIMER PAIRS DESIGNED FOR GENERAL DETECTION OF AND STRAIN CLUSTER IDENTIFICATION AMONG PLANT PATHOGENIC MYCOPLASMALIKE ORGANISMS (MLOs) BY PCR. I.-M. Lee, B.D. Mogen, and R.E. Davis. USDA/ARS, MPPL, BARC, Beltsville, MD 20705.

Several primer pairs for PCR were chosen from an MLO 16S rRNA gene (Lim, P.-O. and Sears, B.B. 1989. J. Bacteriol. 171:5901-5906). These primer pairs were evaluated for detection of MLOs in infected plants. Preliminary results indicated that some primer pairs can be used as general probes for the detection of a broad range of MLOs in periwinkle or other plant hosts, while other pairs can be used as specific probes for identification of MLO strains in the Aster Yellow Strain Cluster.

A303

DISTRIBUTION OF PLANT PARASITIC NEMATODES ON COTTON IN ALABAMA. W. S. Gazaway[1], C. C. Mitchell[2], K. Edmisten[2], and C. Burmester[2], [1]Department of Plant Pathology, and [2]Department of Agronomy and Soils, Auburn University, AL 36849.

A nematode survey was conducted from 1990-1991 in seventeen counties, representing almost 80% of the cotton acreage in Alabama. Root-knot nematode (*Meloidogyne* spp.) was found in 26% of the cotton fields surveyed. Reniform nematode (*Rotylenchulus reniformis*), first reported in one east Alabama location in 1959, now infests 15.5% of the state's cotton fields, indicating that this nematode species is becoming more widely distributed. The highest incidence of reniform nematode was found in central and southern sections of the state. Seventy percent of the fields surveyed in Tuscaloosa county (central Alabama) contained reniform populations (>900/100 cc soil) sufficient to reduce cotton yield. Only 3.8% of the cotton fields survey contained lance nematode (*Hoplolaimus* spp.). Other nematode species, *Pratylenchus* spp. (31%) and *Paratrichodorus* spp. (29%), were found occurring at a higher incidence, but are less damaging to cotton.

A304

POPULATION DEVELOPMENT BY ROOT KNOT NEMATODE ON SOYBEAN DAMAGED BY INSECT-INDUCED DEFOLIATION. J.S. Russin, E.C. McGawley, Dept. Plant Pathology and Crop Physiology, and D.J. Boethel, Dept. of Entomology, Louisiana State University Agricultural Center, Baton Rouge, LA 70803.

Population development by root knot nematode (*Meloidogyne incognita* race 4) on soybean (*Glycine max* cv 'Davis') defoliated by larvae of soybean looper (*Pseudoplusia includens*) was examined in greenhouse studies. Plants were defoliated by larvae for two weeks beginning five weeks after seedlings were transplanted. Four groups of plants were infested with nematodes (5000 eggs/pot) at different times relative to time of insect defoliation. Plants in each group were harvested 28 days after infestation. Nematode infestations occurred at 2-week intervals to allow harvesting of plants at 0, 2, 4, and 6 weeks post-defoliation (WPD). Plants defoliated 45% relative to controls had reduced root and nodule weights at 0 WPD but recovered quickly so that these differences were not detectable at 2 WPD and later. Populations of root knot nematodes were similar on defoliated and control plants at 0 WPD but were greater on defoliated plants at both 4 and 6 WPD. Hatching percentage for eggs produced on these plants also was increased. That these increases were most evident when leaf areas and plant weights had recovered to levels of controls suggests that effects of insect-induced defoliation still were evident in soybean long after plant growth apparently had returned to normal.

A305

TOBACCO CYST NEMATODES CAN STIMULATE YIELD OF FLUE-CURED TOBACCO. C. S. Johnson, Virginia Polytechnic Institute & State University, Southern Piedmont Agricultural Experiment Station, P.O. Box 448, Blackstone, VA 23824.

Plant growth and population densities of tobacco cyst nematode (Globodera tabacum solanacearum, or TCN) were monitored in 42 single row, 40 ft (24 plant) plots throughout the 1991 growing season. Plant height, leaf number, and leaf area data were obtained for flue-cured tobacco cultivar 'Coker 319' at 5 wk intervals from transplanting to topping. Yield, economic value, grade index, average price, % total alkaloids, and % reducing sugars data were also obtained from each plot. Similar agronomic data was obtained and correlated with final TCN population densities in 1990. Initial TCN population densities in 1991 ranged from 3,967 to 59,554 eggs/500 cm^3 of soil. Plant height, leaf number, and leaf area 6 wk after transplanting were negatively correlated with TCN eggs/500 cm^3 of soil in 1991. However, regression models indicated increases in final yield of 5.95 and 7.50 kg/ha per 1,000 TCN/500 cm^3 of soil at the end of the 1990 and beginning of the 1991 growing seasons, respectively. Yield stimulation, rather than suppression, may have been related to weather patterns, particularly rainfall, during 1990 and 1991.

A306

A Brushing Technique for Extracting Nematodes from Citrus Feeder Roots. Mani Skaria, and Nora Solis-Gracia. Texas A&I University Citrus Center, Weslaco, TX 78596.

A simple, rapid technique for extracting citrus nematode, *Tylenchulus semipenetrans*, from citrus feeder roots was developed using a soft toothbrush. Feeder roots from nematode infested trees were washed in water, bleached and then stained with acid fuchsin. The roots, held in place with a forceps were brushed with a soft toothbrush to remove the nematodes. We found that five strokes followed by another five, after the roots were turned over, was enough to extract most nematodes. The number of eggs, larvae and adults were counted easily by this technique, which leaves the nematode intact for counting purpose. This procedure was compared with the maceration-filtration technique using a Waring blender. We found the brushing technique to be useful and fast for our work involving routine assays, and comparison of nematicide efficacy. This technique also be useful for example, in rootstock evaluations for nematode infestation.

A307

DNA AMPLIFICATION FINGERPRINTING (DAF) OF ISOLATES OF FOUR COMMON MELOIDOGYNE SPECIES AND THEIR HOST RACES. T. J. Baum[1], P. M. Gresshoff[2], S. A. Lewis[1], and R. A. Dean[1]. [1]Department of Plant Pathology and Physiology, Clemson University, Clemson, SC 29634-0377 and [2]Plant Molecular Genetics, University of Tennessee, Knoxville, TN 37901-1071.

There is a need for simple, fast, and reliable molecular techniques for identifying Meloidogyne species and races. DNA from isolates of the root-knot nematodes Meloidogyne incognita, M. arenaria, M. javanica, and M. hapla was extracted and purified by CsCl gradient centrifugation. Amplification of DNA was accomplished using arbitrary oligonucleotide primers and the polymerase chain reaction. Amplified DNA fragments were resolved by polyacrylamide gel electrophoresis and visualized by silver staining. Diagnostic DNA banding patterns were obtained for the four major species and several races.

A308

CONTROL OF *MELOIDOGYNE ARENARIA* ON SOYBEAN IN GREENHOUSE TESTS USING POWDERED PINE BARK. N. Kokalis-Burelle, R. Rodríguez-Kábana, C. F. Weaver, and P. S. King. Department of Plant Pathology, Auburn University, Auburn, AL 36849.

Commercially available pine bark mulch was ground into a powder (250 micrometer) and added at rates of 0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, and 5.0% (w/w) to field soil infested with *Meloidogyne arenaria*. Pots with the soil were placed in a greenhouse for 2 weeks, sampled for nematode analysis, and then planted with 'Davis' soybean (*Glycine max*). Preplant samples showed no differences among treatments in the number of *M. arenaria* juveniles in the soil. Six weeks after planting, numbers of *M. arenaria* juveniles in soil decreased linearly with increasing amounts of pine bark. No juveniles were present in soil with 5% pine bark. The number of galls per gram root tissue decreased in proportion to the amount of pine bark added to soil. Gall formation by *M. arenaria* was completely eliminated at the 5% rate. Numbers of saprophagous nematodes were highest in soils with 4-5% pine bark. Pine bark amendments did not affect plant growth.

A309

THE EFFECT OF THE SOUTHERN ROOT-KNOT NEMATODE (*MELOIDOGYNE INCOGNITA*) ON KENAF (*HIBISCUS CANNABINUS*) YIELD. J. A. Veech[1] and C. G. Cook[2], USDA, ARS, [1]Southern Crops Research Laboratory, Rt. 5, Box 805, College Station, TX 77845 and [2]Conservation and Production Research Unit, P.O. Box 267, Weslaco, TX 78596.

In a field heavily infested with the southern root-knot nematode (Meloidogyne incognita), kenaf (Hibiscus cannabinus) yield was severely reduced compared to control plots where the nematode was eliminated from the soil by preplant fumigation with methyl bromide at 1 to 3 lbs per 100 ft^2 and tarping the fumigated rows with 2 mil clear plastic film for 10 days. The rows were planted 1 wk after removing the tarp. After 214 days (4/19/91-11/19/91) in South Texas, plants of Kenaf cv. Everglades 71 were harvested by collecting all of the shoot except leaves. The harvested stems were air dried in the field and then weighed. Yields on fumigated and nonfumigated rows were 11,013 and 4,247 lbs/acre, respectively. The results show that the Southern root-knot nematode can cause severe reduction of kenaf yield, and methyl bromide fumigation can negate that loss.

A310

EVALUATION OF NINE ALFALFA CULTIVARS FOR RESISTANCE TO THE CHRYSANTHEMUM FOLIAR NEMATODE. J. L. Williams[1], F. A. Gray[2], G. D. Griffin[3], and T. E. Wilson[4]. [1]Plant Path. Dept., Univ. of Minn., St. Paul. [2]Plant, Soil, and Insect Sci. Dept., Univ. of Wyo., Laramie. [3]USDA/ARS, Forest and Range Lab., Utah St. Univ., Logan. [4]Farm Seed Res. Corp., Hollister, CA.

Previous reports indicate that the chrysanthemum foliar nematode (CFN), *Aphelenchoides ritzema-bosi*, occurs in combination with the alfalfa stem nematode (ASN), *Ditylenchus dipsaci*, in stem bud tissue of alfalfa plants. Seedlings, inoculated at planting, and 6-week-old plants of nine alfalfa cultivars with varying degrees of ASN-resistance were evaluated for reaction to a mixed population of both nematodes. Percent CFN of total nematodes recovered, decreased in seedling tissue, but increased in older plant tissue; whereas, percent ASN decreased or was constant in either tissue. Older plants had 19% and 34% CFN in ASN-resistant and susceptible cultivars, respectively. This indicates that field selection for resistance to ASN may have increased resistance to both ASN and CFN in alfalfa.

A311

IMPORTANCE OF SOIL TEXTURE ON THE PATHOGENICITY OF PLANT PARASITIC NEMATODES TO RANGE GRASSES. Griffin, G. D., and K. H. Asay. USDA ARS, Forage and Range Research, Utah State University, Logan, UT 84322-6300.

Soil texture affected the host-parasite relationship of four plant parasitic nematode species on crested wheatgrass. The root lesion nematode, Pratylenchus neglectus was affected less by soil texture than were three ectoparasitic nematode species, Merlinius brevidens, Quinisulcius acutus, and Xiphinema americanum. All nematode species were more pathogenic in Kidman fine sandy loam than in Logan fine silty soil. Pratylenchus neglectus was least affected by soil texture, and was the most virulent while X. americanum was the least virulent and was most affected by soil texture. Pratylenchus neglectus had the greatest nematode reproduction while X. americanum population failed to increase in any soil texture on any cultivar. Hycrest, a hybrid crested wheatgrass, was more tolerant to the four nematode species than were its parental cultivars, Fairway and Nordan.

A312

CONTROL OF ROOT-KNOT DISEASE COMPLEXES ON FLUE-CURED TOBACCO WITH SOIL FUMIGATION AND PLASTIC MULCH. B. A. Fortnum and M. P. Pullen, Clemson University, Rt. 1, Box 531, Florence SC 29501-9603.

Infection of tobacco roots by Meloidogyne spp. increases the severity of granville wilt and blackshank diseases. The use of plastic mulch to increase the efficacy of multipurpose fumigants was evaluated in fields containing M. arenaria + Pseudonmonas solanacearum or M. incognita + Phytophthora parasitica. High density, photodegradable, and standard polyethylene mulches were evaluated in combination with Telone C17 (1,3-D + chloropicrin, 98 L/ha) or Terro-O-Gas 67 (Mbr + chloropicrin, 255 kg/ha) in replicated field trials during 1990 and 1991. Use of plastic mulch in combination with multipurpose fumigant enhanced root-knot nematode control over fumigant applications without plastic mulch. Use of plastic mulch and multipurpose fumigants, in fields containing M. arenaria + Pseudomonas solanacaearum, enhanced yields over fumigated plots without plastic mulch.

A313

FOREST FUNGI USED BY THE INDIGENOUS PEOPLES OF WESTERN NORTH AMERICA. R. A. Blanchette, Department of Plant Pathology, University of Minnesota, St. Paul, MN 55108.

Basidiocarps associated with native American cultural properties and raw materials used for paint, dyes, tinder, etc. were identified from several museum collections in the United States and Canada. The most commonly identified fungus, *Fomitopsis officinalis*, was used by many different tribes over large geographical areas. It was used by interior groups, such as the Blackfeet and Cree, as a medicine, in sacred religious bundles, in shaman bundles, on necklaces with human scalps and as fungus beads to ward off illness. Coastal groups (e.g. Tlingit) carved sporophores of *F. officinalis* and used them in society rituals as objects of supernatural power and to guard the shaman graves after death. *Echinodontium tinctorium* was used as a paint and face protector, and when charred in a fire and mixed with tallow it was used to prevent snow blindness and protect people against insects and the elements. *Phaeolus schweinitzii* was utilized as tinder, and *Phellinus igniarius* used by the Inuit in a mixture with chewing tobacco.

A314

TIMBER MANAGEMENT PRACTICES AFFECT FLOWERING DOGWOOD SIZE DISTRIBUTION AND ANTHRACNOSE SEVERITY. K. O. Britton, USDA Forest Service, Green Street, Athens, GA 30602.

Timber management plots at Coweeta Hydrologic Laboratory, near Otto, NC, harvested by clearcutting (in 1939 and 1962) or by thinning (30-35% basal area removed in 1955-65) and undisturbed control plots were surveyed in 1991. Dogwood anthracnose severity was estimated as percent leaf area infected (LAI) and percent branch dieback (DB) in 15 0.08-ha plots of each harvest type. Thinned plots had the most even tree size distribution, with 30% of dogwoods < 2.5" in basal diam., 30% 2.5-3.5" and 40% > 3.5" in basal diameter. Despite a higher proportion of large trees, thinned plots were the most severely diseased, with 31% mean LAI and 62% DB. Dogwoods in undisturbed plots (no timber harvested since 1927) were bimodally distributed into two size classes, with 66% < 2.5" basal diam. and 34% > 3.5", and 22% mean LAI and 38% DB. Plots clearcut in 1939 and 1962 had 51% of dogwoods < 2.5" basal diam., 29% 2.5-3.5", and 20% > 3.5", the lowest mean LAI of 15%, and DB of 44%.

A315

INFLUENCE OF PERIODIC WATER STRESS ON PITCH CANKER DISEASE IN RESISTANT AND SUSCEPTIBLE SLASH PINE FAMILIES. G.M. Blakeslee, W.E.Lante, and J.E.Allen. School of Forest Resources and Conservation, Univ. of Florida, Gainesville, FL 32611

Pitch canker (PC) induced shoot dieback (SD) following deliberate inoculation with Fusarium subglutinans was followed on ca 450 one-year-old slash pine seedlings representing five families each of known PC resistant (R), intermediate (I), and susceptible (S) genotypes. Following transplanting, all trees were grown under well-watered conditions in large sand beds for 6 mo after which periodic water stress (PWS) (max -1.34 MPa) was induced in half of the trees by reduced irrigation. Routine irrigation of the remaining trees constituted the non-stressed (NS) (max -.56 MPa) treatment. PWS increased SD (% trees) in all genotypes yet the relative resistance rankings were retained (R< I< S). Significant increases in SD were observed in the PWS treatment for both the S (SD 93 vs 67%) and R (SD 48 vs 21%) families; effects on I families were less defined. These results support the hypothesis that pitch canker development in slash pine is responsive to water stress in the host.

A316

ANATOMY OF DOGWOOD FLOWERS, LEAVES AND TWIGS AFTER INFECTION BY DISCULA DESTRUCTIVA REDLIN. C. H. Walkinshaw. USDA, Forest Service, Southern Forest Experiment Station, 2500 Shreveport Highway, Pineville, LA 71360.

This study is part of a continuous effort to understand the mortality of dogwood caused by Discula destructiva Redlin--a disease that kills invaded cells and tissues. Specimens taken from infected flowers, leaves and twigs have few fungal hyphae. More hyphae were seen near veins in certain leaves and in parts of flowers that surround ovules. Sporulation was observed in all tissues and was most abundant in leaves with discrete lesions between veins. Movement of the fungus from leaf veins to twigs caused severe distortion of cortical and vascular tissues. The pith was usually destroyed. Judging from variation in leaf symptoms and their histology, at least two types of D. destructiva exist along the Blue Ridge Parkway near Asheville, NC. Veinal necrosis with interveinal spots seems to be caused by virulent form of the pathogen.

A317

POSSIBLE EVOLUTIONARY RELATIONSHIPS IN THE HETEROBASIDION ANNOSUM COMPLEX. W.J. Otrosina, T.E. Chase, F.W. Cobb, Jr., and K. Korhonen. USDA Forest Service, PSW Research Station, Albany, CA 94701; South Dakota State Univ., Brookings; Univ. of Calif., Berkeley; Finnish For. Res. Inst., Vantaa, Finland.

Cluster analyses and canonical discriminant analyses were conducted on allozyme data from 109 European and North American "S", "P", or "F" group isolates of H. annosum. European and N. American groups are genetically diverged from each other to varying degrees. The "F" group appears to have emerged more recently than the other groups. Based upon differing host associations, host specificity, and amount of genetic divergence, the European "S" and "P" intersterility groups may have evolved more recently than the N. American "S" and "P" groups. Host specificity may have evolved on certain species associations present in the Trans-Arctic geoflora during the early to mid-Tertiary, while genetic divergence associated with intersterility barriers may have evolved as a consequence of changing host associations resulting from southward migrations of forests in the latter portion of the Tertiary.

A318

ASSESSMENT OF *CYLINDROCARPON* AND *FUSARIUM* ROOT INFECTION OF DOUGLAS-FIR SEEDLINGS HARVESTED FROM REFORESTATION SITES IN S.W. BRITISH COLUMBIA. P.E. Axelrood[1], W.K. Chapman[2], G.M. Shrimpton[3] and D.B. Trotter[3]. [1]B.C. Research, Vancouver, B.C. V6S 2L2; [2]Ministry Of Forests, Williams Lake, B.C. V2G 1R8; and [3]Ministry Of Forests, Surrey, B.C. V4P 1M5.

Poor performance of Douglas-fir seedlings has been observed on several reforestation sites planted in 1987. Root die back of seedling stock used for planting was reported for some sites. A study was done to determine if root rot fungi, common in conifer container nurseries, were associated with seedlings sampled from four year old affected plantations. The prevalence and/or intensity of *Cylindrocarpon* root infection was significantly greater for planted seedlings compared to naturally regenerating (natural) seedlings on five of the seven reforestation sites. Furthermore, *Cylindrocarpon* infection of planted seedlings was highest in roots sampled closest to the seedling root plug and decreased as the sample was collected further away from the plug. Root infection of natural seedlings did not follow any trend. The prevalence of *Fusarium* root infection was not significantly different for planted and natural seedlings and root infection levels were generally low. Natural seedlings had significantly more lateral roots and the presence of a well developed tap root compared to planted seedlings.

A319

GENETIC CONTROL AND CONSEQUENCES OF VEGETATIVE COMPATIBILITY IN *HETEROBASIDION ANNOSUM*. E. M. Hansen, J. Stenlid, and M. Johansson, Dept. of Botany and Plant Pathology, Oregon State University, Corvallis, OR 97331, and Swedish University of Agricultural Sciences, Uppsala, Sweden.

Vegetative compatibility (VC) was studied *in vitro* by pairing dikaryons of the root rot fungus *Heterobasidion annosum*. Incompatible reactions were characterized by a narrow gap between the interacting colonies that remained free of aerial mycelium. Submerged hyphae from the opposing colonies mingled freely in the gap, however. Both compatible and incompatible reactions were observed. The proportion of compatible reactions between dikaryons differing at only one, sib-related, nucleus averaged 12% in the basidiospore families studied, suggesting that vegetative compatibility (VC) is controlled by genes at three or more loci. Vegetative mycelia of *H. annosum* were composed of mixtures of homokaryotic and heterokaryotic hyphae. In incompatible VC reactions, homokaryotic hyphae met and fused in the gap between the incompatible mycelia. The resultant "gap dikaryons" often represented a recombined genotype, with nuclei from both parents. This novel form of genetic assortment has important implications for the maintenance of pathogen fitness during the colonization of stumps and wounds.

A320

GENETIC VARIATION IN PRE-EPIDEMIC AND POST-EPIDEMIC LIVE OAK POPULATIONS AFFECTED BY OAK WILT. B.K. Bellamy, B.A. McDonald, and D.N. Appel. Department of Plant Pathology and Microbiology, Texas A&M University, College Station, TX 77843

Typically, a low proportion (approx. 4 to 26%) of diseased live oaks (*Quercus fusiformis*) affected by oak wilt in Texas survive indefinitely. Two to three years following infection, the pathogen (*Ceratocystis fagacearum*) cannot be isolated from these surviving trees. Horizontal starch gel electrophoresis of foliar extracts was used to examine the genetic variation in both unaffected, pre-epidemic live oaks (n = 112) at the perimeter of an oak wilt focus and surviving, post-epidemic trees (n = 109). The two populations were found to be essentially identical (Nei's "D" = 0.002 and F_{ST} = 0.004) by interpretation of isozyme phenotypes of four polymorphic loci (PGM-1, PGM-2, PGI-2, and MDH-3) and two monomorphic loci (PGI-1and MDH-1). However, significant differences in allele frequencies between the two populations were found at the PGM-1 and PGI-2 loci. Also, heterozygosity in the post-epidemic surviving trees was lower than in the pre-epidemic population at the PGM-1, PGM-2 and PGI-2 loci. No correlation was established between isozyme phenotype and resistance. Population differences are hypothesized to result from a population bottleneck or selection for resistant clones.

A321

INCIDENCE OF MELAMPSORA RUST IN A SEEDLING PLANTATION OF HYBRID POPLAR. T. Hsiang and G.A Chastagner. Environmental Biology, University of Guelph, Guelph, Ontario N1G 2W1, and Washington State University Research and Extension Center, Puyallup, WA 98371.

Over 3700 hybrid poplar seedlings were surveyed for incidence and severity of Melampsora rust in fall of 1988, 1989 and 1990. The taxonomic lines included: *Populus trichocarpa*, *P. maximowiczii*, and *P. deltoides*. Only families with some *P. trichocarpa* parentage showed susceptibility to *Melampsora occidentalis*. Twelve crosses of *P. maximowiczii* x *P. maximowiczii* showed no rust at all. In three-way crosses between *P. maximowiczii* and hybrids of *P. trichocarpa* x *P. deltoides* (6 crosses), less than 1% of the seedlings had light rust on some leaves. In 21 crosses of *P. trichocarpa* x *P. maximowiczii*, over 80% of the seedlings showed no rust while less than 1% showed heavy rust. For pure *P. trichocarpa* seedlings from 28 crosses, 2% had no rust, 16% had light rust, and over 80% had medium to heavy rust. Female *P. trichocarpa* parents from the dry continental climate east of the Cascade Mountains in Washington State conferred much greater rust susceptibility to their progeny, when grown in a west-side maritime climate, than west-side parents.

A322

DETECTING VERTICILLIUM WILT ON *Fraxinus pennsylvanica*. C. L. Ash and D. W. French. Department of Plant Pathology, University of Minnesota, St. Paul, MN 55108.

Verticillium dahliae was isolated from green ash (*Fraxinus pennsylvanica*) and velvet leaf (*Abutilon theophrasti*) in Minnesota nurseries. Scorch, dieback and premature defoliation of green ash in nurseries, thought to be caused by abiotic conditions, were due to *V. dahliae* (Vd). Symptoms resembled those of wilt diseases; however, vascular discoloration was not observed. A survey of 24 symptomatic ash trees was made in August 1990. Petiole isolations from scorched leaves on alcohol agar amended with potassium thiocyanate resulted in Vd recovery 54% of the time. Recovery from branches was less (42%). Presence of plant parasitic nematodes or Vd in adjacent soils was not associated with the presence of disease. In the greenhouse, Vd isolates from green ash, velvet leaf and maple (*Acer spicatum*) used to inoculate green ash and velvet leaf, were reisolated from diseased plants.

A323

Indication of toxin activity by Mycosphaerella dearnessii in needles of Pinus taeda. F.F. Jewell, Sr., School of Forestry, Louisiana Tech University, Ruston, LA 71272.

Needles exhibiting symptoms of Mycosphaerella dearnessii Barr (=Scirrhia acicola(Dearn.)Siggers) collected from >20 yr Pinus taeda L. were examined by light microscopy. A uniform reaction confined to the mesophyll cells and associated resin ducts was exhibited and delimited by the borders of symptom areas in the needles. The reaction was exhibited as a collapse of the mesophyll cells and abnormalities in resin ducts: tanned duct parenchyma with no cell dissolution; degradated epithelial cells; and occlusion (observed in normal appearing tissue also). Tanned duct cells often extended beyond the symptom area. In symptomatic areas, epithelial cells exhibited dissolution which commonly extended >470 μ distal to symptom borders. Hyphae were absent in duct tissue in or distal to symptom areas, indicating the possible production of an exotoxin or exoenzyme by M.dearnessii causing the cellular abnormalities in the ducts distal to symptomatic tissue.

A324

CLONES OF RADIATA PINE EXHIBIT DIFFERENTIAL SUSCEPTIBILITY TO WESTERN GALL RUST IN THE FIELD. D. R. Vogler, B. B. Kinloch, Jr., W. J. Libby, F. W. Cobb, Jr., and T. L. Popenuck. Dept. of Plant Pathology, 147 Hilgard Hall, University of California, Berkeley, CA 94720.

Ortets (seedlings) of radiata pine (Pinus radiata D. Don) planted in a common garden experiment at Lafayette, CA in 1982 and exposed to natural inoculum of western gall rust (WGR; Peridermium harknessii J. P. Moore) exhibited a range of susceptibility to infection over the ensuing decade. To test the consistency and repeatability of host response, we field-tested ramets (rooted cuttings) of clones derived from both field-susceptible and field-resistant ortets. Clones were chosen to represent each of the five native radiata populations, as well as U.S. mainland-Mexican island hybrids and Australia-New Zealand selects. Two ramets of each of 24 clones were planted in blocks in each of four plantations at Lafayette, CA in 1988, and observed for three years. Clonal response to WGR was generally consistent between the 1982 and 1988 experiments, and highly consistent within the 1988 experiment. Differences among clones were dramatic, with some ramets having few or no galls adjacent to others with 300 or more.

A325

EFFECT OF SORGHUM DEVELOPMENTAL STAGE AND GENOTYPE ON ANTHRACNOSE STALK ROT DEVELOPMENT. R. Bandyopadhyay and G. C. Bergstrom, ICRISAT, Patancheru, Andhra Pradesh 502 324, India, and Dept. of Plant Pathology, Cornell University, Ithaca, NY 14853.

Little is known about the anthracnose stalk rot (ASR) of sorghum caused by *Colletotrichum graminicola*. We investigated the effect of host ontogeny on susceptibility in the sorghum genotype Shallu in the greenhouse by inoculating plants at four growth stages: one wk before panicle initiation (PI), PI, flowering, and soft dough. The third internode above the brace roots of each plant was injected with a conidial suspension (10^5 conidia per ml) through a fresh wound. ASR severity was assessed in the pith 48 days after inoculation by splitting the stalk and measuring the spread of symptoms (maximum vertical distance between inoculated point and discoloration in pith) and percentage of each internode discolored. Maximum ASR developed in plants inoculated at the soft dough stage (spread 20 cm, mean discoloration per internode 44%) and the least ASR in plants inoculated before PI (spread 2.8 cm, discoloration per internode 22%). We also evaluated 12 cultivars for ASR resistance at flowering and found that ORO-G-XTRA, DeKalb X-977, and Sureno were resistant, whereas Malisor 84-7, SRN 39, and DeKalb M-565 were susceptible. It appears that host ontogeny and genotype influence ASR development in sorghum.

A326

EVALUATION OF STENOCARPELLA MAYDIS STALK ROT BY PITH DISCOLORATION AND RESISTANCE TO PRESSURE. M.A. Morant, and H.L. Warren. Department of Agriculture, University of Maryland Eastern Shore, Princess Anne. MD 21853, and Department of Plant Pathology, Physiology and Weed Science, VPI & SU, Blacksburg, VA 24061.

Effect of nitrogen and date of inoculation on stalk rot was evaluated in maize hybrids B73 x Mo17 and Pioneer 3732. Severity measured by pith discoloration, was greatest if hybrids were inoculated 2 wk prior to anthesis and decreased proportionately when inoculated at 2 wk post anthesis, midsilk and 4 wk post anthesis, respectively. As N rate increased from 67 to 240 Kg N/ha, pith discoloration decreased, but was hybrid dependent. B73 x Mo17 was more severely discolored. Severity measured by stalk resistance to pressure, however, indicated that internode 3 was most susceptible to stalk rot and more so in B73 x Mo17.

A327

ENZYME IMMUNOASSAY FOR DETECTION OF *THIELAVIOPSIS BASICOLA*. B. A. Holtz, A. E. Karu, and A. R. Weinhold. Department of Plant Pathology, 147 Hilgard Hall, University of California, Berkeley, CA 94720.

Field isolates of *Thielaviopsis basicola*, the causal agent of black root rot of cotton, were grown in Czapek Dox Broth amended with dialyzed carrot extract. Soluble protein extracts of chlamydospores and mycelium were used to raise polyclonal mouse ascites antibodies. The IgG antibody fraction was purified and biotin-labeled to devise a fungal capture "sandwich" enzyme immunoassay (EIA). The EIA detected both brown and gray cultural types of *T. basicola* and had negligible crossreactivity with other soilborne fungi commonly found in cotton field soil. The minimum detection limit of the EIA was approximately 1 nanogram of fungal protein. *T. basicola* could be detected in cotton roots 2 days after inoculation. At this time initial symptoms were apparent.

A328

STORABILITY OF DRY MYCELIAL PREPARATIONS OF SCLEROTINIA MINOR. H. A. Melouk and C. Bowen. USDA-ARS and Department of Plant Pathology, Oklahoma State University, Stillwater, OK 74078-9947.

Mycelial mats of S.minor produced in shake cultures of potato-dextrose (PD) broth were harvested and gradually dried with polyethylene glycol and anhydrous $CaSO_4$ (Phytopathology 80:1015). Dry mycelial mats were suspended over $CaCl_2$ solutions in sealed flasks to attain relative humidities (RH) of 40 and 80% at 22±2C. Viability of mycelia during storage was determined weekly by plating 25 fragments ($2mm^2$) on PD agar medium containing 100 ug/ml streptomycin sulfate. Mycelia remained viable for up to 10 weeks when stored at 40% RH. Viability of mycelia was reduced by 20% at 3 weeks and 100% at 6 weeks when stored at 80% RH. Dry mycelia of S. minor remained viable for up to six months of storage in a desiccator containing anhydrous $CaSO_4$.

A329

QUANTITIES OF *VERTICILLIUM DAHLIAE* IN SHOOTS OF A RESISTANT AND A SUSCEPTIBLE CULTIVAR OF TOMATO. W. H.Brandt. Dept. of Botany and Plant Pathology, Oregon State University, Corvallis, 97331

In the field, *Verticillium dahliae* proliferates more in a susceptible species of mint than it does in a resistant species. Does *V. dahliae* do likewise in cultivars of the same species? Young plants of two cultivars of tomato, Willamette (susceptible to Verticillium wilt), and Willamette VF (resistant to Verticillium wilt), were planted in a field infested with *V. dahliae*. After 10 wk, thin slices of stem tissue, taken 5-10 cm from the soil line were fragmented in an Omnimixer. Number of propagules was ascertained by a dilution plate technique using improved ethanol-streptomycin agar. *V. dahliae* did not proliferate in the resistant cultivar (33 propagules/g fresh wt) as much as it did in the susceptible cultivar (3738 propagules/g fresh wt). The same lack of proliferation occurred in the resistant cultivar in greenhouse tests where the pathogen was introduced through severed roots. After 5 wk, *V. dahliae* averaged 670 propagules/g fresh wt in Willamette VF stems, but averaged 21,428 propagules/g fresh wt in stems of Willamette. Thus resistance to Verticillium wilt in the tomato cultivar Willamette VF appears to result from suppressing proliferation of the pathogen rather than from interferring with entry of the pathogen into the vascular system.

A330

UTILIZATION OF A DIP-INOCULATION METHOD FOR DETERMINING PATHOGENICITY IN *COLLETOTRICHUM MAGNA* AND *C. ORBICULARE*. S. Freeman and R. J. Rodriguez. Dept. of Plant Pathology, Univ. of California, Riverside, CA 92521.

A rapid bioassay was developed for determining pathogenicity of *Colletotrichum magna*, utilizing cuttings and whole plants. The results corresponded well to disease responses observed by the standard leaf-inoculation methods. Mortality in cuttings occurred 48-72 h after inoculation. The method proved reliable for assessing susceptibility and resistance of cucurbit cultivars to *C. magna* and *C. orbiculare*, and enabled the screening of over 300 uv-irradiated mutants. Isolate HU 25, selected by this method, was nonpathogenic on watermelon seedlings, but induced a hypersensitive response when conidia were assayed on cotyledons. HU 25 colonized host tissue although plants remained healthy contrasting with mortality caused by wild type isolates. The banana-specific pathogen, *C. musae*, failed to cause disease and was also unable to colonize plant tissue.

A331

NUCLEAR BEHAVIOR DURING THE EARLY STAGES OF BASIDIOSPORE GERMINATION IN CRONARTIUM QUERCUUM f. sp. FUSIFORME. P. Spaine and S. Kaneko, USDA Forest Service, Green Street, Athens, GA 30602. Forestry and Forest Products Research Institute, Kukizaki, Inashiki-gun, Ibaraki-305, Japan.

Nuclear behavior in basidiospores was studied during germination on glass slides and pine seedlings with DAPI stains. After meiosis occurred in the basidium, a mitotic division took place within each basidiospore. This binucleate condition is short lived and had not been reported previously. One nucleus remained sharp and condensed while the other enlarges and eventually dissipated leaving a mononucleate condition. Within 1 hr after basidiospore discharge, one of the two nuclei of most basidiospores began to degenerate, and more than 93% of germ tubes by direct germination were mononucleate. During indirect germination, nuclear degeneration occurred without exception, however, mitotic division in the secondary basidiospores resulted in two nuclei. Nuclear movement from basidiospores into germ tubes occurred more rapidly on the pine seedlings than on glass slides.

A332

HOST SPECIALIZATION IN CUCURBIT ISOLATES OF OLPIDIUM RADICALE. R. N. Campbell and S. T. Sim, Department of Plant Pathology, University of California, Davis, CA 95616.

The host range and specificity of nine single-sporangial isolates of Olpidium radicale from France, California, Canada, and Brazil was tested by comparing their reproduction on seedlings. The isolates which had been trapped in cucurbit hosts were classified either as cucumber, melon, or squash strains because each isolate multiplied abundantly (8- to 54-fold increase in zoospores at 14 days) only on one of these hosts and poorly or not at all on the other two hosts. All isolates multiplied moderately on watermelon. All isolates grew in mung bean but seldom released zoospores. The isolates infected but did not grow in timothy, red clover, lettuce, or cauliflower. The cucurbit isolates of O. radicale have a narrow host range compared to red clover isolates that infect all these hosts. Their host specificity may be important in the specificity of virus transmission.

A335

EFFECT OF DATE OF PLANTING ON SEVERITY OF FROGEYE LEAF SPOT AND GRAIN YIELD OF SOYBEAN. C.N. Akem and K.E. Dashiell. Int'l Institute of Tropical Agriculture, Ibadan, Nigeria.

The influence of soybean (Glycine max) planting date on seasonal epidemics of frogeye leaf spot caused by Cercospora sojina was evaluated on two susceptible and one resistant soybean cv., (Samsoy 1, TGx 849-313D and TGx 996-26E) in field trials in Nigeria. Four field plantings were made at 14-day intervals from late May to mid July. For each planting date, half of the plots received 2 foliar applications of benomyl and the other half was untreated. Frogeye disease severity (DS) ratings were taken at R3 to R4, and grain yield for each plot was measured at harvest. There was a significant (P>0.05) increase in DS between the first and third planting on the susceptible cvs, and a corresponding yield reduction averaging 13% and 31% for the treated and untreated plots, respectively, with each 2-week delay beyond the first planting date. Under Nigerian conditions an increase in frogeye leaf spot severity can be expected with delayed planting of soybean after June 1, and this can result in a significant loss of crop yield.

A336

RESPONSES OF SOYBEAN PLANTS AND ENDOPARASITIC NEMATODES TO SIMULATED ACIDIC RAIN. S. R. Shafer[1,2], S. R. Koenning[2], and K. R. Barker[2], [1]USDA/ARS and [2]NC State Univ., Dept. of Plant Pathology, Raleigh, NC 27695-7616.

Interactions of rain acidity, soybean plants, and selected nematode species were studied in a greenhouse. Seedlings in pots of non-infested soil or soil infested with eggs of root-knot (Meloidogyne hapla or M. incognita) or cyst (Heterodera glycines) nematodes were exposed beneath spray nozzles 3 times/wk for 8 wk to simulated rain adjusted to pH 5.3, 4.3, 3.3, or 2.3 (2 cm per 1-h exposure). Only pH 2.3 rains caused major changes relative to pH 5.3 rains. Shoot dry weights and numbers of cyst nematode juveniles per pot exposed to pH 2.3 rains were approximately 20% and 10%, respectively, of those exposed to pH 5.3 rains; numbers of root-knot nematode juveniles were low and apparently unrelated to rain acidity. Numbers of eggs (all species) per pot exposed to pH 2.3 rains were $\leq$10% of those recovered from pots exposed to pH 5.3 rains. Dose-response relationships for most variables differed between cyst and root-knot nematodes but were similar for the two Meloidogyne species. Acid deposition can influence nematode-plant interactions, but the level required to cause major changes exceeded that known in the United States.

A337

EFFECT OF OZONE ON THE RATE OF POPULATION INCREASE OF SPIDER MITES ON WHITE CLOVER. A. S. Heagle and R. L. Brandenberg, USDA, ARS, Plant Pathology Dept., and Entomology Dept., North Carolina State University, Raleigh, NC, 27695.

Effects of ozone (O_3) on two-spotted spider mites (*Tetranychus urticae* Koch) were determined for an O_3-sensitive (NC-S) and an O_3-resistant (NC-R) clone of white clover (*Trifolium repens* L.). Clover was exposed to O_3 before and after mite infestation. Foliar injury and mite populations were measured at different times after infestation. At 10 days after infestation in the first greenhouse experiment, injury was greater on NC-S than on NC-R, but mites were not affected. After 17 days, the total mite population (eggs, nymphs and adults) on NC-S was 0.97, 1.19, and 1.64 times greater at 53, 77, and 100 ppb O_3 (6h day^{-1} means), respectively, than at 10 ppb. The comparative values on NC-R were 0.47, 0.26, and 0.00. Similar trends were observed in two additional greenhouse experiments and in a field experiment. Differences in leaf areas and shoot weight were discounted as reasons for differences in mite populations. Because O_3 caused a greater population increase on NC-S than on NC-R, the effect appears to be mediated through O_3 effects on host plants. Results suggest that the amount of pesticides required to control spider mites may be related to tropospheric O_3 concentrations.

A338

EFFECTS OF CO_2 ENRICHMENT ON MICROBIAL POPULATIONS IN THE RHIZOSPHERE AND PHYLLOSPHERE OF COTTON. G.B. Runion[1], E.A. Curl[2], R. Rodríguez-Kábana[2], P.A. Backman[2], H.H. Rogers[1], and B.E. Helms[2]. [1]USDA/ARS National Soil Dynamics Lab, Auburn, AL 36830 and [2]Department of Plant Pathology, Auburn University, AL 36849.

Cotton (*Gossypium hirsutum* L.) plants were exposed to elevated (550 $\mu L\ L^{-1}$) or ambient (360 $\mu L\ L^{-1}$) levels of atmospheric CO_2 and to wet (100% of evapotranspiration replaced) or dry (67% of ET replaced) soil moisture treatments within a free-air CO_2 enrichment system in Maricopa, AZ. Foliar and soil+root samples were collected in June and August, 1991. Foliage and rhizosphere soil samples were analyzed for bacteria or fungi using dilution plating. Bulk soil from the root zone was analyzed for populations of nematodes, microarthropods and *Rhizoctonia* using various extraction methods. A dehydrogenase assay for total microbial respiration and a bioassay for cotton root-infecting organisms were also conducted using root-zone soil. Effects of CO_2 on mycorrhizal colonization of cotton roots is being evaluated. Microbial respiration and populations of saprophagous nematodes were increased under elevated CO_2 at both sampling dates; other trends (P=0.10 to 0.20) were observed for CO_2 and soil moisture treatments. Effects of elevated atmospheric CO_2 on plant-microbe interactions could have profound influence on the productivity of agro-ecosystems and deserves further research.

A339

DEGRADATION OF THE HERBICIDE PROPANIL BY FUSARIUM SPECIES. R. E. Hoagland, R. M. Zablotowicz and H. K. Abbas. USDA-ARS, Southern Weed Science Laboratory, Stoneville, MS 38776.

Fusarium are dominant soil and rhizosphere fungi with a diverse metabolic potential. We assayed a collection of Fusarium isolates (F. moniliforme, F. oxysporum, F. semitectum and F. solani) for their ability to detoxify the herbicide propanil [N-(3,4-dichlorophenyl)propanamide] via hydrolytic amide cleavage. Arylacylamidase (E.C.3.5.1.a) activity, based upon formation of dichloroaniline (DCA), was determined from cell-free extracts of sonically disrupted hyphae. At least 70% of the isolates tested exhibited propanil-arylacylamidase activity ranging from 0.4 to 8.2 units (1 unit=1.0 nmole of DCA formed/mg of soluble protein/hr). Enzyme activity was observed in all four species, but most prevalent in F. solani isolates assayed (92%). These results indicate that most Fusarium isolates possess levels of arylacylamidase capable of biotransformation of propanil to non-phytotoxic products.

A340

FUNGICIDE SPRAY DRIFT AS MEASURED WITH ELECTRON BEAM ANALYSIS. C. R. Krause[1], R. D. Brazee[1], D. L. Reichard[1], R. D. Fox[1], C. Tappan[1], and S. A. Svensson[2]. U.S. Department of Agriculture (USDA), Agricultural Research Service (ARS), Application Technology Research Unit, OARDC/OSU, Wooster, OH 44691, and Swedish University of Agricultural Sciences, Alnarp, Sweden.

Quantitative data are lacking on the fate of downwind fungicide spray drift. With microclimatic data being recorded, $Cu(OH)_2$ (50 WP) in H_2O was applied with a cross-flow fan orchard sprayer with drift sampled by carbon-coated stubs(inert sample surfaces) and detached crabapple leaves at 1.5 m, 15 m, and 30 m from the point of application, each at 1 m elevation. Electron beam analysis(EBA) was used to characterize and determine sizes and distribution of $Cu(OH)_2$ droplets/particles. Average drop diameter and quantity decreased significantly with distance. EBA will provide data on morphology and transport behavior of fungicide sprays needed to improve efficacy and reduce environmental pollution.

A347

INCOMPATIBILITY OF NITRATE-NONUTILIZING MUTANTS OF CRYPTOSPORIOPSIS CURVISPORA AND C. PERENNANS. F.M. Dugan, R.G. Roberts and G.G. Grove. Washington State University Tree Fruit Research Center, 1100 N. Western Ave., Wenatchee WA 98801.

Nitrate-nonutilizing mutants were generated from 17 isolates of Cryptosporiopsis curvispora, cause of apple anthracnose, and from 24 isolates of C. perennans, cause of perennial canker. Mutants of the former species were paired with each member of pairs of mutants that demonstrated intra- and/or inter-isolate complementation within the latter species. Complementation occurred abundantly within and between isolates of C. perennans, rarely within and between isolates of C. curvispora, but not between species. Complementation occurred mostly between but sometimes within nitrogen source utilization phenotypes. Most phenotypes were characteristic of one or the other species.

A355

IDENTIFICATION OF AN *rcsB*-LIKE LOCUS IN *Erwinia amylovora*. W. Chun[1], A. Mendoza-Herrera[2], and A. K. Chatterjee[3]. [1]Plant Pathology Division, University of Idaho, Moscow, ID 83843, [2]National Center for Nitrogen Fixation, Quernavaca Morelos, Mexico, [3]Dept. of Plant Pathology, University of Missouri, Columbia, MO, 65211.

A positive regulator of EPS production (*rcsA*)from *Erwinia amylovora (Ea)*was previously isolated and characterized. A large subclone from *Ea* containing both *rcsA* and *rcsB* complementing function was mutagenized with λTn*5*. Tn*5* insertions in *rcsA* failed to complement mutations in both *rcsA* and *rcsB* of *E. coli*. Insertions in a region approximately 400 bp from *rcsA* of *Ea* complemented mutations in *rcsA* but not *rcsB* in *E. coli*. Subclones containing this *rcsB*-like region and not *rcsA* did not complement *rcsB* mutations in *E. coli*. Due to the polar effect of insertions in *rcsA* on *rcsB* activity and inability of *rcsB* subclones to function normally, the regulation of these genes in *E. amylovora* may be different from that in *E. coli*.

A356

CHARACTERIZATION OF AVIRULENCE GENE D HOMOLOGUES FROM PSEUDOMONAS SYRINGAE PV. LACHRYMANS. I. Yucel and N.T. Keen. Department. of Plant Pathology, University of California, Riverside, CA, 92521.

A 5.6 kb *Hin*dIII fragment containing avirulence gene D (*avr*D) from *Pseudomonas syringae* pv. *tomato* has been shown to be conserved in several other *Pseudomonas syringae* pathovars. In addition to the 5.6 kb *Hin*dIII fragment, *Pseudomonas syringae* pv. *lachrymans* also contains a ca. 3.8 kb *Hin*dIII fragment that strongly hybridizes with an *avr*D-specific probe. To further elucidate this unique *avr*D homologue pattern, we molecularly characterized the 5.6 and 3.8 kb *Hin*dIII fragments from *Pseudomonas syringae* pv. *lachrymans*. The 5.6 kb fragment was found to reside on the largest indigenous plasmid occurring in this pathovar, while the 3.8 kb fragment was located on a smaller indigenous plasmid. The sequence of the homologue on the 3.8 kb *Hin*dIII fragment shares 97% and 85% amino acid identity with the homologues from *Pseudomonas syringae* pathovars *tomato* and *phaseolicola* respectively. These fragments were further characterized to determine if one or both harbor a functional copy of *avr*D.

A357

PLASMID AND TRANSPOSON-MEDIATED STREPTOMYCIN RESISTANCE IN PHYTOPATHOGENIC AND EPIPHYTIC GRAM-NEGATIVE BACTERIA. C.-S. Chiou and A. L. Jones, Dept. of Botany and Plant Pathology and The Pesticide Research Center, Michigan State University, East Lansing, 48824

Most streptomycin-resistant strains of *Erwinia amylovora* and epiphytic gram-negative bacteria isolated from Michigan apple orchards exhibited streptomycin phosphotransferase activity in an in vitro enzyme assay and their DNA hybridized with SMP3, a portion of streptomycin resistance genes from *Pseudomonas syringae pv. papulans* strain Psp36 from New York. The resistance genes (*str*) in *E. amylovora* were located on a 34-kb self-transmissible plasmid pEa34 that could be transferred by conjugation in the laboratory from *E. amylovora* to *Escherichia coli* and *P. s. papulans*. The nucleotide sequences of the *str* genes cloned from *E. amylovora* and *P. s. papulans* Psp36 were identical to the *strA/strB* genes previously found on the nonconjugative plasmid RSF1010. The *strA/strB* genes in *E. amylovora* were carried by a class II transposon. This 6.7-kb transposon has an 81-bp inverted repeat on both ends; *tnpA*, a gene encoding a transposase of 961 amino acids; *tnpR*, a gene encoding a resolvase of 181 amino acids; the *strA/strB* genes; and a 1.2-kb insertion sequence that was absent in *P. s. papulans* Psp36. A 5-bp direct repeat was generated on the target DNA in the process of transposition. DNA from *P. s. papulans* Psp36 and from 149 of 156 strains of resistant epiphytic bacteria that reacted to SMP3 hybridized to a 3.2-kb DNA probe consisting of nucleotide sequences from *tnpA* and *tnpR*. Thus, the transposon was common in most bacteria with *strA/strB*. Transposition and conjugation are the likely mechanisms for the widespread distribution of *strA/strB* among gram-negative bacteria in the apple orchard.

A358

IDENTIFICATION OF PLANT PATHOGENIC XANTHOMONAS SPECIES AND PATHOVARS BASED ON AMPLIFIED DNA FRAGMENTS RELATED TO HRP GENES OF XANTHOMONAS CAMPESTRIS PV. VESICATORIA. R. P. Leite Jr., R. E. Stall, and U. Bonas*. Department of Plant Pathology, University of Florida, Gainesville, FL 32611, and *Institut für Genbiologische Forschung Berlin GmbH, 1000 Berlin 33, Germany.

The DNA sequence variation in the hrp (hypersensitivity reaction and pathogenicity) gene cluster of several species and pathovars of Xanthomonas was examined by analysis of enzymatically amplified DNA fragments. The DNA sequence of the hrp gene cluster identified in X. campestris pv. vesicatoria (Bonas et al., 1991. Mol. Plant-Microbe Interact. 4: 81-88) was used to design primers that specifically amplify portions of this gene cluster. The fragments amplified from different strains of Xanthomonas were digested with frequent-cutting restriction endonucleases and the restriction pattern for each fragment was analyzed to determine the presence of polymorphism and strain specific variation. Amplification of hrp related DNA was obtained for every strain of X. fragariae and 22 pathovars of X. campestris, but not for X. maltophilia, or for opportunistic xanthomonads. Restriction endonuclease digestions of the amplified DNA fragments had variation in the restriction patterns that allowed distinction of the different species and pathovars tested. On the other hand, the hrp fragments amplified from strains of different groups of X. campestris pv. vesicatoria, pv. citrumelo, and pv. citri had no variation within each group.

A359

RFLP ANALYSIS OF THE *HRP* REGION OF *PSEUDOMONAS SYRINGAE* PV. *TABACI* USING *HRP* SEQUENCES OF *PSEUDOMONAS SYRINGAE PV. PHASEOLICOLA*. B. K. Scholz, J. L. Jakobek, and P. B. Lindgren, Dept. of Plant Pathology, NC State Univ., Raleigh 27695-7616.

A gene cluster cloned from *Pseudomonas syringae* pv. *phaseolicola* is required for pathogenicity and the ability to elicit the hypersensitive response. These genes have been designated *hrp* (for hypersensitive response and pathogenicity). Southern blot analysis has shown the *hrp* region of *P. s.* pv. *phaseolicola* to have significant sequence homology to *P. s.* pv. *tabaci* (*Pst*), suggesting that these bacteria have common *hrp* genes. To help understand how the *hrp* gene cluster varies within a pathovar, restriction fragment length polymorphism (RFLP) analysis was used to study 24 *Pst* isolates representing several geographical locations. Southern blot analysis of 10 genomic restriction digests were conducted using *hrp* sequences from *P. s.* pv. *phaseolicola* NPS3121 as hybridization probes. This analysis has shown the *Pst* isolates to be uniform across all restriction sites examined with the exception of *Pst* Br2 which has an identical RFLP pattern to *P. s.* pv. *phaseolicola* NPS3121. We are continuing to study the diversity within these *Pst* isolates by amplifying specific regions of the *hrp* cluster using PCR, followed by restriction site analysis of the amplified product.

A360

IDENTIFICATION OF A LOCUS THAT ACTS IN TRANS TO STIMULATE PHENAZINE GENE EXPRESSION IN *PSEUDOMONAS AUREOFACIENS* 30-84. L. S. Pierson, III and V. D. Keppenne. Department of Plant Pathology, University of Arizona, Tucson, AZ 85721.

A promoterless lacZ cartridge was inserted in frame into phzB, a chromosomal gene involved in the production of phenazine-1-carboxylic acid in *Pseudomonas aureofaciens* 30-84. Additional regions of the 30-84 genome were introduced in trans into this phzB::lacZ reporter strain (30-84Z) and their effect on phenazine gene expression measured. One cosmid, pLSP259, which contained the phenazine biosynthetic locus and additional upstream regions, acted in trans to stimulate *B*-galactosidase expression. Initial deletion analysis indicated a region upstream of the phenazine biosynthetic genes was responsible for increasing expression of the phzB::lacZ fusion. To determine whether this trans-activation was due to the production of a positive activator or the titration of a negative repressor, $P_{1.1}$, a promoter believed to be involved in phenazine gene expression, was introduced in trans into 30-84Z. The presence of additional copies of $P_{1.1}$ resulted in lower levels of *B*-galactosidase, indicating it was titrating a positive activator. We have named the gene responsible for this activation phzA and have localized it to a 2.7 kb PstI fragment.

A361

MOLECULAR CHARACTERIZATION OF *IS801*, A TRANSPOSABLE ELEMENT FROM *PSEUDOMONAS SYRINGAE* PV. *PHASEOLICOLA*. G. Richter[1], K. Westerstrahle[2], M. Romantschuk[2], and D. Mills[1]. [1]Department of Botany and Plant Pathology, Oregon State University, Corvallis 97331, and [2]Department of General Microbiology, University of Helsinki, SF-00300 Finland.

IS801, a 1512 base pair transposable element previously isolated from strain LR781 of *Pseudomonas syringae* pv. *phaseolicola*, is unusual among prokaryotic transposable elements in that it neither possesses terminal repeats nor generates target sequence duplications. Cloned copies of *IS801* have been shown to transpose in *Escherichia coli recA* hosts. Sequence analyses of insertion junctions from at least six independent transposition events indicate that *IS801* has moderate specificity for insertion and does not duplicate its target sequence. It has sequence and target selection similarities to *IS91*, an element that also inserts without target duplication. Mobilization of a modified version of *IS801*, by expression of the putative transposase *in trans*, has been achieved in *E. coli*.

A362

AROMATIC AMINOTRANSFERASE GENES FROM AN INDOLEACETIC ACID-PRODUCING *ERWINIA HERBICOLA* STRAIN. E. Clark, M. Brandl, and S. E. Lindow. Dept. of Plant Pathology, University of California, Berkeley CA 94720.

Conversion of L-tryptophan (TRP) to indole-3-pyruvic acid, catalyzed by aromatic aminotransferases, is the first step in the biosynthesis of indole-3-acetic acid (IAA) from TRP in epiphytic *Erwinia herbicola* strain 299R. At least 3 aminotransferases (AT) are capable of deaminating TRP *in vitro*. Two AT genes, termed *aatA* and *aatB*, were cloned which direct the synthesis of enzymes possessing both *in vitro* and *in vivo* aromatic AT activity. Both genes were insertionally inactivated using the transposable reporter gene system Tn3-Spice. The native *aatA* and *aatB* genes were replaced via homologous recombination with *aatA-inaZ* and *aatB-inaZ* gene fusion constructs, in which *inaZ* expression is a reporter for *aatA* and *aatB* activity. Inactivation of *aatA* results in isoleucine, leucine, and valine auxotrophy, indicating its primary function to be that of a branched-chain AT. $AatB^-$ mutants are prototrophs. $AatA^-$ mutants show decreased IAA production when cultured in TRP-amended medium; $AatB^-$ mutants are unaffected in IAA production. $AatA^-$ mutants showed a reduced population size on bean leaves relative to 299R; the population size of $AatB^-$ mutants was the same as 299R. Both *aatA-inaZ* and *aatB-inaZ* gene fusions show 10^4-fold higher ice nucleation activity on leaves than on Luria agar.

A365

LOCATION OF VICTORIN BINDING PROTEINS IN OAT TISSUE DETERMINED BY POLYCLONAL ANTI-VICTORIN ANTIBODY. K. Akimitsu[1,2], L. P. Hart[1,2], and J. D. Walton[1,3]. [1]Department of Botany and Plant Pathology, [2]Pesticide Research Center, and [3]DOE-Plant Research Laboratory, Michigan State University, East Lansing, MI 48824.

In vivo and in vitro covalent binding of victorin was detected on the same proteins in susceptible and resistant cultivars of oats using western blotting assays and polyclonal anti-victorin antibody. For the localization of victorin binding proteins in oat tissues, homogenates of dark-grown oat shoots were separated in a 20 to 45 % sucrose continuous gradient, the fractions mixed with 1 μg/ml of victorin, and covalent binding sites detected by western blotting. The 100 kD victorin binding protein was located in the 36 % to 44 % sucrose fractions which contain plasma membrane and mitochondria. Golgi and ER membranes were in different fractions as determined by marker enzyme assays. Victorin binding on 65 kD and 45 kD proteins was detected in all fractions. One μg/ml of victorin had no effect on the enzyme activities of the membrane marker enzymes; glucan synthetase II, cyt-C oxidase, NADH-cyt-C reductase, and IDPase. Further characterization of the location of victorin binding is proceeding by using an aqueous two phase method.

A366

CONSTITUTIVE AND REGULATED EXPRESSION OF A CHITINASE/LYSOZYME ISOZYME IN CABBAGE VARIETIES RESISTANT AND SUSCEPTIBLE TO BLACK ROT. K.M. Dodson[1], J.J. Shaw[2] and S. Tuzun[1], [1]Department of Plant Pathology, and [2]Department of Botany and Microbiology, Auburn University, AL 36849.

Resistant (Hancock, HC) and susceptible (Perfect Ball, PB) 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 HC, which was upregulated in tissue after bacterial colonization. In contrast, CHL2 accumulated in PB only after symptom development. Lysozyme activity assay results paralleled the Western blot data. Therefore, constitutive expression of this chitinase/lysozyme (CHL2) is suggested as a mechanism of black rot resistance.

A367

DIFFERENTIAL ACCUMULATION OF CHITINASES, β-1,3-GLUCANASES AND OSMOTINS IN TOMATO VARIETIES RESISTANT AND SUSCEPTIBLE TO *ALTERNARIA SOLANI*. C. B. Lawrence and S. Tuzun, Department of Plant Pathology, Auburn University, AL 36849-5409.

Differential accumulation of three antifungal proteins, chitinases (CHL), β-1,3-glucanases (BGL) and osmotins (OS) were studied in tomato varieties susceptible (Piedmont) and resistant (NCEBR1, NCEBR2 and 71B2) to A. solani, the causal agent of early blight. Six-week-old greenhouse grown plants were inoculated with a conidial suspension (7000 spores/ml) of A. solani. Samples were collected at different time intervals (days) after inoculation (DAI), and proteins were extracted with neutral and acidic buffers. SDS-PAGE and Western blot analysis indicated the presence of four CHL, three BGL and one OS isozyme(s) in the susceptible and an additional BGL and OS isozyme in resistant varieties. Among the four CHL isozymes, CHL3 was present one DAI only in resistant varieties, whereas, this isozyme appeared in the susceptible variety 6-9 DAI. Other CHL isozymes were present in all varieties at 3 DAI; however, increased levels of CHL1 and CHL2 were detected at this time interval only in resistant varieties. The induction pattern of CHL3 appears to be correlated with the presence of the resistance gene.

A368

DETECTION IN BRASSICA NAPUS OF AN INHIBITOR OF POLYGALACTURONASE OF LEPTOSPHAERIA MACULANS, CAUSAL AGENT OF BLACKLEG DISEASE OF CANOLA. S. L. Annis and P. H. Goodwin. Dept. of Environmental Biology, University of Guelph, Guelph, Ontario. N1G 2W1.

Polygalacturonase produced by Leptosphaeria maculans is believed to be important in the interaction between this fungus and its host, canola. An inhibitor of polygalacturonase of L. maculans has been found in crude extracts of canola. Up to 85% of the activity of the fungal polygalacturonase could be inhibited by plant extracts. The inhibitory activity varied with the age of the plant and the type of tissue. Preliminary characterization of the inhibitor showed that its activity was not affected by boiling or autoclaving. The inhibitory activity was decreased by dialysis (membrane cut-off 12,000-14,000 MW) and by EDTA (a chelator of cations). The inhibitor appears to be a low-molecular weight, heat-stable compound. Cations appear to be important for the inhibitory activity. The relationship between the amount of inhibitor and the resistance of varieties of canola and other Brassica species to L. maculans is being investigated.

A369

A LOW MOLECULAR WEIGHT, HEAT STABLE, SUPPRESSOR OF ACTIVITY OF NON-SPECIFIC ELICITORS OF *CLADOSPORIUM FULVUM*. H. Lu and V. J. Higgins, Department of Botany, University of Toronto, 25 Willcocks St., Toronto, Ontario, Canada, M5S 3B2

It is assumed that suppression of the activity of non-specific elicitors (NSE) from fungal cell walls is required in establishment of basic compatibility in the *Cladosporium fulvum*-tomato system. A suppressor of NSE-induced necrosis on tomato was partially purified from intercellular fluid (IF) obtained from *C. fulvum*-infected, or healthy, leaves. The activity was stable to protease, glucosidase, galactosidase, laminarinase, periodate, heat, acid and base. After treatment of IF with urea, protease, or heat, the suppressor was lost on dialysis (MWCO 3.5 kda), suggesting a loose association with proteins. On a Sephadex G-25 column, the active peak corresponded with the presence of carbohydrate molecules. Suppressor activity was lost when IF was mixed with inactivated NSE. Enzymes in IF that degrade NSE seem to be involved in a slower form of suppression.

A370

TERPENOID PHYTOALEXINS: ULTRASTRUCTURAL AND PHYSIOLOGICAL EFFECTS ON *VERTICILLIUM DAHLIAE*. M. E. Mace and R. D. Stipanovic, USDA, ARS, Route 5, Box 805, College Station, Texas 77845.

Toxic effects of the cotton terpenoid phytoalexins desoxyhemigossypol (dHG) and desoxymethoxyhemigossypol (dMHG) on conidia of the defoliating strain (V-44) of *Verticillium dahliae* were determined. Extensive rupture of the plasmalemma occurred after exposure of conidia to 15 µg/ml of dHG for 4 hrs. Similar treatment of conidia with dMHG at 30 µg/ml caused only scattered swelling of the plasmalemma. Potassium ion leakage was an additional indicator of plasmalemma damage by dHG and dMHG. Maximum potassium losses equal to about 80% of the potassium losses in boiled controls occurred after 2 hr exposure of conidia to 15 and 30 µg/ml of dHG and dMHG, respectively. The percentage of conidia killed during the 6 h potassium loss studies by dHG and dMHG were 98% and 92.5% at 0.5 h and 100% and 98.1% at 4 h, respectively. The concurrent large potassium loss and rapid death of conidia indicate that plasmalemma damage is an essential component in the toxicity of these phytoalexins.

A371

EXPRESSION OF CECROPIN B CONTROLLED BY PLANT INDUCIBLE PROMOTERS IN TRANSGENIC TOBACCO PLANTS CONFERS ENHANCED RESISTANCE TO *PSEUDOMONAS SOLANACEARUM*. Y. Huang, J.H. McBeath, H. Lockwood, and L. Owens. University of Alaska, Department of Plant and Animal Science, Fairbanks, AK 99775

The function of promoters of phenylalanine ammonia-lyase (PAL) from *Arabidopsis* and proteinase inhibitor II (PiII) from potato in response to *P. solanacearum* infection was investigated in transgenic tobacco plants carrying gene fusions of PAL-beta-glucuronidase (GUS) and PiII-GUS. Activation of the PAL gene occurred in a localized area and induction of PiII exhibited a systemic response. Each of the two promoters was fused to the gene encoded for cecropin B, a lytic peptide that confers anti-bacterial activity. To facilitate secretion of the cecropin B into the extracellular fluid, the N-terminal signal sequence of barley alpha-amylase was fused to the upstream of the cecropin B gene by PCR. The triple chimeric gene fusions were cloned into a binary vector and transformed into tobacco plants. Several transgenic lines were identified with resistance to *P. solanacearum* infection as evaluated by stem and root inoculation. Molecular characterization of the transgenic lines will be discussed.

A373

PATHOTYPES OF COCHLIOBOLUS SATIVUS ON BARLEY. T. G. Fetch, Jr.[1], B. J. Steffenson[1], and J. D. Franckowiak[2]. [1]Dept. of Plant Pathology and [2]Dept. of Crop and Weed Sciences, North Dakota State Univ., Fargo, ND 58105

In 1990, a number of 2-rowed barley breeding lines were severely affected by a leaf-spotting disease at Prosper, ND. Yield losses exceeding 35% occurred on lines previously considered resistant to leaf-spotting pathogens. The spot blotch pathogen, Cochliobolus sativus, was identified as the causal organism from laboratory and greenhouse experiments. A field isolate (SB90) of C. sativus from a susceptible two-rowed genotype was compared with an isolate collected in 1985 (SB85) for virulence expression on selected barley lines. Distinct differences in virulence were observed between the two isolates in field tests in 1991. Genotypes ND10277, ND12708, ND12715, ND12719, ND12720, ND12721, and cv. Bowman (all previously considered resistant to C. sativus) were susceptible (disease severity = 50-95%, infection response = moderately susceptible-susceptible) to SB90, and moderately resistant (10-25%, MR-MS) to SB85. Genotypes ND5883, ND12437, and Norbert (all previously considered susceptible to C. sativus) were moderately resistant (15-30%, MR-MS) to SB90, and susceptible (30-90%, MS-S) to 85SB. These data indicate that two distinct pathotypes of C. sativus are present in eastern ND. Evaluation of barley germplasm against both pathotypes is essential for the development of cultivars resistant to the spot blotch pathogen.

A374

GERMINATION OF CONIDIA OF *PHOMA PROBOSCIS* ON LEAVES OF *CONVOLVULUS ARVENSIS*. D. K. Heiny, Department of Plant Pathology, University of Arkansas, Fayetteville, AR 72701.

Growth of field bindweed (*Convolvulus arvensis* L.) is suppressed by the pathogenic fungus *Phoma proboscis* (*Phytopathology* 81:905-909 [1991]). Temperatures of 20-24 C are optimum for disease development, which is limited at 32 C. To investigate the relationship between temperature and conidial germination, field bindweed plants and water agar plates were inoculated with conidia of *P. proboscis* and incubated in dew at 24 C or 32 C. Surface impressions of variously aged leaf samples were made after 6, 12, and 24 h of dew and stained with cotton blue. Mean percent germination of conidia was lower on leaf surfaces than on water agar and declined with increasing leaf maturity at both 24 C and 32 C. Percent germination (>60%) on the youngest

leaves at 24 C was greater than at 32 C (<20%) after 24 h of dew. This suggests that differential germination of conidia is a factor in the differential disease development at the two temperatures.

A375

VIRULENCE TYPES AND PATHOTYPE EVOLUTION OF *ERYSIPHE GRAMINIS* F. SP. *HORDEI* IN TUNISIA. A.H. Yahyaoui[1], and M. Reinhold[2]. [1] E.S.A.K., Kef, Tunisia. [2] Montana State University, Bozeman, MT 59717.

Powdery mildew (*Erysiphe graminis* f. sp. *hordei*) has not been considered a significant factor in barley production in Tunisia in the past. This situation might change due to a new awareness of the disease, which is commonly observed in the major barley growing areas. Among the known resistance genes and gene combinations, only a few were effective against mildew isolates collected from Tunisia over the past three years. The virulence spectrum of each isolate was determined on a differential set in the seedling stage. Barley genotypes having Mla7+Mla4, Mla10, Mla4, Mla9+Mla4, Mla5 and Mla(Ru1) genotypes were effective against eleven isolates of *E. graminis* collected from different regions in Tunisia in 1991. The virulence patterns of the isolates differed between sites and changes were observed at all collection sites. Barley cultivation practices in Tunisia might favor the development of new virulence types. An effective use of available resistance genes requires careful monitoring of the fungal virulence patterns in the area.

A383

GENETIC DIVERSITY OF *RHIZOCTONIA SOLANI* ANASTOMOSIS GROUP 1. Z. L. Liu and J. B. Sinclair, Department of Plant Pathology, 1102 S. Goodwin Ave., University of Illinois at Champaign-Urbana, IL 61801-4709

Population diversity of *Rhizoctonia solani* AG 1 was studied by isozyme and DNA polymorphism using 60 isolates from various geographic and host origins. Using ISG 2D61 (AG 2-2) as an outgroup, five distinct populations, 1A (23 isolates), 1B (4 isolates), 1C (15 isolates), 1D (16 isolates), and 1E (2 isolates) were differentiated based on binary characters of isozyme alleles and DNA restriction sites by numerical cladistic analysis. Isozyme phenotypes and DNA restriction maps of PCR-amplified mitochondrial and ribosomal RNA genes were constructed for each of the five groups. The groups were distinguished from one another by at least one restriction site in addition to the varied lengths of PCR-amplified DNA fragments. Many isozyme alleles at various loci and restriction sites were shown as useful molecular markers for populations. Each of the five groups was proposed as a genetically distinct intraspecific group of AG 1. The relationship of the five groups with previously described subgroups was shown.

A384

GENETIC DIVERSITY OF *RHIZOCTONIA SOLANI* ANASTOMOSIS GROUP 2. Z. L. Liu and J. B. Sinclair, Department of Plant Pathology, 1102 S. Goodwin Ave., University of Illinois at Champaign-Urbana, IL 61801-4709

Population diversity of *Rhizoctonia solani* AG 2 was studied by isozyme and DNA polymorphism using 70 isolates from various geographic and host origins. Using ISG 1C19 (AG 1 IC) as an outgroup, five distinct populations, 2A (9 isolates), 2B (13 isolates), 2C (22 isolates), 2D (14 isolates), and 2E (12 isolates) were differentiated based on binary characters of isozyme alleles and DNA restriction sites by numerical cladistic analysis. Isozyme phenotypes and DNA restriction maps of PCR-amplified ribosomal RNA genes were constructed for each of the five groups. The groups were distinguished from one another by at least one restriction site in addition to the varied lengths of PCR-amplified DNA fragments. Many isozyme alleles and restriction sites were shown as useful molecular markers for population studies. Each of the five groups was proposed as a genetically distinct intraspecific group of AG 2. The relationship of the five groups with previously described subgroups based on anastomosis frequency was shown.

A385

A 1.4 Kb SEQUENCE HOMOLOGY IN THE 3' NONCODING REGIONS OF BLUEBERRY LEAF MOTTLE NEPOVIRUS RNA 1 AND RNA 2: POSSIBLE MECHANISMS FOR MAINTAINING IDENTITY. J. W. Bacher[1], D. S. Warkentin[1,2], D. C. Ramsdell[2] and J. F. Hancock[1]. [1]Department of Horticulture and [2]Department of Botany and Plant Pathology, Michigan State University, East Lansing, Michigan, 48824.

The 3' ends of RNA 1 and RNA 2 of blueberry leaf mottle nepovirus have been cloned and their cDNA sequences have been determined. The 3' terminal -1400 nt of both RNAs are identical and are apparently noncoding. This precise conservation between the 3' ends of the genomic RNA components has been reported for other nepoviruses as well as for other multicomponent RNA plant viruses. The possible roles of RNA recombination and selection pressure are discussed in relation to the conservation of these 3' end sequences.

A386

MOLECULAR EVOLUTION OF ICE NUCLEATION: A PHYLOGENETIC APPROACH. A. R. Edwards and C. S. Orser, Department of Bacteriology and Biochemistry, University of Idaho, Moscow ID 83843.

Bacterial ice nucleation activity can be traced to the product of a single gene, ina. The ice nucleation (Ina^+) phenotype is narrowly distributed in bacteria, occurring in strains of six species but absent from closely related species, indicating that its evolution has been unusual. This research attempts to unravel the evolutionary history of ina by using a phylogenetic approach. Absence of the ice nucleation gene from closely related bacterial species was confirmed by Southern blot. Sequence data from 16S ribosomal RNA genes of four Ina^+ strains was generated and used in a detailed phylogenetic analysis to infer the evolutionary relationships of the species. From this data we were able to construct a phylogenetic tree of the species. A similar analysis was performed on ina sequence data. Incongruence exists between the two trees, suggesting that the ina gene and the species have followed separate evolutions.

A387

ISOLATES OF *Glomerella* (*Colletotrichum*) FROM MAIZE AND SORGHUM ARE DISTINCT SPECIES. Lisa J. Vaillancourt and Robert M. Hanau, Dept. of Botany & Plant Path., Purdue Univ., W. Lafayette, IN 47907

Morphological and genetic characteristics of *Glomerella* (*Colletotrichum*) from maize and sorghum were compared. The teleomorph of sorghum isolates was similar to *Glomerella graminicola*, the teleomorph of *Colletotrichum* from maize. Mating tests demonstrated that *Glomerella* from sorghum and maize were not interfertile. Small but consistent differences in the morphologies of spores and appressoria of isolates from maize and from sorghum were observed which agreed with earlier reports. DNA fingerprints, detected as restriction fragment length polymorphisms of mitochondrial DNA and random amplified polymorphic DNA (RAPD) produced from nuclear DNA by the polymerase chain reaction, reliably and unambiguously distinguished isolates of *Colletotrichum* from maize and sorghum. Analysis of similarity of the RAPD fingerprints indicated that maize and sorghum isolates of *Colletotrichum* are only about 45% similar (+/- 10%), representing distinct and separate genetic lineages. Hence, isolates of *Colletotrichum* from maize and sorghum are sibling species since they are morphologically very similar but reproductively completely isolated.

A390

APHID PERFORMANCE DIFFERENCES ON VIRUS-INFECTED POTATOES: COMPARISON BETWEEN VECTOR-DEPENDENT AND VECTOR-INDEPENDENT VIRUSES. S.J. Castle, and P. H. Berger. Dept. of Plant, Soil, & Entomological Sciences, Univ. of Idaho, Moscow, ID 83843.

Virus infection of plants can have either positive, negative or neutral effects on the performance (i.e., growth, reproduction and survival) of phytophagous insects. It is possible that this effect may correlate with the relationship between virus and vector. This hypothesis was tested using three viruses infecting potato which have different associations with the aphid, *Myzus persicae*. Mean relative growth rate, intrinsic rate of increase, and longevity were greater for *M. persicae* when feeding on plants infected with the persistent-circulative potato leafroll virus (PLRV) vs. plants infected with the noncirculative potato virus Y (PVY), the nonvectored potato virus X (PVX), or healthy potato. In feeding preference studies, aphids showed a strong preference for PLRV-infected plants when given a free choice between the various treatments

A391

FATE OF PLANT VIRUSES IN LEAVES AFTER DEPOSITION BY LEAF-FEEDING BEETLES. **T.K. Field, R.C. Gergerich, C.A. Patterson, and K.S. Kim. Dept. of Plant Pathology, Univ. of Arkansas, Fayetteville 72701.**

Fluorescent-antibody labelling was used to detect plant viruses within bean leaves after virus deposition by Mexican bean beetles previously fed purified virus (15-20 mg/ml). Three days post-feeding, southern bean mosaic virus, a beetle-transmitted virus, was detected in veins leading from the feeding wound, and primary infection sites occurred in mesophyll cells associated with these veins. Two non-beetle-transmitted viruses, tobacco ringspot and tobacco mosaic, were detected only on the edges of feeding wounds at 3 days post-feeding. However, at 4-12 hours post-feeding, all 3 viruses were found in veins leading from the feeding site, although the non-beetle-transmitted viruses were found in fewer veins and were not detected far from the feeding wound. These results suggest that non-beetle-transmitted viruses introduced by beetle feeding are translocated in veins to a limited extent and are then degraded.

A392

BIOLOGICAL CHARACTERIZATION OF PATHOTYPES OF BLACKEYE COWPEA MOSAIC AND COWPEA APHIDBORNE MOSAIC POTYVIRUSES. M. Bashir and R. O. Hampton, Dept of Botany and Plant Pathology, Oregon State University, Corvallis, OR 97331-2902.

Fifty cowpea genotypes were tested as differential hosts, respectively, of 32 and 50 seed-borne isolates of blackeye cowpea mosaic (BlCMV) and of cowpea aphidborne mosaic (CAMV) potyviruses. These genotypes partitioned viral isolates into eight (8) BlCMV pathotypes and 13 CAMV isolates pathotypes. Genotype-pathotype interactions included two types of immunity, asymptomatic infection (tolerance), and susceptible-sensitive responses. Based on these interactions, the following 13 cowpea genotypes are proposed as differential hosts for BlCMV or CAMV: TVU 109P2, TVU 347, TVU 408P2, TVU 410, TVU 1000, TVU 1016-1, TVU 2740, UCR 7964, UCR 8517, Worthmore, Snapper, Knuckle Purple Hull, and Texas Pinkeye. Sources of genetic immunity to each viral pathotype also were identified. Seven genotypes were immune to all identified BlCMV pathotypes: TVU 2657, TVU 3433, Purple Hull Pinkeye BVR, White Acre BVR, Serido, Texas Cream No. 8, and Big Boy. Two genotypes, TVU 401 and TVU 1582, were immune to all identified CAMV pathotypes. Three other genotypes, Purple Hull Pinkeye, White Acre BVR, and Knuckle Purple Hull, were immune to all CAMV pathotypes except CAMV-Mor.

A393

BASAL LAMINA AS A SELECTIVE BARRIER ASSOCIATED WITH VECTOR-SPECIFIC TRANSMISSION OF LUTEOVIRUSES BY APHIDS. F. E. Gildow, and S. M. Gray. Dept. of Plant Pathology, Penn State University, University Park, PA, 16802, and USDA/ARS, Dept. Plant Pathology, Cornell University, Ithaca, NY 14853.

Virus-free *Sitobion avenae* and *Rhopalosiphum padi* were allowed to feed 2 wk on oats infected with the MAV or RPV isolates of barley yellow dwarf luteoviruses. When the accessory salivary glands (ASG) were examined by transmission electron microscopy, virions of both transmissible MAV and nontransmissible RPV were observed in high concentrations embedded in the basal lamina surrounding the ASG of *S. avenae*. In *R. padi*, virions of transmissible RPV, but not of nontransmissible MAV, were observed in the ASG basal lamina (BL). Infectious MAV was detected in hemolymph from MAV-fed *R. padi* and MAV was observed in the hindgut by EM, indicating no gut barrier to MAV acquisition. Purified MAV at 75 or 100 ug/ml was microinjected into the hemocoel of aphids which were fed 24 - 48 hr on oats and examined by EM. In 2 experiments, MAV was observed in the ASG basal lamina of 10 of 10 *S. avenae* and in 0 of 10 *R. padi* examined. In a third experiment, MAV was observed concentrated at 2 - 8 virions/ um BL and in the ASG in 6 of 7 *S. avenae*, but not in any of 7 *R. padi*, *R. maidis*, *Schizaphis graminum*, or *Metopolophium dirhodum* examined. Results support the hypothesis that the BL surrounding the ASG may act as a selective barrier determining access of some luteoviruses to the ASG cell membrane.

A394

METHODS FOR THE DETECTION OF THE WALNUT AND THE BIRCH STRAIN OF CHERRY LEAFROLL VIRUS. M. J. Borja[1], Flora Sanchez[2], and F. Ponz[2]. [1]University of California, Berkeley, California 94720 and [2]CIT-INIA, Madrid, Spain.

Three methods were considered for the detection of cherry leafroll virus: ELISA, dot-blot and and reverse transcriptional - polymerase chain reaction (RT-PCR). Dot-blot and RT-PCR were carried out in crude plant extracts without any further RNA purification. Dot-blot hybridization using a ^{32}p-labelled DNA probe was as sensitive as previously reported ELISA results for cherry leafroll virus detection. The most sensitive method was RT-PCR, which amplified a specific fragment of 448 bp from the 3' untranslated region of both viral genomic RNAs. RT-PCR was used to detect cherry leafroll virus in infected walnut buds and twigs. The birch strain of CLRV was also detected by RT-PCR.

A395

PROPERTIES OF A CRYPTIC VIRUS FROM PEPPER (Capsicum annuum) R. A. Arancibia, and R. A. Valverde. Dept. of Plant Pathology and Crop Physiology, Louisiana Agricultural Experiment Station, Louisiana State University Agricultural Center, Baton Rouge, 70803.

A cryptic virus was partially purified from apparently healthy Capsicum annuum cv. Jalapeno M. The virus was designated pepper cryptic virus-1 (PCV-1). Spherical particles of about 30 nm in diameter were obtained from partially purified virus preparations. Virions consisted of one protein coat of about 45 kDa and two dsRNA components of approximately 1.0 and 0.85 x 10^6 daltons. PCV-1 was not serologically related to three other previously described cryptic viruses (beet cryptic virus 1, carnation cryptic virus, and white clover cryptic virus 1). The virus does not appear to induce detectable symptoms on Jalapeno M pepper. PCV-1 was transmitted 100% maternally and about 30% paternally. However, it could not be transmitted to a virus-free Jalapeno M line by grafting or mechanical inoculations.

A396

EFFECT OF TEMPERATURE ON A GLOXINIA ISOLATE OF TOMATO SPOTTED WILT VIRUS. R. H. Lawson, M. M. Dienelt and H. T. Hsu, USDA-ARS, Florist and Nursery Crops Laboratory, Beltsville, MD.

A gloxinia isolate of an impatiens strain of tomato spotted wilt virus (TSWV-Igg) typically forms chain-like aggregated and unaggregated nucleocapsid protein and amorphous and paracrystalline inclusions in infected cells. Enveloped virions are rare. We have observed a difference in cytopathology and serological reactivity of TSWV-Igg infected Nicotiana benthamiana plants grown at two different temperatures. Infected plants grown at 21/18 C, light/dark, showed the cytological features previously reported. These cytopathological features remained constant after more than 10 passages at this temperature. In contrast, inoculated plants transferred to 27/24 C light/dark showed an increased number of encapsulated virions, an absence of filamentous inclusions and a change in the appearance of the inclusion protein after the third passage. The change remained stable at this temperature after 10 passages. TSWV-I antiserum reacted strongly in ELISA tests with extracts from plants grown at 21/18 C but not from plants grown at 27/24 C after several passages. This change in ELISA reactivity is correlated with a disappearance of aggregated nucleocapsid protein and the appearance of enveloped virions.

A397

IDENTIFICATION, DISTRIBUTION, AND SEED-TRANSMISSION OF COWPEA VIRUSES IN SENEGAL. M. Ndiaye, M. Bashir, K. Keller, and R. Hampton, USDA ARS, Department of Botany and Plant Pathology, Oregon State University, Corvallis, OR 97331-2902.

Viral diseases of cowpea were surveyed in Senegal during the rainy season of 1990 and 1991. Sixty plants with virus-like symptoms were sampled among five cowpea-producing regions and tested against antisera to seven seed-borne viruses by DAC-ELISA. The following viruses were detected: cowpea aphid-borne mosaic potyvirus (CAMV) alone in 35/60 samples; cowpea severe mosaic comovirus (CSMV) with cowpea mottle carmovirus (CMtV) in 1/60; and southern bean mosaic sobemovirus (SBMV) with CAMV in 1/60. Two pathotypes of a potyvirus unrelated to either CAMV or blackeye cowpea mosaic viruses were detected alone or together in 21/60 samples, using DAC-ELISA and a potyvirus-selective monoclonal antibody (II-197, Wang & Mink). These potyvirus pathotypes were accompanied by CSMV in another 4/60 samples, and occurred in each sampled region of Senegal. The virus attacked principally new Senegalese CAMV-resistant cowpea genotypes. Several cowpea genotypes resistant to both viral pathotypes were identified. This is a second and verifying report of CSMV in Senegal cowpeas (Hampton, et al, Seed Sci & Technol, 1992).

A398

SEED-TRANSMISSION INTERACTIONS OF PEA SEEDBORNE MOSAIC POTYVIRUS PATHOTYPES P1 AND P4. P. D. Kohnen and R. O. Hampton, Department of Botany and Plant Pathology, Oregon State University, Corvallis, OR 97331-2092.

Pisum resistance to pea seedborne mosaic potyvirus (PSbMV) pathotypes P1 and P4 is conferred by separate genes, sbm-1 and sbm-4, respectively. P1 and P4 seed transmission rates (STR) differ significantly in cultivars susceptible to both pathotypes, i.e., genotype Sbm-1/-,Sbm-4/-. Pathotype P1 STRs in eight selected pea cvs varied from 5 to 33%; those of P4 ranged from 0 to 0.7% in the same cvs. These cultivars were accordingly inoculated with individual or combined pathotypes, to investigate their interactions in seed transmission. Resulting seedlings were assayed for P1 and P4 seed-transmissibility, by means of ELISA and pathotype-specific PCR. P4 was seed-transmitted in the presence of P1 at rates of 0 to 2%, as determined by pathotype-specific DAC-ELISA. Limited PCR data (i.e., 3/10) suggest that P4 is seed-transmissible with P1 at even higher rates than indicated by enhanced-ELISA determinations. The survival and dissemination potentials of P4 (now known to occur in only a few exotic Pisum sativum landraces) could therefore be increased in natural mixed infections with P1.

A399

A SMALL RNA ASSOCIATED WITH THE LACK OF SEED TRANSMISSION OF TOBACCO STREAK ILARVIRUS ISOLATE MEL F IN BEAN. Walter, M. H., Wyatt, S. D. and Kaiser, W. J., Department of Plant Pathology, Washington State University, Pullman WA 99164-6430.

A lack of seed transmission of tobacco streak virus (TSV) Mel F (seed transmitted in *Phaseolus vulgaris* L., var. Black Turtle Soup I: BTS, at less than 0.5%) was linked to a small RNA (approx. 300 bases), called "RNA F5". RNA F5 was detectable as encapsidated RNA from Mel F infections, but not from sub-isolate Mel FS (F-Seed) infections. Mel FS originated as a rare seed transmitted sub-isolate of Mel F in BTS and was subsequently transmitted to approximately 30% of seedlings. Except for seed transmission rate and RNA F5, isolates Mel F and Mel FS appeared equal in terms of RNA content, symptomology, serology and sucrose density gradient profiles. RNA F5 was only detected as encapsidated RNA from Mel FS infections on *Chenopodium quinoa* Willd. that had been established by co-inoculation with RNA F5 and Mel FS RNA. Similar infections on BTS were associated with a decrease in seed transmission of Mel FS from approximately 30% to 0%. RNA F5 appeared similar in size to some plant virus satellite RNAs, displayed little sequence identity to Mel FS RNA by cDNA/RNA hybridizations and appeared to delay and attenuate systemic symptoms in co-inoculations with TSV Mel F genomic RNAs.

A400

APHID TRANSMISSION OF MIXED AND REASSORTED CUCUMOVIRUS GENOMIC RNAs. K. L. Perry. Cornell University, Plant Science Center, 147 Biotechnology Building, Ithaca, NY 14853-2703 U.S.A.

Virus transmissions with the aphid Myzus persicae were performed using plants co-infected with 2 cucumoviruses, namely, an aphid-transmissible strain of tomato aspermy virus (V-TAV) and a very poorly aphid-transmissible strain of cucumber mosaic virus (M-CMV). Five of the aphid-transmitted progeny virus (3.7%) induced symptoms distinct from both parental viruses. Northern hybridization analysis of RNAs from these novel progeny demonstrated all of the RNA profiles to be characteristic of pseudorecombinants, i.e., viruses with reassorted genomic RNAs. The two larger RNAs 1 and 2 originated from V-TAV, while RNA 3 was derived from M-CMV. A more sensitive RNase protection assay analysis revealed the presence of a minor population of V-TAV derived RNA3. A bias against the encapsidation of minor populations of RNAs by the M-CMV coat protein was observed, suggesting specificity or competition with regard to the encapsidation of cucumoviral RNAs in vivo. This study demonstrates that insect vectors can mediate the establishment of pseudorecombinants with mixed populations of RNA 3s.

A401

ADDITIONAL SUBGENOMIC RNA OF PRUNUS NECROTIC RINGSPOT ILARVIRUS (PNRSV).
B. Di Terlizzi, L. J. Skrzeczkowski and G. I. Mink. Istituto Agronomico Mediterraneo, Bari, Italy; Institute of Biochemistry and Biophysics PAN, Warsaw, Poland; and Washington State University IAREC, Prosser, WA 99350.

Four RNAs are usually extracted from capsids of Prunus necrotic ringspot virus (PNRV). We found an additional small (ca. 180-210 nucleotides) subgenomic RNA (RNA-5) present in extracts from several strains of the virus. Unlike the subgenomic RNA-4, RNA-5 does not activate infectivity of mixtures that contain the three genomic RNAs (RNA-1 + RNA-2 + RNA-3). RNA-5 is always produced following infection of susceptible hosts by mixtures containing RNA-1 + RNA-2 + RNA-3 + RNA-4. Results from hybridization studies suggest that RNA-5 has partial sequence homology with the other four RNAs. RNA-5 is preferentially encapsidated in the slowest electrophoretic capsid component which contains primarily RNA-1.

A402

ANALYSIS OF TRANSGENIC TOBACCO LINES EXPRESSING DIFFERENT FORMS OF THE COAT PROTEIN GENE OF TOBACCO ETCH VIRUS IN THEIR RESISTANCE TO VIRUS INFECTION.
L. Silva-Rosales [1,2], J.A. Lindbo [1], and W.G. Dougherty [1]. [1] Department of Microbiology. Oregon State University. OR 97331 USA. [2]CINVESTAV -IPN Irapuato, Mexico.

Transgenic tobacco (*N. tabacum* cv. Burley 49) lines were obtained which express either full length, amino-, carboxy, or amino- and carboxy-terminally truncated versions of the tobacco etch virus (TEV) coat protein (CP). One of the carboxy terminal truncations represents the elimination of the C-terminal 118 amino acids (ΔC118). Analysis of lines containing this transgene revealed TEV-specific RNA could be readily detected; however, truncated CP could not. Plants derived from these lines displayed variable symptoms. All transgenic plants were analyzed for alteration in symptoms after challenge with TEV, PVY, and TVMV delivered via mechanical inoculation or aphid vectors. Resistance was evident for TEV isolates; however, broad spectrum resistance to other potyviruses , reported in other systems, was not observed.

A403

NATURAL OCCURRENCE AND ECOLOGICAL RELATIONSHIPS OF WHITE LUPIN MOSAIC POTYVIRUS. K. E. Keller and R. O. Hampton, USDA ARS, Department of Botany and Plant Pathology, Oregon State University, Corvallis, OR 97331-2902.

White lupin mosaic potyvirus (WLMV) was isolated from white lupin (Lupinus albus) experimental plantings exhibiting typical bean yellow mosaic (BYMV)-like symptoms. These symptoms were reproducible in L. albus also by inoculations with type isolates of BYMV, pea mosaic virus (PMV), and clover yellow vein virus (CYVV). In host range tests, WLMV behaved like PMV (non-infectious) in most Phaseolus bean cvs and like CYVV (lethal infection) in Pisum, Lens, and Cicer. The name WLMV was assigned after viral distinctions were established by serological and HPLC CP peptide profile analyses (Hampton, et al, 1992). Serological homologues of WLMV were detected in Trifolium hybridum (Montana, Oregon, California), in T. pratense (Oregon), and in Phaseolus bean planted near T. hybridum (Oregon). However, antigen-homologous isolates from these sources did not match WLMV pathology. WLMV was not transmitted through 360 T. hybridum seeds or 338 fababean (Vicia faba) seeds from WLMV-infected mother plants, but was transmitted through one of 86 seeds from infected L. albus cv Astra. L. albus plants were killed by WLMV unless > 20 days old when inoculated.

A404

BIOTYPIC CHARACTERIZATION OF BEMISIA TABACI POPULATIONS BASED ON ESTERASE PROFILES, DNA FINGERPRINTING, VIRUS TRANSMISSION, AND BIOASSAY TO KEY HOST PLANT SPECIES. J.K. Brown, S. Coats, Dept. of Plant Sciences, Univ. of AZ, Tucson, AZ 85721; I. D. Bedford, P.G. Markham, Dept. Of Virus Res., John Innes Inst. Norwich, Norfolk NR4 7UH, UK and J. Bird, College of Agric. Sciences, Univ. of Puerto Rico, Rio Piedras, PR 00928.

Twenty populations of Bemisia tabaci from North and Central America, The Caribbean Basin, West Africa, North Africa, the Middle East, and Asia have been characterized with respect to non-specific esterase profiles, DNA fingerprints, virus transmission, and ability to induce phytotoxic disorders in two indicator plants. Over ten differential profiles were ascertained by esterase analysis, and correspondingly distinct, but characteristic DNA patterns were resolved by PCR amplification of adult whitefly DNA. In virus transmission tests, all populations except those from Benin and Puerto Rico vectored ten candidate geminiviruses tested. Only test populations from Arizona, California, and Florida (USA), those from poinsettia in Puerto Rico, and field populations from Antigua (E. Carib) and Yemen had identical esterase and DNA profiles, vectored all candidate viruses with high efficiency, and induced phytotoxic disorders in indicator hosts. These data suggest that these six populations are genetically homogeneous, and further support the contention that a biologically and genetically identical biotype is now distributed in several world regions.

A405

MODULAR DEMOGRAPHY OF LOTUS CORNICULATUS INFECTED BY RHIZOCTONIA SPP. J.T. English, Dept. of Plant Pathology, Univ. of Missouri, Columbia, 65211.

Rhizoctonia spp. cause foliar and tip blight of L. corniculatus (Birdsfoot trefoil) in temperate regions of the U.S. High rates of leaf mortality may reduce production of seed sufficiently to affect stand persistence. Evaluations of the influence of infection on survival of host leaf modules in central Missouri were made in 1991. Evaluations of the variety "Norcen" showed that all shoots sampled in June or later were affected by blight. Approximately 70% of vegetative nodes on stems and primary lateral shoots supported leaves in early June; the proportion of such nodes declined steadily until fewer than 10% of them supported leaves in early September. Patterns of leaf formation and mortality in variety "Norcen" are being compared to those of other commercial varieties with different canopy architectures.

A406

VIRULENCE OF ISOLATES OF *FUSARIUM* SPP. CAUSING ENDOSEPSIS IN BOTH CULTIVATED AND WILD CAPRIFIGS IN CALIFORNIA. K.V. Subbarao, and T.J. Michailides. University of California, Berkeley/Kearney Agricultural Center, Parlier, CA 93648.

A total of 62 isolates of *Fusarium* collected from both cultivated and wild caprifigs in most fig production areas of California included one isolate of *F. episphaeria*, seven of *F. solani*, and 54 of *F. moniliforme*. These isolates were compared for growth rate, sporulation, temperature optima, and virulence. Virulence was tested by placing 5 µl of a 10^6 conidia/ml suspension of each isolate on a wound made on the surface of caprifigs and incubating at 25 C. Lesion sizes were recorded after 5 days incubation. Growth rates of isolates within species differed significantly and sporulation correlated highly with growth rate. The majority of the isolates had a temperature optimum of 25 C. *F. episphaeria* was moderately virulent; *F. solani* isolates were either virulent or highly virulent; and *F. moniliforme* isolates showed greater variation for virulence. About 11% of the isolates were avirulent, 67% were either weakly or moderately virulent, and 22% were virulent to highly virulent. *F. moniliforme* isolates from wild caprifigs were significantly more virulent than those from cultivated caprifigs but no such differentiation occurred with *F. solani*. Infusion of *F. moniliforme* from wild caprifigs may cause significant long-term problems for the fig industry.

A407

QUANTIFICATION OF DIFFERENCES IN INFECTION EFFICIENCY AMONG *COCHLIOBOLUS HETEROSTROPHUS* GENOTYPES AS A MEANS TO MAP QUANTITATIVE TRAIT LOCI. F.W. Nutter Jr., C.R. Bronson, S.S.A. Rizvi and P.M. Schultz. Department of Plant Pathology, Iowa State University, Ames, IA 50011.

Quantitative trait loci can be identified and mapped by observing the amount of variation in a trait that is associated with each region of the genome. Infection efficiency, a quantitative trait contributing to the aggressiveness of *Cochliobolus heterostrophus*, was estimated by inoculating 2-3 wk old maize seedlings and incubating these at 24°C for wetness periods ranging from 3 to 24 hr. Infection efficiency, defined as the regression coefficient (slope) relating hours of leaf wetness (X) to lesion number/leaf (Y), was significantly higher for isolate HM540 (slope=0.95) compared to laboratory strain

B30.A3.R45 (slope=0.45). Progeny of these genotypes were used in a previous study to prepare an RFLP map (Bronson) and are currently being tested to determine the amount of variation in infection efficiency. The existence of these progeny, and the high variability between parents and progeny, makes the detection of loci for aggressiveness likely.

A408

Relationship between the amount of *Phytophthora* in soil and yield loss from Phytophthora rot of soybean in Ohio. A. F. Schmitthenner, Dept of Plant Pathology, Ohio State Univ., Wooster, OH 44691.

The amount of *Phytophthora* antigen in soil cropped to soybean was evaluated with a *Phytophthora*-specific ELISA kit from Agri-Diagnostics Assoc. in 20 fields in 1989, 63 fields in 1990 and 22 fields in 1991. Significant amounts of > 9 *Phytophthora* units/g soil (equivalent to > 9 culture-produced oospores) were found in 81% of the fields. Yield loss was measured by 1) comparing metalaxyl soil treatment with no treatment in 1989 and 1990; (2) comparing cv. Resnik (resistant) with closely related cv. Asgrow 3127 (susceptible) in 1990 and 1991. Yield loss in fields with *Phytophthora* damage varied from 25% in 1989 (5 fields); to 29% in 1990 (16 fields); to 27% in 1991 (7 fields). Regression of *Phytophthora* units on yield loss was not significant. It was concluded that amount of *Phytophthora* in soil was not useful in predicting the magnitude of yield loss from root rot.

A409

PATTERNS IN AIRBORNE ASCOSPORE COUNTS OF MYCOSPHAERELLA FIJIENSIS, CAUSE OF BLACK SIGATOKA ON BANANA. Wayne M. Thal[1], Hans P. Sauter[2], Harvey W. Spurr, Jr.[1] and Teresa Arroyo[3]. [1]USDA-ARS, Oxford, NC 27605 and NCSU, Raleigh, NC 27695; [2]POB 4084, [3]POB 1173 [2,3]San Jose, Costa Rica.

Hourly ascospore counts were collected from June 1984 to August 1991 in a Hirst spore trap near a commercial banana plantation in Carmen, Costa Rica. Diurnal patterns in mean spore concentration were compared for subsets of data under different rain and wind conditions. Daytime ascospore counts were higher than nighttime counts ($P<0.05$). Spore counts generally decreased as rain in the past 24 h increased. Rains of 0.1 to 0.2 mm were generally sufficient to increase spore counts. Rains of at least 1.0 mm were associated with higher spore counts than lighter rains when they followed a dry period of at least 24 h. Weekly spore counts did not differ ($P>0.05$) when grouped based on different rain conditions in preceding weeks.

A410

EFFECTS OF TEMPERATURE, HUMIDITY, AND PRECONDITIONING ON ASCOSPORE DISCHARGE IN *VENTURIA INAEQUALIS*. D.M. Gadoury, A. Stensvand, and R.C. Seem. Department of Plant Pathology, Cornell University, New York State Agricultural Experiment Station, Geneva, New York 14456.

Suppression of ascospore release during nocturnal rain has been described in a number of field studies on *V. inaequalis*, but this phenomenon has not consistently been reproduced under controlled conditions. We constructed a wind tunnel in which light, simulated rain (SR), relative humidity (RH), and temperature during ascospore release was precisely controlled. In 3 hr of SR in darkness followed by 3 hr of SR in light, with RH ≥80% at 20C, *ca* 90% of the ascospores were released in light. However, when RH was <70% during SR, up to 90% of the ascospores were released during darkness. Preconditioning via incubation of moist leaf samples at 20C for 1, 3, 5, or 7 days resulted in 0, 18, 24, or 80%, respectively, of the ascospores being released in darkness during SR and 90% RH. The experimental conditions that caused large nocturnal releases of ascospores were not observed in 6 yr of weather data for April-June. RH during natural rain events was >70%, and periods of leaf wetness >24 hr were coincident with rain events that caused ascospore release. The rate of ascospore release at 2C was <10% of the rate at 20C. The pattern of ascospore release examined at 2 min intervals during 6 hr of SR at 2-20C consisted of a series of peaks lasting 6-16 min, separated by 30-60 min. Maxima of spore releases were often >100 X the level seen in the 6 min preceeding the peak.

A411

EFFECT OF INOCULUM CONCENTRATION ON BROWN ROT DEVELOPMENT IN CHERRIES A. R. Biggs, West Virginia University, University Experiment Farm, Kearneysville, WV, 25430, and J. Northover, Agric. Canada, Research Station, Vineland Station, Ont., Canada, L0R 2E0.

Mature sweet and sour cherries (Prunus avium and P. cerasus) were harvested, arranged in trays, and inoculated without wounding with single drops of up to 10 different inoculum concentrations ranging from $1x10^3$ to $1x10^6$ conidia/ml. After 22 hr wetting, fruits were incubated at 20° C and evaluated daily for 8-9 days postinoculation (DPI). In sweet cherry, lesions (L) and sporodochia (S) first appeared 2 and 4 DPI at > $2x10^4$ conidia/ml and reached 80-100%, but with $1x10^3$ conidia/ml L and S first appeared 4 and 5 DPI and reached only 20-25% at 9 DPI. In sour cherries, L and S appeared 2 and 3 DPI from $2x10^5$ conidia/ml, and with $1x10^3$ conidia/ml, L and S appeared 4 and 5 DPI, respectively. Longer wetting periods of 36 and 48 hr increased infection by low inoculum concentrations, and similar numbers of conidia applied in 3 and 10 μl drops were more infectious than in 30 μl drops.

A412

THE EFFECT OF THE INTERACTION BETWEEN ALTERNARIA MALI AND PANONYCHUS ULMI ON SEVERITY OF ALTERNARIA BLOTCH OF APPLE. *N. Filajdić[1], T. B. Sutton[1], J. F. Walgenbach[2], and C. R. Unrath[3]. [1]Department of Plant Pathology, [2]Department of Entomology, and [3]Department of Horticulture. North Carolina State University, Raleigh 27695.*

The effect of the interaction between Alternaria mali Roberts and European red mite, Panonychus ulmi Koch on apple trees (Malus x domestica 'Oregon II') was studied during the summer of 1991 in western North Carolina. Disease incidence and severity, defoliation, yield, fruit drop, and fruit characteristics such as length, diameter, weight, firmness, soluble solids, and percent red color were examined at combinations of two disease levels and three mite infestations. At the low disease level, disease incidence, severity and defoliation were greater in the high mite infestation treatment. Defoliation was greater in the moderate mite infestation treatment. Yield, fruit length, diameter, weight, and soluble solids were significantly decreased in treatments with high disease and high mite infestation. At low disease levels, soluble solids content decreased and fruit drop increased in the high mite infestation treatment.

A413

PRESYMPTOMATIC DETECTION AND QUANTITATION OF *SEPTORIA NODORUM* AND *SEPTORIA TRITICI* WITH IMMUNODIAGNOSTICS.
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E. I. Du Pont De Nemours and Company, Agricultural Products, Stine-Haskell Research Center, Stine-PCC, P.O.Box 30, Newark, DE 19714

Microtiter plate enzyme-linked immunosorbent assays (ELISA), which detect the economically significant wheat disease Septoria, have been developed. Polyclonal antibodies specifically detect soluble, extracellular or surface antigens of the fungi *Septoria nodorum* and *Septoria tritici*, the causative agents of Septoria. Although both fungal species have been assigned to the same asexual taxonomic genus, *S.nodorum* has been found to be immunologically distinct from *S.tritici*. The lack of cross-reactivity between antibodies and antigens of the two species has necessitated the development of two separate assays to accurately diagnose Septoria. Data obtained from field studies demonstrated the usefulness of the immunodiagnostics to detect disease presymptomatically, and to effectively monitor and quantitate disease distribution and progression.

A414

DEVELOPMENT OF FIELD AND LABORATORY IMMUNO-DIAGNOSTIC KITS FOR DETECTION AND DIFFERENTIATION OF SEPTORIA NODORUM AND SEPTORIA TRITICI IN WHEAT. F. P. Petersen, J. H. Rittenburg, T. R. Joaquim, 1S. A. Miller, and G. D. Grothaus. Agri-Diagnostics Associates, 2611 Branch Pike, Cinnaminson, NJ 08077. 1The Ohio State University-OARDC, Wooster, OH 44691.

Hybridoma cell lines, Sen1 5C6 and Set3 7B5, were identified as secreting monoclonal antibodies (Mabs) that were, respectively, S. nodorum- and S. tritici-specific. Sensitivity and specificity against a panel of fungi comprising 14 isolates of S. tritici, 43 isolates of S. nodorum and 85 isolates of taxonomically related and nonrelated fungi were determined in an indirect- or DAS-ELISAs. In DAS-ELISA, Sen1 5C6 reacted strongly ($O.D._{405nm}$ > 1.5) with 41 of 43 isolates of S. nodorum and it only cross-reacted with S. avenae var. triticea. In an indirect ELISA, Set3 7B5 reacted strongly ($O.D._{405nm}$ = > 1.5) with 12 of 14 isolates of S. tritici but neither cross-reacted with isolates of S. nodorum nor with 85 other nontarget isolates. Each Mab has been formatted as the capture antibody in a 96-well laboratory immunoassay and a 10-minute, Rapid On-site field assay. Peroxidase conjugated-polyclonal antibodies raised against S. nodorum and S. tritici were used as the second antibody. Both formats were evaluated in the USA and Europe under lab and field conditions and are seen as valuable tools for accurate diagnosis of Septoria leaf spot of wheat.

A415

CHARACTERISTICS OF ANTISERA AGAINST ENDOPHYTES FROM RYEGRASS, FESCUE AND HORDEUM. R.E. Klein, W.J. Kaiser, and S. Clement. Dept. of Plant Pathology and USDA-Regional Plant Introduction Station, Washington State University, Pullman, 99164.

Antisera against mycelial preparations (M) of endophytes isolated from ryegrass (R), fescue (F) and Hordeum (H) and against culture supernatant fractions (S) of the R and H endophytes were tested in indirect ELISA and dot immunoassay against homologous and heterologous antigens. In both assays, antisera against FM, HM. and HS reacted with FM, FS, HM, and HS but not with RM or RS. RM antiserum reacted with RM, RS and HM but not with FM, FS, or HS. Similarly, RS antiserum reacted with RM, RS, and HS but not with FM, FS, or HM. Indirect ELISA with plant samples was unsuccessful but DAS-ELISA with HS antiserum detected endophytes in plants of F and H but not in R. Other researchers have reported no serological difference among endophyte isolates from R and F.

A416

APPLICATION OF THE POLYMERASE CHAIN REACTION (PCR) FOR THE DETECTION OF *XANTHOMONAS CAMPESTRIS PV. CITRI.* J.S. Hartung [1] J. F. Daniel[2], and O.P. Pruvost[3]. USDA-ARS-Plant Sciences Institute, Beltsville, MD[1]; ORSTOM, Montpelier, FRANCE[2]; CIRAD/IRFA, La Reunion, FRANCE[3].

Plasmid pFL62.42 contains a 4.2 Kb *Bam* HI fragment cloned from plasmid DNA of strain XC62 of *Xanthomonas campestris pv. citri* (*Xcc*) and was shown to be a highly sensitive and specific hybridization probe for *Xcc*. Plasmid pFL1 contains a 711 base pair *Eco* RI fragment from pFL62.42 and was shown to give slightly more specific results. Both plasmids detected 44/44 pathotype A strains of *Xcc* originally isolated in 15 countries. Nearly all strains of pathotypes B and C were also detected with these probes. Neither probe reacted with strains of *X. campestris* isolated from symptomless *Citrus* nor with 56 strains of *X. campestris* associated with citrus bacterial spot disease (CBS) in Florida citrus nurseries nor with bacteria from other genera. The sensitivity in dot blot assays using chemiluminescent detection was approximately 2-7 ng DNA/spot. Nucleotide sequence information is being used to design primers in order to apply the polymerase chain reaction for the detection of *X. c. citri.*

A417

DETECTION OF CUCURBIT VIRUSES BY ENZYME-LINKED IMMUNOSORBENT ASSAY ON NITROCELLULOSE MEMBRANE (NCM-ELISA). D. C. Custer, J. J. Cho, D. E. Ullman, G. C. Wall, L. S. Yudin. University of Hawaii, Honolulu 96822 and University of Guam, Mangilao, 96923.

Zucchini yellow mosaic virus (ZYMV), cucumber mosaic virus (CMV), papaya ringspot virus (PRSV), watermelon virus 2 (WMV-2) and squash mosaic virus (SqMV) were detected in sap extracts from plant tissue by a direct double antibody sandwich on nitrocellulose membranes (NCM-ELISA) with polyclonal antibodies. NCM-ELISA sensitivity was somewhat less than DAS-ELISA. NCM-ELISA kits, prepared in Hawaii, successfully detected cucurbit viruses from samples blotted in Guam and returned to Hawaii for processing. Samples from Guam were predominantly infected with PRSV. All five virus controls were detected after shipping and on NCM stored locally at room temperature for 8 weeks prior to processing. Stability of NCM-ELISA at room temperature and during shipping suggest this technology will provide a simple tool for diagnosing cucurbit viruses from remote locations, particularly when quarantine limitations or long shipping times prohibit shipment of plant samples for DAS-ELISA testing.

A418

THE INTRODUCTION OF THE EURASIAN POPLAR LEAF RUST FUNGUS, MELAMPSORA LARICI-POPULINA, INTO NORTH AMERICA. G. Newcombeand G.A Chastagner, Washington State University, Puyallup Research and Extension Center, Puyallup, WA 98371.

Melampsora larici-populina, native to Eurasia, was found in late autumn 1991 in hybrid poplar plantations along the lower Columbia River near Woodland, WA and Scappoose, OR. Both the uredinial and telial stages fit the literature description of M. larici-populina. Urediniospore germination, after incubation at 5,10,15,20 and 25C, was optimal at 10C, approximating what has been observed in Europe for M. larici-populina. When challenged with 3 different monouredinial isolates (i.e., 1 from Woodland and 2 from Scappoose), 7 native Populus trichocarpa clones, eight P. trichocarpa x deltoides hybrid clones, P. nigra cv. italica and P. x euramericana cv. I-488 were susceptible, whereas three other P. trichocarpa x deltoides hybrid clones, P. alba and three P. deltoides clones were resistant. Thus, host range is also as expected for M. larici-populina.

A419

EFFECTS OF INFECTION AGE/PLANT CONDITION AND STORAGE ON DETECTION OF *PHYTOPHTHORA PARASITICA* IN INOCULATED AZALEA ROOTED CUTTINGS. J. M. Mullen, A. K. Hagan, N. K. Burelle, and B. J. Jacobsen. Dept. of Plant Pathology, Auburn University, AL 36849.

Healthy azalea rooted cuttings inoculated with a zoospore suspension (450 zoospores/plant) of *Phytophthora parasitica* were tested for Phytophthora infection using ELISA (Agri-Diagnostics Associates) and PARPH selective culture medium 1,2,4, and 5 weeks after inoculation. Moderately and severely diseased plants (2 and 5 wks, respectively) were subjected to 6 different storage conditions and then checked for Phytophthora. Both detection techniques confirmed the presence of Phytophthora in moderately and severely diseased plants tested immediately upon removal from the greenhouse, after 1 wk storage at 23°C in plastic bags, and after 2 wk in plastic at 5°C. After 1 wk at 29°C in plastic, ELISA results were negative and culture work gave variable results. After 2 wks in plastic at -17°C and after 1 wk in a dried condition at 23°C, ELISA gave positive results whereas culture results were variable.

A420

EFFECTS OF STORAGE TEMPERATURE AND INFECTION AGE ON DETECTION OF TOMATO SPOTTED WILT VIRUS L-STRAIN IN TOMATO LEAVES. D. K. Carey, J. M. Mullen, and R. T. Gudauskas, Department of Plant Pathology, Auburn University, AL 36849.

Greenhouse-grown 'Marglobe' tomato plants were mechanically inoculated with TSWV-L 4 weeks after transplanting. After 5 to 9 weeks, leaves from symptomatic plants testing positive for TSWV-L were stored at various temperatures and tested periodically by ELISA (Agdia, Inc.). TSWV-L was detected through 21 days storage at -17 or 5 C and through 6-7 days at 23, 27, or 32 C. ELISA values declined significantly after 5 days at 38 C. Infectivity was lost by 1 day at 38 C and after 1 day at 27 or 32 C based on local lesion assays on petunia. ELISA values for symptomatic plants in the greenhouse fluctuated but were positive for TSWV-L throughout the testing period.

A421

PATHOGENICITY CHARACTERIZATION AND SEASONAL PATTERNS OF ASCOSPORE RELEASE OF *LEPTOSPHAERIA MACULANS* ASSOCIATED WITH BLACKLEG OF CANOLA IN KENTUCKY. D. E. Hershman, D. M. Perkins and P. R. Bachi. Department of Plant Pathology, University of Kentucky, P.O. Box 469, Princeton, KY 42445.

During 1989-1991, 38 isolates of *Leptosphaeria maculans* were collected from canola (*Brassica napus* var. *oleifera*) diseased by blackleg. The isolates were evaluated for aggressiveness on cotyledons of *B. napus* differential cultivars Westar, Quinta and Glacier. 79.0% and 18.4 % of the isolates belonged to the highly aggressive groups PG-4 and PG-3, respectively. None belonged to PG-2 and 2.6% belonged to the weakly virulent PG-1. Seasonal periods of ascospore release by *L. maculans* (PG-4), harbored in canola stubble, were determined using a rotating drum spore sampler. Ascospore release begins in June about the time of harvest of the diseased canola crop. Release in July and August is variable, being either high or low. Release increases in September and continues at high levels through March or April, depending upon the year. Ascospore release from second-year stubble is greatly reduced compared with first-year stubble. The period of spore release from second-year stubble is more restricted, with significant release occurring December through February. Spore release from third-year stubble is extremely low, but a few spores continue to be released even after 34 months.

A422

FUNGI ASSOCIATED WITH ROOT ROT OF WHEAT IN THE NILE VALLEY, DELTA REGION AND NEW LAND AREAS OF EGYPT. H.T. Wilkinson and W.L. Pedersen, H.M. Fouly, and M.M. Abdel-Kader, University of Illinois, Urbana, IL, 61801 and Cairo University, Egypt.

The development of new agricultural land, that is separate from the Nile valley and delta areas in Egypt, involves the transportation of water and organic materials to virgin sand soils. Difficulties associated with timing of planting, flood irrigation and fertilization have produced an increased occurrence of soil-borne diseases of winter wheat. More than 30 fields with various soil types, cropping histories, and management programs were sampled by extracting seedlings with soil attached to roots. Plants roots were washed in tap water, examined for lesions, and surface sterilized with NaOCl and plated on acidified PDA. Currently, eight genera of fungi have been isolated. Fusarium, Rhizoctonia, and Gaeumannomyces spp. have been associated with the most severe root rots. Differences in susceptibility have been observed on greenhouse inoculated Egyptian wheat cultivars.

A423

EARLY LEAFSPOT OF PEANUT: EFFECT OF SODIC WATER ON DISEASE DEVELOPMENT. D. M. Porter and F. J. Adamsen. USDA-ARS, Tidewater Agricultural Experiment Station, Suffolk, VA 23437, and U.S. Water Conservation Laboratory, Phoenix, AZ 85040.

Water type (non-sodic; sodium absorption ratio of 3.0 and sodic; sodium absorption ratio of 103) and irrigation method (sprinkle and trickle) influenced the severity and incidence of early leafspot (Cercospora arachidicola Hori) in field plots of peanuts (Arachis hypogaea). Leaflet infection, the number of leaflets with leafspot lesions and defoliation percentages were usually greater in plots receiving sodic irrigation water. Disease development was greater in sprinkler irrigated plots than in trickle irrigated plots. Sodium levels were highest in plant tissues (leaf, stem and seed) of sprinkler irrigated plants using sodic water than in sprinkler irrigated plants using non-sodic water. Sodium levels in plant tissues were always higher in sprinkler irrigated plots than in trickle irrigated plots.

A424

EFFECT OF SEEDING RATE AND ROW SPACING ON RHIZOCTONIA LIMB ROT AND YIELD IN FLORUNNER AND AGRATECH 127 PEANUT. T. B. Brenneman, Dept. of Plant Pathology, University of Georgia, Tifton, Georgia 31793.

Florunner and AgraTech 127 peanut were planted at 67,100 and 134 kg seed/ha in irrigated fields in 1990 and 1991. The test was duplicated in adjacent fields with either a 91.4 cm row spacing or an alternating 76.2 x 106.7 cm spacing. All plots were treated with PCNB (5.6 kg/ha) and traveled by tractor every 2-wk to apply chlorothalonil (1.3 kg/ha). Paired plots were treated or nontreated with flutolanil (3.4 kg/ha). Higher seeding rates produced taller plants but did not affect white mold (WM), limb rot (LR) or yield. Florunner yielded more and had less LR than AgraTech 127. Flutolanil reduced WM and LR but had little effect on yield. LR was more severe in the outer (traveled) versus the inner side of the bed, but correlations with yield and crop value were lower. Results in adjacent tests with different row spacings were similar.

A425

EFFECTS OF SOUTHERN STEM ROT AND RHIZOCTONIA LIMB ROT ON PEANUT YIELD. J. C. Jacobi and P. A. Backman, Department of Plant Pathology, Auburn University, AL 36849-5409.

The relationships between yield reductions and incidence of southern stem rot (*Sclerotium rolfsii*) and Rhizoctonia limb rot (*Rhizoctonia solani* AG-4) were examined under field conditions during 1990 and 1991, on the susceptible peanut cultivar Florunner. Crop rotation and fungicide treatments were arranged in a split-plot randomized complete block design to provide a range of disease levels. Whole plot treatments consisted of various duration crop rotations involving corn (*Zea mays*) and bahiagrass (*Paspalum notatum*) with peanut. Subplots were either treated with Tebuconazole, for control of southern stem rot and Rhizoctonia limb rot, or left untreated. At harvest, southern stem rot incidence ranged from 2 to 48 disease loci per 30 m of row; Rhizoctonia limb rot incidence ranged from 0 to 18 lesions per 5 lateral limbs. A multiple regression equation using incidence of southern stem rot and Rhizoctonia limb rot as independent variables explained 75% of the variation in yield when data from both years were pooled. Predicted yield losses were -1.05% per southern stem rot locus and -2.03% per Rhizoctonia limb rot lesion. Future work will include model validation with independent field data.

A426

FUNGAL INTERACTIONS IN SEED ROT AND DAMPING-OFF OF SHRUNKEN-2 (sh_2) SWEET CORN. R.E. Baird[1], J.K. Pataky[2], and D.M. Huber[1]; [1]Botany & Plant Pathology Dept., Purdue Univ., SWPAC, Vincennes, IN 47592 and [2]Plant Pathology Dept., Univ. of IL, Urbana-Champaign, IL 61801

Seed rot and damping-off of sh_2 sweet corn hybrids were evaluated in laboratory, greenhouse, and field tests. *Penicillium oxalicum* was the most frequently isolated fungus from the inner seed coat, endosperm, scutellum, and embryo of all the three vigor groups of sh_2 sweet corn. *Aspergillus niger, Fusarium oxysporum, F. moniliforme, Rhizopus arrihzus, R. niger* and *R. oryzae* were also isolated. An unidentified bacterium commonly isolated from all seed tissues was inhibitory to *P. oxalicum* and several of the other fungi in culture. Isolates of *R. arrihzus, F. oxysporum* and *A. niger* added to soil enhanced seed germination and growth of all three vigor groups in greenhouse pathogenicity trials. These fungi also enhanced seedling vigor in the presence of *F. moniliforme,* and fungal x hybrid differences were noted. Additional trials are in progress with these and various chemical and biological agents to reduce seed rot and damping-off.

A427

CORN YIELD LOSSES TO MODERATE SEVERITIES OF GRAY LEAF SPOT IN OHIO. P. E. Lipps and L. V. Madden, Ohio State Univ., Wooster, OH 44691.

Yield losses to gray leaf spot (GLS), caused by Cercospora zeae-maydis, were determined using three corn hybrids at two locations in 1990 and one in 1991. Varying disease severities were obtained by inoculating once, twice or treating plots with Benlate 50DF. For the three experiments, the mean percentage leaf area affected on three leaves (ear leaf, third leaf above and below ear leaf) of plants in inoculated plots was 9.3 to 16.0% by early scenescence and yield was 12.1 to 13.2% lower in plots inoculated twice than those treated with fungicide. Regression analysis indicated that the relationship between yield and area under the disease progress curve or percentage leaf area affected was significant ($P = 0.04$ to 0.0001), but R^2 values were low ($R^2 = 0.19$ to 0.57). Thus, although GLS reduced yield, disease severity did not provide a precise prediction of the level of yield loss.

A428

RACE DETERMINATION OF PHYTOPHTHORA PARASITICA VAR. NICOTIANAE COLLECTED FROM TOBACCO IN GEORGIA. A. S. Csinos, Department of Plant Pathology, Coastal Plain Exp. Stn., University of Georgia, Tifton, GA 31793-0748.

Tobacco black shank incited by Phytophthora parasitica var. nicotianae (Breda de Hann) Tucker continues to be a serious soilborne pathogen of tobacco in Georgia. Diseased tobacco samples were collected from across the Georgia tobacco growing region in both 1990 and 1991. Isolations were made from the samples and stored in culture until the fall of each of those years. Race determination and virulence was determined for each of the isolates by stem inoculating tobacco cultivars Coker 371-Gold, Speight G-70 and K-326 and the breeding line 1071. Thirteen and thirty-three isolates were collected in 1990 and 1991 respectively from tobacco farms across the tobacco growing area of Georgia. All 1990 isolates were race 0. However, six of the thirty-three isolates in 1991 were determined to be race 1. These preliminary results suggest that the development of race 1 is widespread in the state of Georgia.

A429

POPULATION DYNAMICS OF ALFALFA FOLIAR PATHOGENS IN IOWA. F.W. Nutter Jr. and S.S.A. Rizvi. Department of Plant Pathology, Iowa State University, Ames, IA 50011.

The seasonality of alfalfa foliar pathogens in Iowa was determined by establishing research plots at 4 locations: Ames, Ankeny, Chariton, and Knoxville. Leaf samples containing at least 25 lesions or blotches were sampled at 7-day intervals from each of four replications per site beginning in early May and ending in late September. To facilitate pathogen identification, samples were incubated at 23°C for 48 hours to induce sporulation. The frequency of pathogens was then recorded and expressed as a percentage of total lesions sampled. Nine fungal pathogens and one bacterial pathogen were found. *Phoma medicaginis* and *Xanthomonas campestris* were predominant early in the season; *Stemphylium botryosum, Pseudopeziza medicaginis, Leptosphaerulina briosciana, Cercospora medicaginis,* and *Colletotrichum dematium* in mid- to late-season; and *Leptrotrochila medicaginis, Uromyces striatus* and *Stagonospora meliloti* in late season samplings. Yield reduction at Ankeny and Chariton due to foliar diseases ranged from 17 to 31 percent.

A431

PEPPERMINT PERFORMANCE WITH RESPECT TO VERTICILLIUM WILT AND CULTURAL PRACTICES (ROTOTILLING VS PROPANE FLAMING). F.J. Crowe and J. Debons, Oregon State University-COARC, Madras, OR, 97741.

Large fumigated field plots were infested in 1989 with 0, 0.01, 0.1, 1.0 or 5.0 propagules of Verticillium dahliae per gram of soil. A moderately resistant peppermint rootstock was planted in the spring of 1990. The effect of subunit cultural treatments (post-harvest propane flaming vs spring rototilling) on Verticillium wilt and peppermint performance was determined in 1991. Plots were evaluated for percent ground cover, disease severity, and yield. Spring ground cover of mint in the flamed+high inoculum density treatment was significantly lower ($P=0.04$) (28.5%) compared to an average of 78% for all flamed+lower inoculum density treatments. In contrast, ground cover in all tillage plots averaged > 95% across all tillage treatments. At harvest in 1991, mint growth had compensated and foliage covered bare areas in all treatments. In plots with highest inoculum density, disease severity was significantly greater ($P=0.05$) in the tillage compared to the flamed treatment; however, yield was significantly higher ($P=0.05$) in the flamed compared to the tillage treatment. In plots without inoculum and at the lowest level of initial inoculum, subplots having either cultural treatment yielded equally. Significance levels for interactions between inoculum density and cultural treatments were $P=0.03$, 0.06 and 0.08 for ground cover, disease severity and yield, respectively. Interestingly, yield was significantly higher ($P=0.05$) in all infested compared to noninfested treatments, with best yields obtained for treatments with low and intermediate infestions, in which little or no wilt symptoms occurred. Changes in inoculum density from initial levels will be dicussed.

A432

EFFECT OF IN-SEASON FLOODING ON WHITE ROT OF GARLIC AND SURVIVAL OF SCLEROTIUM CEPIVORUM. F.J. Crowe and J. Debons, Oregon State University-COARC, Madras, OR, 97741.

In June 1989 an 8 ha field of garlic in central Oregon had one or more expanding patches of diseased garlic per 1.5 m of planted row. The field was diked with noninfested soil and continuously flooded for 21 wk beginning 25 June. Water depth was 12 cm at one end and 1 m at the other end of the field slope. Soil temperature, and garlic and sclerotia survival were monitored monthly from June 1989 until 22 November 1989. Inoculum survival also was evaluated in the spring of 1990. Although sclerotia had developed prior to flooding in areas of early pathogen activity, disease development and pathogen reproduction immediately ceased with flooding. At all water depths, soil temperature at 6 cm decreased from 20-25 C in late June to 10 C 21 wk later. Sclerotia survival was determined by plating on water agar after surface sterilization and cracking the rind, then observing characteristic growth of the pathogen. For all water depths, survival was 100, 35, 19, 12, 5 and 1% at 0, 2, 6, 8, 10 and 12 wk after flooding. Decay of dead sclerotia began to be evident by week 16, which confounded continued determination of the proportion of sclerotia which survived beyond this period. All submerged garlic were dead within 1 mo of flooding but a few aerial bulblets of garlic had already developed and survived the flooding event. In spring 1990, inoculum density was <0.001 viable sclerotia/ml of soil in the areas of early pathogen reproduction in 1989.

A433

EFFICACY OF IMAZAQUIN SEED TREATMENT FOR THE CONTROL OF STRIGA GESNEROIDES AND ALECTRA VOGELII IN COWPEA (VIGNA UNGUICULATA). D. K. Berner, A. E. Awad, and E. I. Aigbokhan, International Institute of Tropical Agriculture, Ibadan, Nigeria.

The herbicide imazaquin was tested for efficacy in Striga gesneroides and Alectra vogelii control when applied as a cowpea (Vigna unguiculata) seed treatment. Cowpea seeds, soaked for 5 minutes in 0.35% solution of the ammonium salt of imazaquin, were dried and planted into pots infested with 3,000 germinable S. gesneroides or A. vogelii seeds. After 74 d treated plants had an average of 0.06 and 0.63 attached S. gesneroides and A. vogelii, respectively, per host plant. Untreated controls averaged 4.84 and 5.13 attachments per plant. There were no emerged parasites in the treated pots. A total of 54 emerged S. gesneroides and 71 emerged A. vogelii were found in the untreated controls. Treated cowpea yielded 73% more biomass than the untreated. Observations in vitro indicated post-germination demise of both parasites. Treated seed continued to germinate S. hermonthica. Field trials on minimum necessary concentrations are underway.

A434

HYPHAL ADHESION AND SIDEROPHORE PRODUCTION IN THE INHIBITION OF *PHYTOPHTHORA PARASITICA* BY *PSEUDOMONAS* SPP. FROM CITRUS. C.-H. Yang, D. A. Cooksey, J. A. Menge, and J. K. Turney, Dept. of Plant Pathology, University of California, Riverside 92521.

Pseudomonas fluorescens 09906 and *P. putida* 06909 suppressed root rot of citrus caused by *Phytophthora parasitica*. Both bacteria adhered to the hyphae of the fungus and inhibited its growth *in vitro* and on citrus roots. A mycelial column assay was developed to measure adhesion of the bacteria to the fungus and to enrich for adhesion-defective mutants from pools of Tn*5* mutants. The adhesion-defective mutants recovered were all nonmotile (Mot$^-$) and lacked flagella. More than 65% of wild-type cells of both bacterial strains adhered to the mycelial column, but less than 14% of Mot$^-$ mutant cells adhered. Mot$^-$ mutants of both bacterial strains had a reduced ability to inhibit growth of the fungus *in vitro*. In addition, Tn*5* mutants of both bacterial strains that were defective in siderophore production had a reduced ability to inhibit growth of the fungus *in vitro*.

A435

BIOLOGICAL CONTROL OF POTATO SCAB WITH SUPPRESSIVE ISOLATES OF *Streptomyces*. Daqun Liu and Neil Anderson, Department of Plant Pathology, University of Minnesota, St. Paul, MN, USA.

Two suppressive isolates of *Streptomyces* spp. from potato scab research plots produced bacteriocin-like reactions to pathogenic isolates of *Streptomyces scabies*. A three-year field experiment to control scab on potato cv. Norchip tubers was made using 12L pots set into the soil. The two suppressive isolates were grown on vermiculite plus oatmeal broth and added to scab conducive soil at 1, 5, and 10% (v:v). In 1989, both 1% treatments gave significant control compared to the nonamended check and the 5 and 10% treatments. However, in 1990 and 1991 all three treatments gave the same highly significant level of disease control based on disease severity ratings. The suppressive isolates did not affect yield and have been identified as *Streptomyces scabies* strain PonR and *Streptomyces fulvoviolaceus* strain PonSSII. Isolates of *Streptomyces* spp. selected on the basis of bacteriocin tests against *S. scabies* have the potential for biocontrol of the potato scab disease.

A436

SELECTING ISOLATES OF STREPTOMYCES SUPPRESSIVE TO *Streptomyces scabies*. Daqun Liu and Neil A. Anderson, Department of Plant Pathology, University of Minnesota, St. Paul, MN 55108, U.S.A.

A total of 93 isolates of *Streptomyces* spp. were obtained from potato tubers grown in five different fields and tested for bacteriocin activity against pathogenic *Streptomyces scabies* strain RB311. Twenty-four isolates produced more bacteriocin against RB311 than the control, suppressive isolate PonSS11. Of the 24 isolates, six had superior bacteriocin activity in tests against six additional pathogenic *S. scabies* isolates from Minnesota. These six isolates also gave significant scab control in field pot tests and on leaf bud tubers in the greenhouse. The following criteria were used to further test the above 24 suppressive isolates: 1) non pathogenic on leaf bud tubers, 2) good bacteriocin producers against 17 *S. scabies* isolates, 3) good competitors against 17 *S. scabies* isolates, and 4) compatible with each other in co-plating tests. Of the suppressive isolates tested, only two met all four selection requirements as potential biocontrol agents against the potato scab disease.

A437

EFFECT OF NATURALLY OCCURRING ANTAGONISTIC BACTERIA ON THE BACTERIAL RING ROT DISEASE OF POTATO. P. Gamard and S.H. De Boer. Agriculture Canada, 6660 N.W. Marine Drive, Vancouver, B.C. V6T 1X2.

Naturally occurring bacteria antagonistic to *Clavibacter michiganensis* subsp. *sepedonicus*, the causal agent of bacterial ring rot of potato, were isolated from potato tuber surfaces. Isolates were screened for biological control potential in a greenhouse on micropropagated potato plantlets inoculated with *C.m. sepedonicus*. Three isolates, that gave complete control of bacterial ring rot during the first 2-3 weeks of plant growth were field tested. For field experiments, antagonists were either inoculated on tuber seed-piece surfaces after tubers had been vacuum infiltrated with *C.m. sepedonicus*, or on whole seed tubers prior to inoculation with a ring rot contaminated knife. Inoculation with any of the antagonistic bacteria increased plant stand by at least 50% when seed tubers were inoculated by vacuum infiltration. Antagonistic isolate, 16C, tentatively identified as *Arthrobacter protophormiae* on the basis of its fatty acid profile, significantly reduced the number of plants and tubers with symptomatic and symptomless ring rot infections. It was also detected with a monoclonal antibody on progeny tubers grown in the field from tubers inoculated with 16C. Strain 16C, when applied in an alginate bead carrier, survived at high population levels for at least two weeks on the surfaces of tubers planted in soil in the greenhouse.

A438 Withdrawn

A439

INTERNAL AND EXTERNAL COLONIZATION OF CUCUMBER BY BACTERIA WHICH INDUCE SYSTEMIC RESISTANCE TO *COLLETOTRICHUM ORBICULARE*. G. Wei, J. W. Kloepper, and S. Tuzun, Dept. of Plant Pathology, Auburn University, AL 36849-5409.

Select bacterial strains, which demonstrated induced systemic resistance against *Colletotrichum orbiculare* in cucumber, were evaluated for colonization on and in roots, and inside stems using spontaneous rifampicin-resistant mutants. With seed treatment, populations on roots decreased about 0.5 log units 1 day after planting (DAP), increased 0.3 - 1.6 log units at 3 DAP, and steadily decreased to log 4.4 - 4.9 cfu/root system at 21 DAP. Some strains were recovered from inside surface-disinfested roots; however, none of the inducing strains was found in leaves challenged with *C. orbiculare*. The internal colonization potential was further assessed using injections of cotyledons and petioles. With cotyledon-injections, no injected bacteria were detected in petioles of any subsequently sampled leaves. With petiole-injections, one strain moved 6 - 8 cm above the injection point, while all other strains were confined to the site of injection. Thus, while some bacteria which induce systemic resistance may have limited internal colonization, they do not translocate and colonize challenged leaves, suggesting that neither competition nor antagonism was the principal determinant of the observed protection.

A440

INDUCTION OF SYSTEMIC RESISTANCE AGAINST CUCUMBER MOSAIC VIRUS BY SEED INOCULATION WITH SELECT RHIZOBACTERIAL STRAINS. L. Liu, J. W. Kloepper, and S. Tuzun, Department of Plant Pathology, Auburn University, AL 36849-5409.

Strains of plant growth-promoting rhizobacteria (PGPR) which previously demonstrated induced systemic resistance (ISR) against *C. orbiculare* in cucumber were examined for their ability to cause ISR against cucumber mosaic virus (CMV). Seeds were treated with two strains of PGPR or water, and cotyledons were challenge inoculated with extracts from CMV-infected leaves or water. The percent of plants which developed CMV symptoms was compared among plants treated at planting with two PGPR strains and nontreated controls. In 5 repeating experiments, treatment with one PGPR strain resulted in a significant reduction in the mean percent symptomatic plants 7 d after challenge with CMV compared to controls, while one strain had a nonsignificant effect on symptoms. Protected plants failed to develop mosaic symptoms during the experimental period, which contrasts with previously reported results for classical ISR where protection

against CMV was evident as a delay in symptom development. The finding that PGPR strains which cause ISR against a fungal pathogen also protect against a virus strongly suggests that the principal determinant of protection is not an antifungal bacterial metabolite. The effects of PGPR-mediated ISR on viral multiplication and infectivity are being determined.

A441

COMPARISON OF BIOCHEMICAL RESPONSES IN CUCUMBER SYSTEMICALLY PROTECTED AGAINST *COLLETOTRICHUM ORBICULARE* BY PRIOR LEAF INOCULATION WITH THE PATHOGEN OR SEED TREATMENT WITH RHIZOBACTERIA. G. Wei, S. Tuzun, and J.W. Kloepper, Dept. of Plant Pathology, Auburn University, AL 36849-5409.

Cucumber plants (cv. 'Straight Eight') were systemically protected against *C. orbiculare* by either prior inoculation of the first leaf with the same pathogen or seed treatment with 3 strains of plant growth-promoting rhizobacteria (PGPR), resulting in reduced lesion development of *C. orbiculare* on the second leaf (*Phytopathology* 81:1508-1512). Enzyme assays for peroxidases and chitinases were conducted to compare the biochemical responses between pathogen- and PGPR-mediated induced systemic resistance (ISR). Enzyme activity assays and IEF-gel electrophoresis for peroxidases and Western blot analysis for chitinases indicated an early increase in all parameters of both enzymes in plants induced with *C. orbiculare*. In contrast, reductions in basal and post-challenge levels of both enzymes occurred in plants induced with PGPR. These observations suggest that the 2 enzymes may not have a determinant role in PGPR-mediated ISR, and therefore, distinct mechanisms may operate with ISR by PGPR. Work is underway to determine transcriptional and translational changes occurring during PGPR-mediated ISR.

A442

EFFECTS OF ENVIRONMENTAL PARAMETERS ON INDUCTION OF SYSTEMIC RESISTANCE IN CUCUMBER TO *COLLETOTRICHUM ORBICULARE* BY PLANT GROWTH-PROMOTING RHIZOBACTERIA (PGPR). G. Wei, J. W. Kloepper, and S. Tuzun, Department of Plant Pathology, Auburn University, AL 36849-5409.

Experiments were conducted to determine if the level of PGPR-mediated induced systemic resistance (ISR) was affected by various environmental parameters, including soil moisture, fertility, and temperature. Three soil moisture regimes ("dry": -105 mbar, "normal": -80 mbar, and "wet": -53 mbar), 4 levels of soluble fertilizer (0, 315, 630, and 945 ppm N), and 2 temperatures (constant 25 and 35 C) were evaluated for effects on ISR following seed treatment of cucumber with PGPR and subsequent challenge inoculation with *C. orbiculare*. ISR by PGPR was strongly affected by each parameter. With normal soil moisture, all 4 test PGPR strains caused significant reductions in mean necrotic area of lesions compared to noninduced controls; however, only 1 strain in dry soil and 2 strains in wet soil caused ISR. The level of ISR increased with increasing N-levels from 0 to 315 ppm but decreased with N-levels above 315 ppm. All 4 strains caused ISR at 25 C but not at 35 C. In contrast, ISR resulting from prior inoculation with *C. orbiculare* was generally not affected by changing environmental parameters.

A443

ROLE OF HCN IN SUPPRESSING SEPTORIA TRITICI BLOTCH AND LEAF RUST OF WHEAT BY Pseudomonas putida STRAIN BK8661. M. Flaishman[1], Z. Eyal[1], A. Zilberstein[1], C. Voisard[2], and D. Haas[2]. [1]Dept. of Botany, Tel Aviv University, Israel and [2]Dept. of Microbiology, ETH, Zurich, Switzerland.

Strain BK8661 of P. putida suppressed symptoms (90%) on wheat seedlings incited by Septoria tritici and Puccinia recondita BK8661 excreted the siderophore pseudobactin BK8661, antibiotics, and HCN. HCN suppressed conidial and mycelial growth of S. tritici and germination of urediospores of P. recondita. HCN overproducing strains were constructed by integrating the HCN biosynthetic genes from P. fluorescens strain CHAO into the genome of BK8661, and into two pleiotropic Tn5 BK8661 mutants (pseudobactin-, antibiotics-), which exhibited limited antagonistic activity. Integration of the HCN genes enhanced HCN production 10-20 fold on synthetic media. The pleiotropic, HCN overproducing mutants improved (20-45%) the ability to suppress symptoms of leaf rust and Septoria tritici blotch. No improvement in the suppression of symptoms was expressed by the HCN overproducing BK8661 derivatives. Hydrogen cyanide (HCN) production by Pseudomonas spp. may be involved in suppressing pathogens on the wheat foliage.

A444

Influence of Clipping Recycling on Disease Indicence in Three Turfgrass Species. P. F. Colbaugh, B. W. Hipp and T. Knowles. Texas Agric. Expt. Station. TAMU Res. & Extn. Center, Dallas, TX 75252.

Mowing practices were investigated as a means of recycling turfgrass clippings in situ and reducing the burden of clipping disposal in metropolitan areas. During 1989 and 1990 two types of mowing operations were used on St. Augustinegrass, tall fescuegrass and bermudagrass field plots to determine their influence on fungal disease severity from June to September. Field plots receiving 0, 0.6, 1.2, and 1.8 kg N/92.5 m^2 were mowed weekly using a mulching mower with clippings returned or a standard rotary mower with clippings bagged and removed. Over a two-year period, damaging fungal diseases were not observed with either of the mower regimens. Fungal disease severity was more related to seasonal environmental conditions than mowing practices on field plots. Leafspot symptoms were generally less on mulching mower plots

A445

BINUCLEATE RHIZOCTONIA ISOLATES CONTROL PYTHIUM AND RHIZOCTONIA SOLANI, AND PROMOTE SEEDLING GROWTH. A.R. Harris, D.A. Schisler, and M.H. Ryder. CSIRO Division of Soils, Private Bag 2, Glen Osmond, S.A. 5064, Australia.

Two binucleate *Rhizoctonia* isolates from nursery potting media were assayed for biocontrol of damping-off diseases in seedlings grown in pasteurized potting media with added pathogens. Both isolates reduced death caused by *Pythium ultimum* var. *sporangiiferum* in seedlings of bell pepper and celosia at least as well as the fungicide propamocarb. In the presence of a pathogenic isolate of *R. solani* anastomosis group (AG) 4, they reduced damping-off and increased shoot dry weights of bell pepper and celosia seedlings, in most cases more than did the fungicides quintozene or propamocarb. In potting medium infested with AG 4, viola and petunia seedling survival, but not shoot weights, were increased significantly by either binucleate *Rhizoctonia* isolate. *R. solani* AG 8 did not reduce bell pepper shoot dry weights in the presence of either binucleate *Rhizoctonia* isolate. For pasteurized potting medium without added pathogens, one binucleate *Rhizoctonia* isolate increased shoot dry weights of bell pepper in three of four experiments, the other in two of four experiments.

A446

EVALUATION OF PSEUDOMONAS SPP. AS BIOLOGICAL CONTROL AGENTS FOR PYTHIUM ROOT ROT OF GREENHOUSE EUROPEAN CUCUMBERS. L. Rankin, T. Zhou, and T. Paulitz. McGill University, Macdonald Campus, Ste. Anne de Bellevue, Quebec Canada H9X 1C0

Two isolates of Pseudomonas corrugata (Pc13 or 35) and three isolates of P. fluorescens (Pf15, 16, or 27) were evaluated for their ability to control Pythium root rot of European cucumbers (Cucumis sativus L. cv. Corona) grown in a rockwool hydroponic system. Seven-week-old plants were set onto rockwool slabs and treated with water or 200 ml (10^6 cells/ml) of Pc13 or 35 or Pf15, 16 or 27. Six days later, half of the plants were inoculated with 10,000 zoospores of Pythium aphanidermatum (Pa) strain 186. In a spring crop, Pa-inoculated plants treated with Pc13 or Pf15 produced fruit yields equal to 92% and 74% respectively of the control (no Pa, no bacteria). Pa-inoculated plants without bacteria yielded only 46% of the control. In a fall crop, Pa-inoculated plants treated with Pc13 or Pf15 yielded 52% and 47% of the control, compared to Pa-only treatment, which yielded 12.5% of the control. In the fall crop, treatment with Pf15 alone (no Pa) increased shoot dry weight compared to the non-treated control.

A447

BIOLOGICAL CONTROL ACTIVITY OF TILLETIOPSIS SPP. AGAINST SPHAEROTHECA FULIGINEA ON GREENHOUSE CUCUMBERS. E.J. Urquhart [1], J.D. Menzies [2] and Z.K. Punja [1]. [1]Dept. of Biological Sciences, Simon Fraser University, Burnaby, B.C., Canada V5A 1S6 and [2]Agriculture Canada Research Station, Box 1000, Agassiz, B.C., Canada V0M 1A0.

Powdery mildew, caused by Sphaerotheca fuliginea, is a major problem on greenhouse grown cucumbers in British Columbia. The efficacy of the yeast Tilletiopsis was evaluated for biological control of this disease. Two local isolates, Albion and LGG, were evaluated for their response to environmental factors and efficacy in reducing mildew sporulation. Growth was not significantly different between 15 and 30 C but was reduced at 3 C. Increasing the osmotic potential with PEG 8000 in liquid suspension culture reduced biomass and blastospore production. Tilletiopsis blastospores were grown in shake culture in a medium containing 2.5% D-glucose, 1.0% peptone and 0.1% yeast extract. This inoculum was applied weekly for 3 weeks to heavily infected cucumber plants at 1 x10^8 spores/mL. Both Albion and LGG isolates reduced the mildew conidia density by up to 18-fold compared to the control, which was 3-fold lower. SEM examination of treated mildewed leaves indicated that hyphae and conidiophores appeared shrunken and collapsed. The role of hydrolytic enzymes in biocontrol activity is being investigated.

A449

CHARACTERIZATION AND SEQUENCE OF THE COAT PROTEIN CISTRON OF APPLE MOSAIC VIRUS. P.J. Shiel and P.H. Berger, Plant Pathology Division, PSES, University of Idaho, Moscow, ID 83843.

Components of apple mosaic ilarvirus (ApMV) were separated by sucrose density gradient centrifugation and then cloned into pBluescript following random primed reverse transcription. The coat protein (CP) open reading frame was identified by sequence data collected from independent clones, results from western blots of bacterial cultures, and direct protein microsequencing of the native viral CP. The CP cistron encodes an open reading frame of 705 bases, which translates to an amino acid sequence of 26,600 daltons. The CP is not related to alfalfa mosaic virus or tobacco streak virus coat protein at the nucleic acid level. The virion does not completely dissociate in SDS-PAGE, instead appearing as several concatemeric bands from n=1 to at least n=8. This effect is even more pronounced in western blots, with the antibodies reacting to all bands. This effect is not observed with alfalfa mosaic virus prepared and electrophoresed under the same conditions. Apple mosaic virus CP has a high cysteine content at both the amino- and carboxy-termini, which may be partially responsible for the effect. In addition, the high cysteine content and virion resistance to dissociation may be related to particle structure and/or stability.

A450

STRUCTURE OF THE L (POLYMERASE) PROTEIN GENE OF SONCHUS YELLOW NET VIRUS. T. Choi[1], S. Kuwata[2], E. V. Koonin[3], L. A. Heaton[4], and A. O. Jackson[4]. [1]University of California, Berkeley, California 94720; [2]Japan Tobacco, Inc., Yokohama, Japan; [3]National Institutes of Health, Bethesda, MD 20894; and [4]Kansas State University, Manhattan, KS 66506.

The complete nucleotide sequence of the L protein gene of sonchus yellow net virus (SYNV), a plant rhabdovirus was determined by dideoxynucleotide sequencing of cloned cDNAs derived from the negative strand genomic RNA. The L protein gene is composed of 6401 nucleotides (nt) located between positions 7158 to 13558 relative to the 3' end of the genomic RNA. Sequence analysis suggests that the complementary mRNA contains a 44 nt untranslated 5' leader sequence preceding an open reading from of 6348 nucleotides that is capable of encoding a polypeptide of 2116 amino acids with a deduced molecular weight of 241,569 daltons. The L protein is positively charged, has a high proportion of the amino acids Leu and Ile and contains putative polymerase and RNA binding domains. Extended alignment of the SYNV L protein amino acid with those of other non-segmented negative strand RNA virus polymerases reveals conservation of sequences within 12 blocks that appear sequentially along the protein.

A451

EXPRESSION OF FOREIGN GENES BY TOMATO BUSHY STUNT VIRUS AND ASSOCIATED DEFECTIVE RNAS. H. B. Scholthof, T. J. Morris, and A. O. Jackson. University of California, Berkeley, CA 94720.

Tomato bushy stunt virus (TBSV), the type member of the tombusvirus group, is a small isometric virus with a single, positive sense RNA genome of ca. 4800 nucleotides. The virus genome contains five genes: two 5' proximal genes (p33 and p92) involved in replication, are followed by the coat protein gene, which is translated from a subgenomic mRNA (sgRNA), and two nested 3' genes (p19 and p22) translated from a second sgRNA. Mutational analyses, using *in vitro* generated transcripts from infectious cDNA clones, demonstrate that the capsid protein is dispensable for systemic infection of plants. The coat protein gene could be replaced to yield efficient expression of a reporter gene in protoplasts and inoculated leaves. The 3' nested genes could also be replaced for high levels of reporter gene expression in protoplasts but not in plants; thus confirming that at least p19 or p22 is involved in systemic movement. Defective RNAs, with most of p92 deleted and with a reporter gene fused with p19, had high levels of replication and reporter gene expression upon co-transfection with wild-type transcripts. These results demonstrate that foreign genes can be efficiently expressed by TBSV and that defective RNAs and subgenomic promoters can be used to study TBSV mediated activation of gene expression.

A452

ANALYSIS OF CIS-ACTING ELEMENTS REQUIRED FOR REPLICATION OF BARLEY STRIPE MOSAIC VIRUS RNAS. H. Zhou, R. D. K. Donald, and A. O. Jackson. University of California, Berkeley, California 94270.

Barley stripe mosaic hordeivirus (BSMV), has a tripartite genome with RNAs designated α, ß, and γ. To investigate the cis-acting sequences involved in the replication of these RNAs, sequential deletions were constructed in full-length biologically active cDNA clones and the replicative abilities of RNA transcripts derived from these clones were investigated in barley protoplasts that had been coinoculated with wild type RNAs α and γ. All tested deletions in the RNAα open reading frame (ORF) abolish accumulation of progeny RNAs, in contrast to RNAs ß and γ which can tolerate extensive deletions in each ORF. The ßa (capsid protein) ORF can be deleted and the ßb, ßc and ßd ORFs can be individually or collectively deleted without obvious deleterious effect on the replication of RNAß. Although the intergenic region between ßa and ßb is dispensable for replication of RNAß, small deletions within this region reduce RNA accumulation by at least 10 fold. Deletions within the first 570 nt of the 5' terminus of RNAγ also abrogate detectable RNA accumulation in protoplasts, but sequences corresponding to the central and carboxy-terminal regions of the γa protein, the γa-γb intergenic region and the γb ORF are totally dispensable for replication and accumulation of RNAγ.

A453

CAULIFLOWER MOSAIC VIRUS IS CAPABLE OF RECOMBINATION WITH TRANSGENIC NICOTIANA BIGELOVII THAT CONTAIN CAMV CODING SEQUENCES. W.M. Wintermantel and J.E. Schoelz. Dept. of Plant Pathology, University of Missouri, Columbia, MO 65211

We have previously shown that gene VI of CaMV strain D4 determines systemic infection of N. bigelovii. Recently, we expressed the D4 gene VI product in N. bigelovii and demonstrated that CaMV strain CM1841 is capable of recombination with the gene VI coding sequence present in the transgenic plants. CM1841 is unable to systemically infect nontransformed N. bigelovii, but transgenic plants inoculated with CM1841 virions developed a systemic mosaic. Nucleotide sequencing of the CM1841 virus was recovered from systemically infected leaves of transgenic plants revealed that the gene VI coding sequence of the virus corresponded to that of gene VI of D4, which is the viral sequence expressed by the transgenic plants. We are now attempting to use the technique of agroinfection to inoculate CM1814 viral DNA to transgenic N. bigelovii. A double length CM1841 clone has been inserted into the Agrobacterium vector pKYLX7 and the ability of this construct to infect transgenic N. bigelovii that express the D4 gene VI product is being evaluated.

A454

EXPRESSION OF POTYVIRUS HELPER COMPONENT USING RECOMBINANT BACULOVIRUS. D. W. THORNBURY, J. F. J. M. VAN DEN HEUVEL, J. A. LESNAW, AND T. P. PIRONE. UNIVERSITY OF KENTUCKY, LEXINGTON, KY 40546.

Two constructs, one representing the first three genes (34K, HC-PRO, 42K) of the tobacco vein mottling virus (TVMV) genome and the other representing the helper component (HC) gene of potato virus Y (PVY) were individually cloned into transfer vectors which mediate insertion of foreign genes into the polyhedrin gene of *Autographa californica* nuclear polyhedrosis virus (AcMNPV). Both constructs were designed for expression of discrete proteins rather than fusion products. Recombinant baculovirus was isolated from non-occluded plaques and used to infect *Spodoptera frugiperda* cells, and the HC-specific products produced in insect cells were compared with HC purified from virus-infected plants. Western blot analysis showed that an authentic-size TVMV-HC polypeptide was produced in cells infected by the TVMV recombinant baculovirus and that PVY-HC recombinant baculovirus infected cells produced an authentic-size PVY-HC polypeptide.

A455

COMPARISON OF PROTEIN COATS OF NL-3 AND TYPE STRAINS OF BEAN COMMON MOSAIC VIRUS (BCMV). J.L. George, M.J. Sparks, J.E. Knesek.
Texas Woman's University, P.O. Box 23971, Denton, Tx. 76204

The protein coats of NL-3 and Type strains of bean common mosaic virus (BCMV) were compared. The viral strains were purified from BCMV infected Phaseolus vulgaris tissue and the protein coat was analyzed on a denaturing SDS polyacrylamide gel system. The Type strain protein coat was found to migrate as two bands in the gel, whereas the NL-3 strain exhibited a single band in the gel, intermediate in size between the two Type strain protein coat bands. The N-terminus amino acid sequences for each of the viral coat proteins have been determined by protein microsequencing.

A456

EVIDENCE FOR ANTIGENIC AND SIZE HETEROGENEITY AMONG THE P1 PROTEINS OF CERTAIN ZUCCHINI YELLOW MOSAIC VIRUS (ZYMV) ISOLATES. G.C. Wisler, E. Hiebert, and D.E. Purcifull. University of Florida, Gainesville, FL 32611.

An aphid transmissible isolate of ZYMV from Florida (ZYMV-FL/AT) was cloned in lambda gt11 and lambda ZapII. Clones representing the 5'-terminal region of the genome were selected using antisera to the P2 proteins of tobacco vein mottling virus and papaya ringspot virus-type W (PRSV-W). The 5'-coding region (P1) was amplified by anchored PCR and cloned in the pETH expression vector (McCarty et al., 1991, Cell 66:895-905). The expressed 34-kDa P1 protein was used for polyclonal antiserum production. Antiserum to P1 of ZYMV reacted in Western blots to proteins (c. 33-35-kDa) in extracts from pumpkin singly infected with 12 ZYMV isolates from Florida and one from Italy, but not with one from Reunion Island (ZYMV-RU). Extracts from ZYMV-RU infected plants, however, reacted with ZYMV-FL/AT capsid and cylindrical inclusion protein antisera and PRSV-W amorphous inclusion protein antisera. The P1 antiserum reacted with proteins of c. 35-kDa and c. 33-kDa of a mild and a severe ZYMV-FL isolate, respectively, and this difference in size corresponded to a c. 60-bp difference in the P1 coding regions amplified by RNA-PCR. Analysis of P1 proteins may be useful in distinguishing ZYMV isolates.

A457

APPARENT CONFORMATIONAL DIFFERENCES AMONG VIRIONS OF SYMPTOM-MODULATING TURNIP CRINKLE VIRUS COAT PROTEIN MUTANTS. **L. A. Heaton, Department of Plant Pathology, Kansas State University, Manhattan, KS 66506.**

Several small, spherical RNA plant viruses swell under conditions that deprotonate carboxyl groups and/or remove divalent cations. Swollen turnip crinkle carmovirus (TCV) and tomato bushy stunt tombusvirus particles migrated more slowly than contracted particles during agarose gel electrophoresis (AGE). Swollen TCV particles also stained more intensely with ethidium bromide than did contracted particles. Symptom-modulating coat protein mutants of TCV were assayed by AGE. One mutant was like the wild-type virus, two mutants appeared to be always swollen and less stable at elevated pH, and one mutant appeared to be always contracted and more stable at elevated pH. A fifth mutant was a mixed population of swollen and contracted particles. The possible correlation between the symptoms elicited by each mutant and its apparent conformation will be discussed.

A458

ANALYSIS OF SEED TRANSMISSIBILITY USING INFECTIOUS TRANSCRIPTS OF PEA SEEDBORNE MOSAIC POTYVIRUS. E. Johansen, P. D. Kohnen, W. G. Dougherty, and R. O. Hampton. Departments of Microbiology and Botany & Plant Pathology, Oregon State University, Corvallis, OR 97331.

P1, P2, and P4, the three pathotypes of pea seedborne mosaic potyvirus, are distinguished by their response to resistance conferring *Pisum sativum* genes *sbm-1*, *sbm-2*, *sbm-3*, and *sbm-4*. These pathotypes are also differentiated serologically, by symptomatology, and by seed-transmission rates in specific pea cultivars. A full-length complementary DNA of P1 RNA has been assembled in an *in vitro* transcription plasmid vector and the infectivity of RNA transcribed from this recombinant molecule has been established. Comparisons of virus particle morphology, symptom development, and seed-transmissibility of the transcript-derived virus and the P1 parent isolate are presented. Sequence comparison and replacement studies with P4 are being conducted to identify genomic sequence(s) responsible for phenotypic distinctions between pathotypes P1 and P4.

A459

MOLECULAR CHARACTERIZATION OF LETTUCE INFECTIOUS YELLOWS VIRUS. V.A. Klaassen, M. Boeshore*, and B.W. Falk, Dept. of Plant Pathology, University of California, Davis, CA 95616; and* The Upjohn Company, Kalamazoo, MI 49001.

Lettuce infectious yellows virus (LIYV) is a filamentous virus that causes yellowing symptoms in a wide range of host plants. Based on particle morphology, LIYV has been grouped with the closteroviruses which have monopartite ssRNA genomes. cDNA clones were synthesized and used as hybridization probes to characterize the RNAs present in LIYV-infected plants. Two groups of clones were identified based on their differential hybridization to total and dsRNAs extracted from LIYV-infected tissue. Nucleotide sequence analysis revealed several potential ORFs in each group of clones and indicates that the groups might represent two different RNAs. To determine the number and size of RNAs associated with LIYV-infection, clones from these two groups were used to hybrid-select total RNA from LIYV-infected plants. In addition, immunohybridization was used to analyze the RNAs encapsidated in LIYV virions.

A460

IDENTIFICATION OF SYMPTOM AND RATE-OF-SPREAD DETERMINANTS ON THE GENOME OF THE HOLMES' MASKED STRAIN OF TMV. R.S. Nelson, G. Li, M. Shintaku, The Samuel Roberts Noble Foundation, Plant Biology Division, P.O. Box 2180, Ardmore, OK 73402.

Previously, we have determined that the Holmes' Masked (M) strain of TMV, which is attenuated in symptom type compared with the U_1 strain, is capable of normal rates of replication and cell-to-cell spread in inoculated leaves but is impeded in movement to or accumulation in tissue normally accessible only through phloem transport. Although the impeded long distance movement of the M strain might logically be considered to be the cause of the masked symptoms this may not be true. Chimeric infectious clones have been produced by exchanging segments between M and U_1 cDNAs and used to determine the degree to which these traits are linked. Results indicate these traits can be unlinked but sequencing of the PCR segments has not been completed.

A461

CONSTRUCTION OF A RANDOM CDNA LIBRARY OF SORGHUM CHLOROTIC SPOT VIRUS RNA AND DETERMINATION OF THE NUCLEOTIDE SEQUENCE. Yukio Shirako, Roy C. French and James H. Strauss. California Institute of Technology, Pasadena, CA 91125 and USDA, ARS, University of Nebraska, Lincoln, NE 68583.

A random cDNA library made from unfractionated RNA from purified sorghum chlorotic spot virus (SCSV), a proposed furovirus, was constructed in a plasmid vector. Thirty clones (average insert length ca. 1.6 kb) were randomly selected for sequencing. Amino acid sequences deduced from the assembled SCSV nucleotide sequences were aligned with those encoded in RNA 1 and RNA 2 of soil-borne wheat mosaic virus (SBWMV). Comparisons of open reading frames and predicted amino acid sequences from the two viruses showed that SCSV and SBWMV share the same genome organization. Identities at the amino acid level in the putative RNA replicase, transport protein and capsid protein were 60%, 40% and 45%, respectively. The readthrough region following the capsid protein gene and a small cysteine-rich protein each share less than 20% identity between the two viruses. These results indicate that SCSV is a member of the furovirus group but is distinct from SBWMV.

A462

Molecular Cloning and Nucleotide Sequence of the Coat Protein Gene of the NL-3 and Type Strains of Bean Common Mosaic Virus. M.J. Sparks, J.L. George and J.E. Knesek. Texas Woman's University, P.O. Box 23971, Denton, Tx. 76204

Bean common mosaic virus (BCMV) is a member of the potyvirus group, causing severe damage to leguminous crops. Two strains of the virus, the Type strain and the NL-3 strain were purified and viral RNA extracted. Complementary DNA was synthesized from each viral RNA using Moloney-Murine Leukemia reverse transcriptase and Xho I oligo-dT priming. The single-stranded cDNA was converted into ds DNA by DNA polymerase and directionally cloned into lambda Uni-ZAP XR vector. The coat protein gene of the potyviruses is located at the extreme 3' end of the viral RNA, so clones of approximately 1 kbp were selected and subcloned from each strain. Restriction enzyme mapping indicates differences between the two strains. Furthermore, the subclones from each strain were sequenced by the chain termination method. Results from the nucleotide sequencing indicate that areas of homology and non-homology exist between the two strains.

A463

cDNA SEQUENCE OF BLUEBERRY SHOESTRING VIRUS COAT PROTEIN GENE. D. S. Warkentin[1,2], J. F. Hancock[1], and D. C. Ramsdell[2], [1]Horticulture Department and [2]Botany and Plant Pathology Department, Michigan State University, East Lansing, Michigan, 48824.

A one kb cDNA corresponding to the 3' terminus of blueberry shoestring virus (BBSSV) genomic RNA was cloned into bluescript KS+ vector. The clone was transcribed *in vitro* by T7 RNA polymerase. The *in vitro* transcript was translated *in vitro* by a rabbit reticulocyte lysate. The protein produced was detected by Western blot using rabbit polyclonal antiserum raised against purified BBSSV virions. The cDNA clone was sequenced and compared with coat protein sequences from virus groups with similar physical and biological properties. No homologies were detected.

A467

ULTRAVIOLET LIGHT-INDUCED CHANGES IN VEGETATIVE COMPATIBILITY GROUPS IN *CRYPHONECTRIA PARASITICA*. R. Rizwana and W. A. Powell. Environmental and Forest Biology, State University of New York, College of Environmental Science and Forestry, Syracuse, NY 13210-2788.

The effect of ultraviolet light (UV) on vegetative compatibility (v-c) groups in the fungus *Cryphonectria parasitica* was investigated. When complementary auxotrophic protoplasts made from UV-treated mycelium, from v-c groups that differed in the alleles of a single *VIC* gene, were fused together, heterokaryon formation increased 1000 fold as compared to non-UV-treated controls. Vegetative compatibility tests of auxotrophic single conidial isolates from the resulting prototrophic heterokaryons indicated only one of the two vc groups was maintained. These results could be due to increased parasexuality or possibly mutations in the *VIC* gene. In another experiment, UV-treatments induced instability in vegetative incompatibility within a single v-c group, suggesting that changes in v-c groups were not limited to sexual or parasexual recombination. These experiments indicate that vegetative compatibility in *C. parasitica* is amenable to UV light , which may be important in bringing about the large diversity of v-c groups of this pathogen.

A471

PRODUCTION OF SEXUAL SPORES OF *PHYTOPHTHORA INFESTANS* ON HOST TISSUES AND SUSPENSION CELLS. K.L. Deahl and S.P. DeMuth, USDA, ARS, Vegetable Laboratory, Beltsville Agricultural Research Center, Beltsville, Maryland 20705-2350, and R.J. Young, West Virginia University, Department of Plant Pathology and Agriculture Microbiology, Morgantown, West Virginia 26506.

Although oospore formation in host tissues is thought to be an important stage in the disease cycle of *Phytophthora infestans*, most experiments are conducted on agar media. Therefore, we investigated the sexual reproduction of North American isolates of *P.infestans* on host tissues. Seven to ten days after dual inoculations of A^2 and A^1 compatibility strains, oogonia with amphigynous antheridia were formed in stem, leaf, and tuber tissues of potato; however, a great number of these gametangia lacked mature oospores. Since sexual spores were also difficult to locate in diseased tissues, a method was developed for their production on potato suspension cells. When opposite mating types were paired on sterile suspension cells, abundant oospore formation occurred both intra- and intercellularly. Most of these spores resembled those induced on agar media.

A472

EFFECT OF BUFFER COMPOSITION ON EXTRACTION OF TOTAL PROTEINS FROM *SCLEROTINIA MINOR*. M. D. Bandla, H. A. Melouk and J. L. Sherwood. Department of Plant Pathology and USDA-ARS, Oklahoma State University, Stillwater, OK 74078-9947.

Antigenic preparation often requires extraction and solubilization of total cellular proteins. The influence of a non-ionic detergent and a salt on the efficiency of extraction of total mycelial proteins from *Sclerotinia minor* was examined. Triton X-100 and/or NaCl at various concentrations were added to 0.05 M Tris-HCl (pH 6.8) buffer at 4 C (Canadian Journal of Botany 63:2311). The protein profile was obtained by SDS-PAGE followed by color-based silver staining. Proteins were quantified by densitometry. The concentration and number of proteins were maximized by adding 0.5% Triton X-100 and 0.05M NaCl to the extraction buffer. The addition of Triton X-100 and NaCl increased the efficiency of extraction of total proteins from *S. minor* and other fungi as well.

A473

GROWER BIOASSAY KIT FOR VINCLOZOLIN RESISTANCE IN BOTRYTIS. G. W. Moorman and R. J. Lease, Department of Plant Pathology, The Pennsylvania State University, University Park, PA 16802.

A bioassay kit for greenhouse grower use to test for vinclozolin resistance in B. cinerea consisted of sunflower seeds (Helianthus annuus cv. Sunspot), 2 disposable beakers with soilless potting mix, disposable dropper bottles, plastic bags, and forceps. One bottle contained glucose to yield a 0.1 M solution when the grower added tap water to a designated level. A second bottle contained glucose and vinclozolin (Ornalin 50WP) to yield 1.2 mg vinclozolin/ml of 0.1 M glucose. Four to 6 sunflower seedlings were grown in each beaker until the first true leaves were visible (@ 10 days). One drop of glucose was applied to the apex of each seedling in one beaker and a drop of glucose/vinclozolin to each seedling in the second beaker. Segments Botrytis-infected tissue to be tested were placed in each drop. The beakers were sealed in separate plastic bags with tap water in the bottom and placed in a bright location. Inoculated glucose treated seedlings collapsed and died in 7 days. Vinclozolin resistant Botrytis isolates killed vinclozolin treated seedlings while sensitive isolates did not. Test reliability was verified using isolates identified as resistant or sensitive by other methods. Botrytis isolates from infected sunflower tissue returned by growers were tested for resistance by other methods to verify grower results.

A474

SPECIFIC DNA PROBES TO DIFFERENTIATE HIGHLY AND WEAKLY VIRULENT PATHOTYPES OF LEPTOSPHAERIA MACULANS. B. Xue and P.H. Goodwin, Environmental Biology, Univ. of Guelph, Guelph, Ont. N1G 2W1.

Regions of mitochondrial and chromosomal DNA were sequenced from weakly virulent ('Unity') and highly virulent (LM26, 'Leroy') isolates of L. maculans. The sequence of a portion of the mitochondrial large rRNA gene was highly conserved within L. maculans, but was highly divergent between L. maculans and Aspergillus. Compared to Aspergillus, 'Leroy' and LM26 had an additional 60 bp of AT-rich DNA in common with 'Unity' which also had with an adjacent extra 49 bp. The sequence of the Internal Transcribed Spacer 1 (ITS 1) of the chromosomal rRNA genes differed between 'Unity' and 'Leroy' but less so than between these isolates and other fungi (Neurospora, Thermomyces) and several plant species. ITS 1 of 'Leroy' and LM26 were almost identical. Oligonucleotides were selected from ITS 1 sequences differing between pathotypes and were used in a PCR-based assay to differentiate highly and weakly virulent isolates. This pathotype-specific assay is being applied to detect L. maculans in infected plant tissue.

A475

THE EFFECT OF USING A HORSFALL-BARRATT SCALE ON ACCURACY AND PRECISION OF VISUAL ESTIMATIONS OF POTATO LATE BLIGHT SEVERITY. G. A. Forbes and J. T. Korva. International Potato Center (CIP), P.O. 17-16-129-CEQ, Quito, Ecuador.

Assessments of potato late blight severity of 11 people using direct percentage estimation (DPE) were compared with those of another group of 10 people using a scale with wider intervals in the mid range of severity (25 - 75 % infection), placing it in the class of Horsfall-Barratt scales. The scale we tested is frequently used at the International Potato Center (CIP) for germplasm evaluations. DPE readings had greater variances among assessors at mid range of severity. Transformation of DPE to CIP scale units corrected uneven variances. Direct utilization of the CIP scale in the field, however, did not correct uneven variances, suggesting that people tend to linearize the scale intervals, which are based on logarithms. Therefore, use of the CIP scale resulted in a general loss of precision. Accuracy, measured by comparing observed severity to a non-biased measure of true severity, was significantly greater among assessors using DPE ($P < 0.001$).

A476

A NON-DESTRUCTIVE TECHNIQUE FOR THE ASSESSMENT OF HEALTHY AND DISEASED LEAF AREA. J.T. Korva and G.A. Forbes. International Potato Center CIP, P.O. 17-16-129-CEQ, Quito, Ecuador.

A method for non-destructively assessing diseased and healthy leaf area (LA) was developed. The procedure involves counting the number of leaves in the paths of vertical lines descending from a predefined horizontal grid suspended above the plant. The horizontal projection of leaf area is calculated as:

LA_H = (sum of passes) * (grid row distance) * (grid column distance).

Real LA, if necessary, is estimated by calibration with a destructive method, assuming that the average leaf angle does not vary excessively. Initial use of sunbeams limited the method to low latitudes, around noon. A laser flashlight resulted slow in practice. The best results were obtained by looking through the vertically aligned pairs of holes in a perforated double table, and displacing each leaf, one by one, seen in the center of the exposed area. When tested against a destructive method, the technique worked equally well for healthy and diseased leaf area of potato plants infected by *Phytophthora infestans* (R^2 = 0.92***).

A477

IN VITRO BIOASSAYS OF FUMONISIN PHYTOTOXICITY ON THREE WEED SPECIES Joseph O. Kuti[1] and Hamed K. Abbas[2], [1]Department of Agronomy and Resource Sciences, Texas A&I University, Kingsville, TX 78363 and [2]USDA-ARS, Southern Weed Science Lab., Stoneville, MS 38776.

In vitro bioassays were developed to investigate cellular toxicity of fumonisin B_1, a phytotoxin obtained from *Fusarium moniliforme*, on jimsonweed (*Datura stramonium* L.), hemp sesbania (*Sesbania exaltata* Rydb. ex. Hill) and prickly sida (*Sida spinosa* L.). Cotyledon explants were used to initiate callus culture and leaf tissues were used to produce cell suspension and protoplast cultures. The cultures were incubated in media supplemented with 0-100 ug ml^{-1} of the toxin at 25°C for 0-6 hrs. Callus fresh weight, cell viability and electrolyte leakage of cells and protoplasts were determined after exposure to the toxin. Hemp sesbania and prickly sida were very sensitive to the cytotoxic effect of fumonisin (ED_{50} of < 1 μg ml^{-1}) while jimsonweed was relatively tolerant (ED_{50} of > 5 μg ml^{-1}). Both cell suspension and protoplast assays were more sensitive and reliable than callus assay.

A479

DIFFERENTIATING LEVELS OF BACTERIAL STRIPE RESISTANCE IN WHEAT BY DISEASE REACTION. **Milus, E. A. and Mirlohi, A. F., Dept. of Plant Pathology, University of Arkansas, Fayetteville, AR 72701.**

Disease reactions under controlled conditions were used to evaluate resistance to *Xanthomonas campestris* pv. *translucens* on primary and flag leaves of wheat cultivars. Leaves were inoculated at discrete sites using a syringe tipped with a short length of rubber tubing and scored for disease reaction on a 0-5 scale for primary leaves (based on the amount of watersoaking) and a 0-4 scale for flag leaves (based on the amount of chlorosis and/or watersoaking). On flag leaves of four cultivars there was a quadratic relationship between disease reaction and $\log_{10}$ population of the pathogen. Disease reaction and population estimates gave similar rankings among the cultivars, and Terral 101 was the most resistant. Differences in reaction among ten cultivars were more distinct on primary leaves than on flag leaves, and Terral 101, Twain and Keiser were the most resistant. The inoculation technique and reaction scales should be useful for identifying resistant wheat lines and differences in virulence or aggressiveness among strains of the pathogen.

A480

PROGRESS OF *SEPTORIA NODORUM* INFECTION ON SUSCEPTIBLE AND MODERATELY RESISTANT WHEAT CULTIVARS. **Penix, S. E.[1], Milus, E. A.[1], and Gbur, E. E. Jr.[2], Dept. of Plant Pathology[1] and Agric. Statistics Lab[2], University of Arkansas, Fayetteville, AR 72701.**

Progress of *S. nodorum* infection was monitored throughout the spring on replicated field plots of susceptible (Caldwell) and moderately resistant (Florida 302) soft red winter wheat cultivars in 1990. Samples of leaves from all available positions were rated for predominant symptom type (symptomless, flecks or typical) and percent lesion area and plated to determine whether the samples were infected by *S. nodorum*. Incidence of infection on all leaf positions at various Feekes' growth stages (gs) generally was greater on Caldwell than on Florida 302, and Caldwell had a greater proportion of infection associated with fleck and typical symptoms than Florida 302. Sixty-three percent of all infected samples from Florida 302 were symptomless. Compared to the same leaf position on Florida 302, percent lesion area was greater on flag-2 leaves of Caldwell at gs 9 to 10.5 and on flag-1 leaves of Caldwell at gs 10.5 to 11.

A481

PATHOGENIC VARIABILITY OF *SEPTORIA TRITICI* ON FIVE WINTER WHEAT CULTIVARS. H. U. Ahmed, S. M. Coakley, and C. C. Mundt, Oregon State University, Department of Botany and Plant Pathology, Corvallis, OR 97331-2902.

Fifteen Septoria tritici isolates, including six from California, six from Oregon and three from Texas, were tested for their pathogenic variability on five common winter wheat cultivars grown in Oregon. The five cultivars (Gene, Hill, Malcolm, Madsen and Stephens) were grown in pots in the greenhouse and inoculated with a pycnidiospore suspension at a concentration of 8×10^6/ml 4 wk after seeding. Percent diseased leaf area for 10 plants in 4 replications was recorded 3 wk after inoculation. There were significant varietal differences ($p = 0.0001$), isolate differences ($p = 0.0001$) and variety X isolate ($p = 0.0001$) interactions. The five cultivars fell into three distinct groups: resistant (Gene), intermediate (Hill, Malcolm and Stephens), and susceptible (Madsen). Among the isolates, those from Oregon were more virulent than the isolates from California and Texas, which suggests that the Oregon isolates have better fitness than the others on the Oregon cultivars tested.

A482

CROP MANAGEMENT EFFECTS ON PHYSIOLOGIC LEAF SPOT OF WINTER WHEAT. R.W. Smiley, W. Uddin, P.K. Zwer, L.-M. Gillespie-Sasse, and M.A. Stoltz. Oregon State University, Columbia Basin Agric. Res. Ctr., P.O. Box 370, Pendleton, OR 97801.

Effects of physiologic leaf spot on grain yield are unknown. Objectives of studies from 1990 to 1992 were to quantify disease severity on existing crop management experiments and estimate effects of disease on grain yield. Winter wheat cultivars varied from resistant to susceptible. Severity decreased as nitrogen rate increased and planting date was delayed. Severity was reduced and yield increased by foliar application of urea + calcium monocarbamide monohydrogen chloride, and was unaffected by multiple applications of fungicides. Severity was unaffected by stubble burning and by nitrogen source or application timing. Severity was lowest in a wheat/pea rotation, intermediate in wheat/fallow, and highest in annual wheat. Regression of yield and percentage necrotic leaf area indicated that this leaf spot constrained grain yield by 10%. Etiology of the disease remains unknown.

A483

NITRATES LEFT IN THE SOIL PROFILE AFTER HARVEST OF WHEAT AND BARLEY RELATE TO SEVERITY OF ROOT DISEASES. R. J. Cook, B. H. Ownley, USDA-ARS, Pullman, WA 99164, and P. Rasmussen, USDA-ARS, Pendleton, OR 97801.

Nitrate-N left in the soil after harvest related directly to root disease severity for winter wheat, spring wheat, and spring barley. For the winter wheat, take-all (*Gaeumannomyces graminis* var. *tritici*) was more severe and grain yield averaged 8.2% (600 kg ha^{-1}) less with direct drilled (no-till) than with preplant tillage (both sig. @ $P<0.05$), and the 180-cm soil profile contained 22 kg ha^{-1} more nitrate with no-till than with till, with the difference at 180 cm significant at $P=0.05$. For the spring wheat, yields averaged 2968 kg ha^{-1} and 301 kg residual nitrate-N ha^{-1} remained in the 180-cm profile with Rhizoctonia root rot (*Rhizoctonia solani* AG8), and 5783 kg ha^{-1} with only 87 kg residual nitrate-N ha^{-1} nitrate N in plots with this root rot controlled with methyl bromide (MB). The spring barley also had Rhizoctonia root rot; more residual nitrate-N ($P=0.02$) remained in the top 30 cm of soil when fertilizer shanks were positioned to loosen the soil and place N, P and S 10 cm to one side of the seed than when positioned within each seed row (controls Rhizoctonia root rot); but not in plots with root rot controlled by MB.

A484

BARLY YELLOW DWARF VIRUS TRANSMISSION BY RUSSIAN WHEAT AND GREEN PEACH APHIDS IN MOROCCO. M.EL-YAMANI[1], B.BENCHARKI[1], A. COMEAU[2], and K.MAKKOUK[3], [1] INRA/MIAC, Settat, Morocco, [2] Station de Recherche, Quebec, Canada, [3] ICARDA, Aleppo, Syria.

Transmission of PAV-type of BYDV by 5 populations of Diuraphis noxia and one of Myzus persicae was studied during 1989-92 in Morocco. Aphid transmissions to oat-test-plants and ELISA were the techniques used. Test-plants directly inoculated by aphids collected from diseased cereals yielded 7% transmission by D. noxia and 14% by M. persicae. ELISA performed on single aphids of D. noxia taken directly from diseased cereals showed that 7% carried the virus and 14% acquired it when given a suplementary feeding for 48 hr prior to testing against 16% for M. presicae. The 5 populations of D. noxia transmitted BYDV-PAV from 5 different locations. Compared to 4 other aphid vectors of the virus occurring in the country, D. noxia was least efficient requiring longer access periods of acquisition and inoculation.

A485

C.D.Lamison

DU PONT ADVISOR FIELD TEST FOR CEREAL FOOT ROT

E.I. du Pont de Nemours and Company, Wilmington, Delaware

Du Pont has developed a 10-minute field assay for the semi-quantitative detection of cereal foot rot. Using the same antibodies as the Du Pont Advisor ELISA test, the field test gives similar, but less precise results. Benefits and drawbacks of the two methods are discussed. Results of the field testing in Europe and the US in 1990 and 1991 are presented. The test will be demonstrated at the diagnostics workshop.

A486

EFFECTIVENESS OF QUARANTINES FOR CONTROL OF FLAG SMUT (*Urocystis agropyri*) OF WHEAT. Roland F. Line, USDA-ARS, Washington State University, Pullman, WA 99164-6430

A USA quarantine to prevent flag smut (FS) introduction has existed since 1919 when FS was first discovered in Illinois. Quarantine actions to prevent spread within the USA were also initiated in 1919 but discontinued in 1923 because they were ineffective. FS was undetectable by 1934. FS was reported in the Northwest in 1940. By 1968, it was the most important disease in two counties. By 1973, it was controlled by seed treatment. Resistant cultivars and improved management also controlled FS. FS has not spread to other regions. The "flag smut problem" resulted from growing highly susceptible cultivars in a favorable environment made more favorable by crop management. World-wide, FS is not as damaging as once thought; it occurs only in certain regions and has not spread to other areas because of unique environmental requirements; and it is easily controlled by seed treatments, resistant cultivars, and preventative management. In retrospect, the regulations did not control FS and were and are not necessary.

A490

OBSERVATIONS ON TAXA IN SECTION LEPIDELLA OF THE GENUS AMANITA. R.P. Bhatt and O.K. Miller, Jr. Virginia Polytechnic Institute and State University, Blacksburg, Va. 24061.

Genus Amanita, a well known ectomycorrhizal fungus is common and widely represented in the mixed coniferous and deciduous forests of North America. During the course of present investigations section Lepidella which forms a major component of the genus Amanita has been studied. New information is presented here on the ecology, morphology and distribution of selected taxa within the subgenus Lepidella. The key features of confused and misidentified taxa are discussed.

A499

ENVIRONMENTAL CONDITIONS ASSOCIATED WITH RHIZOCTONIA "WINTER-KILL" OF WHEAT IN INDIANA. D.M. Huber, R.E. Baird and T.S. McCay-Buis. Botany & Plant Pathology, Purdue University, West Lafayette, IN 47907.

A severe November frost throughout the Eastern Cornbelt in 1991 killed leaf tissues of winter wheat and provided a nutrient base for early saprophytic development and infection by *Rhizoctonia solani* (BN). Wheat health continued to deteriorate during the cool, cloudy, wet winter months which were near optimum for fungal development but not conducive for plant growth or photosynthesis in Indiana. Extensive plant kill by *Rhizoctonia* resulted in abandonment of many fields and an estimated loss of over 60% of the winter wheat crop. Although some varietal differences were noted, the most significant reduction in disease severity occurred (50-60% stand) in wheat which was preplant fertilized with cattle manure. Adjacent areas of manured fields which received inorganic fertilizer were severely damaged (0-10% stands). Early seeded wheat and wheat in specific areas of fields were less severely damaged. This resistance to *Rhizoctonia* was associated with higher Zn and carbohydrate levels in plant tissues.

A500

LEVEL OF GRASS IN PASTURE FAILS TO INFLUENCE RHIZOCTONIA (AG8) BARE PATCH IN SUBSEQUENT BARLEY CROP. G.C. Mac Nish, C.K. McLernon, and D.A. Wood, Department of Agriculture, Esperance, Australia, 6450.

Rhizoctonia bare patch, caused by *R. solani* (AG8 - zymogram strains ZG1-1, ZG1-2 , ZG1-4 and ZG2) is an important disease of cereals, lupin(*Lupinus angustifolius* L.) and pasture in the southern parts of the cereal belt of Australia. There has been considerable debate about the importance of grasses in the formation of bare patches in crops following pasture. An experiment is described which tests the hypothesis that a high level of grass in pasture leads to increased bare patch incidence in the subsequent crop. The pasture treatments were : (1) Mixed grass [mainly *Lolium rigidum* Gaud. and *Hordeum leporinum* Link] and broad leaf pasture [mainly *Trifolium subterraneum* L. and *Cryptostemma calendula* Druce] - ungrazed, (2) Pure grass pasture - ungrazed, (3) Pure grass pasture - grazed, (4) Grass free broad leaf pasture - grazed, (5) Plant-free chemical fallow. The incidence of rhizoctonia bare patch in the subsequent barley crop was not significantly affected by any treatment. There was, however, a trend towards more patch in the plant-free treatment , thus the hypothesis was not supported.

A501

CHARACTERIZATION OF PEANUT ISOLATES OF *RHIZOCTONIA SOLANI* FROM GEORGIA. M. A. Cubeta, D. Gonzalez, R. Vilgalys, and T. B. Brenneman*, Dept. of Botany, Duke University, Durham, NC 27706; *Dept. of Plant Pathology, University of Georgia, Coastal Plain Expt. Stn. Tifton, GA 31793.

Isolates of *Rhizoctonia solani* on infected peanut hypocotyls were obtained from 20 different major peanut producing counties in Georgia, and characterized using hyphal anastomosis and rDNA restriction analysis by PCR fingerprinting. Of 50 isolates which were studied, 72% could be assigned to AG-2, 4% to AG-4 HGI, and 4% to AG-4 HGII, while 20% were unassignable to any anastomosis group. Three predominant restriction phenotypes based on rDNA type were identified for the AG-2 isolates, with 14%, 24%, and 34% of the isolates belonging to each class. Sixty additional *R. solani* isolates obtained from peanut hypocotyl, midstem, stem-tip and soil from an individual peanut field in Tift Co. were also studied. Sixty percent of the isolates could be assigned to AG-2, 5% to AG4 HGI, while 35% were unassignable. The 3 predominant restriction phenotypes of the AG-2 isolates identified in the county survey represented 5%, 25%, and 30% of the isolates belonging to each phenotypic class. These results suggest a similar distribution of pathogenic AG-2 and AG-4 types of *R. solani* at each level of sampling. Additional characterization of these *R. solani* isolates using RAPD markers and DNA/DNA hybridization are underway and will also be discussed.

A502

FACTORS INFLUENCING SURVIVAL OF PHIALOSPORES OF *CHALARA ELEGANS* (SYN. *THIELAVIOPSIS BASICOLA*) IN ORGANIC SOIL. S. Chittaranjan and Z.K. Punja, Dept. of Biological Sciences, Simon Fraser University, Burnaby, B.C., Canada V5A 1S6.

Chalara elegans, the causal agent of black root rot on carrots, is widespread among commercial fields in the Fraser Valley of B.C. The influence of moisture level (55±5% constant or gradual drying), host plants (carrot), nonhost plants (onions, rye), fallow soil, flooding of soil at 4 C or 25 C, addition of $CaCO_3$ (1%, w/v) or various air dried plant tissues: rye (0.25%), onions, alfalfa or carrots (0.5%, w/v) on survival of *C. elegans* was determined. Phialospores (endoconidia) from V-8 agar cultures were used as inoculum. Survival in soil (O.M. content 80%, pH 5.2, calcium 9146 ppm) was monitored for 19-20 wk using a semiselective medium. In fallow soil at constant moisture, survival was reduced to 22.9% of the initial inoculum level after 15 wk. Survival with carrot plants, $CaCO_3$, and flooding at 4 C was similar to that in fallow soil. Significant reductions in survival ($P \geq 0.01$) were observed with high temperature flooding (25 C) and with onions (shallot or green) within 5 wk. Enhanced levels of *Penicillium* and *Trichoderma* were isolated from soil planted to onions, and may account, in part, for the reduced survival of *C. elegans*.

A503

INHIBITION OF GERMINATION AND GROWTH OF *THIELAVIOPSIS BASICOLA* BY ALUMINUM AND A CALMODULIN ANTAGONIST. J. R. Meyer, H. D. Shew and U. J. Harrison. North Carolina State University, Raleigh, NC 27695-7616.

The effects of aluminum (Al) on the germination, growth and reproduction of *T. basicola* were tested at levels (0 to 1.1 meq) found in soils conducive and suppressive to black root rot disease of burley tobacco. Germination of endoconidia and chlamydospores was inhibited at Al concentrations >0.5 meq. In carrot agar amended with Al, fungal growth was sparse and spore production was inhibited. The effect of Al was dose-dependent and was greatest at low nutrient levels. A similar series of experiments was conducted with W-7, a calmodulin antagonist. Calmodulin is a calcium binding protein that is denatured by Al ions. Significantly fewer endoconidia germinated and fewer spores were produced on carrot agar amended with W-7 than on unamended carrot agar. The response was dose-dependent and greatest at low levels of nutrients. Results indicate that Al affects calcium regulatory pathways important in vegetative and reproductive growth of *T. basicola*.

A504

NUTRIENT DEPRIVATION AFFECTS RESPONSES OF *PYTHIUM ULTIMUM* SPORANGIA TO GERMINATION STIMULANTS. E. B. Nelson and J. Hsu, Cornell University, Department of Plant Pathology, Ithaca, NY 14853

Sporangia of *P. ultimum* produced on living plant tissues or in lipid-containing media, fail to germinate in response to sugars and amino acids but remain highly responsive to unfractionated seed exudates. Our study was designed to examine nutritional factors regulating these responses. As mycelial cultures aged on media containing living plant tissue or ∝-phosphatidyl choline, glucose-responsive germination of sporangia arising from these mycelia increased from less than 5% at 4 days of age to nearly 100% by 14 days. Responses of sporangia to unfractionated cotton seed exudate remained high (80-100%) throughout the 14-day period. Growth (4 days) of mycelial cultures on media containing decreasing levels of either glucose, asparagine, ammonium sulfate, or ∝-phosphatidyl choline gave rise to sporangia with increasing responses to seed exudate. Germination of sporangia in response to glucose remained low regardless of the concentration of glucose, asparagine, or ammonium sulfate in the medium. Decreasing levels of ∝-phosphatidyl choline in the medium, however, increased the response of sporangia to glucose. Four-day-old sporangia that were unresponsive to glucose became highly responsive after exposure to microbial-induced soil nutrient stresses. Results suggest that culture age and nutrient deprivation can regulate qualitative and quantitative responses of sporangia to germination stimuli.

A505

THE INFLUENCE OF INFECTION BY *PYTHIUM* SPP. ON ROOT SYSTEM MORPHOLOGY OF ALFALFA SEEDLINGS. R.P. Larkin, J.T. English, and J.D. Mihail, Dept. of Plant Pathology, University of Missouri, Columbia 65211.

The effect of infection of alfalfa seedlings by *Pythium* spp. on root morphology was monitored in relation to seed or soil treatment with metalaxyl in greenhouse and field experiments. Root system morphologies were characterized by the abundance, length, and branching pattern of root orders using the morphometric and topological assessment systems. Several *Pythium* spp. isolated from alfalfa roots in the field, including *P. ultimum*, *P. irregulare*, and *P. sylvaticum*, caused damping-off, root necrosis, and reductions in overall root and stem length in pathogenicity tests. Many isolates also caused alterations in root structure and morphology, including reductions in root branching, specific root length, and root system pathlength. Metalaxyl applied to the soil was more effective than seed treatment in reducing infections and alterations in root morphology caused by these pathogens.

A506

INFLUENCE OF FAST-AND SLOW-GROWING *PYTHIUM* SPECIES ON THE HERBICIDAL EFFECT OF GLYPHOSATE ON BEANS. R.C. Descalzo, C.A. Lévesque, Z.K. Punja, and J.E. Rahe, Dept. of Biological Sciences, Simon Fraser University, Burnaby, B.C., Canada, V5A 1S6.

Pythium isolates from roots of glyphosate-treated bean plants grown in different soil types were identified and grouped based on DNA RFLP patterns. Each isolate was tested for growth rate on PDA and pathogenic characteristics on beans. Colony growth rate was evaluated after 24 hours, and pathogenicity was tested by planting bean seeds in infested metro-mix soil medium to determine the rate of pre-emergence damping-off. Representative isolates from each group were compared for their efficiency as glyphosate synergists. This was done by treating 2-week-old bean plants growing in soil medium infested with the respective isolate with six different doses of glyphosate (0, 2, 6, 20, 50, and 150 µg/plant). Two weeks after treatment, plant mortality was counted and the LD_{50} was determined by logistic regression. Pathogenicity tests showed that fast-growing isolates were more virulent than the slow-growing isolates, and lower glyphosate levels were needed to kill plants grown in soil infested with the former than with the latter. These results suggest that growth rates of *Pythium* may reflect their efficiency as glyphosate synergists.

A507

DEVELOPMENT OF A SEROLOGICAL ASSAY TO SELECTIVELY DETECT PYTHIUM ARRHENOMANES. J. W. Hoy, Dept. Plant Path. and Crop Phys., La. Ag. Exp. Sta., La. State Univ. Ag. Center, Baton Rouge, LA 70803.

An indirect ELISA for the selective detection of Pythium arrhenomanes from amongst Pythium spp. isolated from sugarcane roots was developed using antibodies separated from a polyclonal antiserum by affinity column purification. An antiserum was prepared against an isolate of P. arrhenomanes. The I_gG population was passed twice through an agarose column to which antigens of P. catenulatum, P. dissotocum, P. irregulare, and P. spinosum were bound. Antibodies that did not recognize antigens to the other Pythium spp. were collected and used in an indirect ELISA using goat-antirabbit, alkaline phosphatase conjugate. A rapid, strong reaction was obtained with agar colony samples of 21 P. arrhenomanes isolates. Reactions were negative for 5, 2, 6, and 8 isolates of P. dissotocum, P. heterothallicum, P. irregulare, and P. spinosum, respectively. Slow developing reactions were observed for 2 of 7 P. catenulatum and 2 of 6 P. torulosum isolates.

A508

RELATEDNESS OF DISTINCT POPULATIONS OF PHYMATOTRICHUM OMNIVORUM DETERMINED BY GENETIC MARKERS. J. L. Riggs and S. D. Lyda, Dept. of Plant Pathology and Microbiology, Texas A&M University, College Station, TX 77843.

Genetic variation of Phymatotrichum omnivorum was analyzed by restriction fragment length polymorphisms (RFLPs). Total genomic DNA was extracted from 75 P. omnivorum isolates obtained from different hosts and geographic regions. One population was extensively surveyed to detect variation within a single infestation site. The DNA was probed with a Saccharomyces cerevisiae ribosomal DNA (rDNA) probe along with probes originating from a mitochondrial DNA (mtDNA) library of P. omnivorum. A cotton isolate with the distinctive morphological characteristics of P. omnivorum differed from other isolates with respect to RFLP patterns obtained from DNA digested with six restriction enzymes (BAMH I, DRA I, ECO RI, HIND III, MSP I, and PVU II) and probed with the rDNA or mtDNA probes. No variation was observed among cultures from the single focus. Pathogenicity was verified for all isolates in greenhouse tests. To date little genetic variation has been detected among the P. omnivorum isolates examined.

A509

VERTICILLIUM WILT OF PRUNUS ORCHARD TREES CONTROLLED WITH POLYETHYLENE MULCH. J. J. Stapleton[1], E. J. Paplomatas[2], R. J. Wakeman[2], and J. E. DeVay[2], Univ. of California, Kearney Agricultural Center[1], Parlier 93648, and Dept. of Plant Pathology, Davis 95616[2].

First-leaf apricot (Prunus armeniaca) and almond (P. dulcis) trees were planted in soil infested with Verticillium dahliae, and either mulched (solarized) with clear or black polyethylene film, or not mulched, in the San Joaquin Valley during March-August 1990. Mulched trees were not irrigated during the treatment period. Soil temperatures were elevated in mulched treatments. Trees mulched with clear polyethylene did not survive or grow as well as those mulched with black film. Foliar symptoms in experimental trees were reduced 86-100% by film mulches in 1991. Vascular discoloration in trunks and scaffolds were similarly reduced by mulches. Black film mulch was better than clear because Verticillium wilt was controlled but trees were not chronically injured.

A510

EFFECT OF ORGANIC AMENDMENTS AND SOLARIZATION ON PATHOGEN CONTROL, RHIZOSPHERE MICROBIOLOGY, PLANT GROWTH, AND VOLATILES IN SOIL. A. Gamliel and J. J. Stapleton, University of California, Kearney Agricultural Center, Parlier, CA 93648.

Field solarization of composted chicken manure-amended soil gave better control of Meloidogyne incognita and Pythium ultimum and higher lettuce yield than either treatment alone. Numbers of pathogens, as well as of total fungi, were significantly lower in the rhizosphere of lettuce plants grown in chicken manure-amended, solarized soils. In contrast, population densities of fluorescent pseudomonads and Bacillus spp. were higher in plants grown in solarized soil, whether amended or not. When studied in vitro, numbers of P. ultimum in soil amended with composted chicken manure or dried cabbage were reduced by heating, even at sublethal temperatures. Changes in volatile evolution in heated, amended soil may be related to increased biocidal activity.

A511

A QUANTITATIVE METHOD FOR THE RECOVERY OF ASCOSPORES OF *MONOSPORASCUS CANNONBALLUS* FROM FIELD SOIL. M.E. Stanghellini and S.L. Rasmussen. Dept. of Plant Pathology, University of Arizona, Tucson, AZ 85721

Ascospores of *Monosporascus cannonballus* were recovered from naturally-infested field soil as follows: 20 g of soil were placed in a flask containing 200 ml of sterile distilled water (SDW) with a magnetic stir bar and mixed for 5 min. The contents of the flask were passed through nested 75-µm and 38-µm sieves. Material retained on the 38-µm sieve was washed into a centrifuge tube and pelleted at 900 *g* for 4 min. The supernatant was discarded and the pellet was resuspended in 50% sucrose and centrifuged at 900 *g* for 2 min. The supernatant, which contained the ascospores, was decanted onto a 38-µm sieve and ascospores retained on the sieve were washed into a petri dish. The pellet resulting from the first sucrose centrifugation was resuspended in 50% sucrose and the subsequent procedure repeated. Ascospores in the petri dish was counted using a dissecting microscope at 60 X.

A512

MORPHOLOGY AND GERMINATION OF *MONOSPORASCUS CANNONBALLUS* ASCOSPORES. R. Martyn, J. Mertely, M. Miller, C. Katsar, and R. Baasiri. Department of Plant Pathology and Microbiology, Texas A&M University, College Station (77843) and Weslaco (78596), TX.

Monosporascus cannonballus is a recently described ascomycete that causes a root rot/vine decline disease of melons and other cucurbits. It produces a single, large, spherical, jet-black, multinucleate ascospore per ascus, but no conidial stage has been observed. Germination of the ascospores has not been reported. Mean ascospore diameter of five isolates was 42.6-46.5 µm and mean number perithecia/cm^2 *in vitro* ranged from 120-259. The ascospore has an outer, pigmented wall (epispore) and a thick, hyaline inner wall accounting for 15-20% of the diameter. Ascospores have a specific gravity in sucrose of 1.23-1.28. Ascospore germination was observed after heat-treating 6-mo-old spores to 45 C for 10 min, although maximum germination was <0.05%. A 5% Chlorox (0.26% hypochlorite) treatment for 4 min also resulted in some germination, but less than heating. Germination occurred from multiple sites, typical of that reported for *M. eutypoides*, but no germ pores were observed. Inoculation of muskmelon plants with treated spores did not result in any detectable disease symptoms after 75 days, but *Monosporascus* was isolated from roots of 4/10 and 2/10 plants, respectively, for the 45 C heat and 5% Chlorox treatments.

A513

QUANTIFICATION OF *MONOSPORASCUS CANNONBALLUS* ASCOSPORES IN COMMERCIAL MUSKMELON FIELDS IN SOUTH TEXAS. J. C. Mertely, R. D. Martyn, and M. E. Miller, Department of Plant Pathology and Microbiology, Texas A&M University, College Station 77843 and Weslaco 78596, and B. D. Bruton, USDA, ARS, SCARL, Lane, OK 74555.

Monosporascus cannonballus ascospores in soil samples from two adjacent south Texas muskmelon fields with different cropping practices were quantified following the 1991 harvest. Eight transects within a 100 x 50 m area in each field were systematically sampled. In field "A" (bare soil, furrow irrigation, deep tillage, and

crop rotation), five 0-30 cm depth samples were removed per transect. In field "B" (plastic mulch, drip, shallow tillage, and successive annual cropping), five samples at three depths were removed from beds and between beds, for a total of 30 per transect. Average ascospore densities within beds were 3.4, 4.2, and 3.6/g dry wt of soil at 0-10, 10-20, and 20-30 cm, respectively. Corresponding densities between beds were 2.9, 4.3, and 3.1. In-bed spore density was significantly greater than between-bed density only at 0-10 cm ($P=0.03$); however, spore density at 10-20 cm was significantly higher than at 0-10 or 20-30 cm ($P=0.01$ & 0.03, respectively). An average spore density of 2.6 in "A" was significantly lower than in-bed or between-bed averages for "B". These results suggest an association between successive in-bed cropping practices and increased inoculum densities.

A514

WOUND-ASSOCIATED INFECTION BY CYTOSPORA SP. IN ALNUS INCANA, CRATAEGUS DOUGLASII, AND CORNUS STOLONIFERA IN OREGON. G.M. Filip, Dept. of Forest Science, Oregon State Univ., Corvallis, OR 97331, C.A. Parks, and G.L. Starr, Pacific N.W. Res. Stn., USDA For. Serv., 1401 Gekeler Ln., La Grande, OR 97850.

We chose to determine the cause of mortality associated with cankers on stems of dead and dying mountain alder in the Grande Ronde River drainage near La Grande, Oregon. Dieback was absent in associated species of black hawthorn and red-osier dogwood. Cytospora sp. was isolated from necrotic tissue at canker margins. Koch's postulates were conducted by isolating the fungus from cankers on affected alder, inoculating healthy stems in the field, and reisolating the fungus from newly developed cankers. Incidence of infection was significantly higher in alder than in hawthorn or dogwood. Significantly more infection occurred in wounded alder stems than in unwounded stems. Stem wounds can be caused by several factors including large mammals, but mass movement of river ice also causes periodic killing and wounding of trees and shrubs in riparian zones.

A515

TREE VIGOR AND SUSCEPTIBILITY TO INFECTION BY PHELLINUS WEIRII: RESULTS OF FIELD INOCULATIONS. E. M. Goheen and E. M. Hansen. USDA Forest Service, Forest Pest Management, Portland, OR 97208 and Dept. of Botany and Plant Pathology, Oregon State University, Corvallis, OR 97331.

Artificially wounded and unwounded roots of Douglas-fir and mountain hemlock in different vigor classes were inoculated with isolates of Phellinus weirii, the cause of laminated root rot, on 4 study sites in Oregon. Tree vigor differences were determined by computing sapwood basal area to growth ratios. Mycelium grew onto roots from inoculum blocks in 83% of 710 inoculations. After 2-3 years, the extent of ectotrophic growth of P. weirii mycelium ranged from 5.1 cm to 72 cm with an average growth of 33 cm. Within study sites, no significant difference in extent of ectotrophic mycelial growth occurred between trees of different vigor classes. Mycelial growth differed among study sites. P. weirii was isolated from phloem and xylem of successfully inoculated roots. The fungus caused advanced decay of xylem when roots were intentionally or inadvertently wounded during excavation and inoculation.

A516

A SEARCH FOR ANTIFUNGAL COMPOUNDS IN CALLUS TISSUE OF AMERICAN AND CHINESE CHESTNUT CHALLENGED BY CRYPHONECTRIA PARASITICA. Z. Solel, ARO, POB 6, Bet-Dagan, Israel and L. Shain, Department of Plant Pathology, University of Kentucky, Lexington, KY 40546.

American chestnut calli were colonized more quickly and were more intensely discolored than Chinese chestnut calli after inoculation with C. parasitica. Bioassays of methanol and hexane extracts were conducted on thin-layer chromatograms developed with ethyl acetate: methanol: water (3:1:1) and sprayed with conidia of Cladosporium cucumerinum or C. parasitica. Although extracts of both species contained compounds which caused zones of fungal inhibition, clear evidence for the occurrence of a preformed or induced inhibitor(s) which may account for blight resistance of chinese chestnut was not found in this limited study. Bioassay results with the two test organisms differed greatly demonstrating the importance of using the target organism rather than a convenient surrogate for such studies.

A517

ANTHRACNOSE RESISTANCE AMONG *CORNUS* SPECIES. D.A. Brown (1), M.T. Windham (2), and R.N. Trigiano (2). School of Forestry (1), Auburn University, AL, 36849, and University of Tennessee Inst. Agric. (2), Knoxville, TN 37901-1071.

Dogwood anthracnose, caused by *Discula destructiva*, poses a significant threat to flowering dogwoods (*C. florida*). Seven *Cornus* species, including *C. alternifolia*, *C. amomum*, *C. controversa*, *C. florida*, *C. kousa*, *C. kousa* cv 'Chinensis,' *C. mas*, and *C. sericea* were placed in a disease resistance trial at Ozone, Tennessee. Trees were placed under native flowering dogwoods with *Discula* anthracnose and rated weekly for disease development using a modified Horsfall-Barrett assessment scale. As symptoms developed, samples were taken for laboratory confirmation of *D. destructiva*. Greatest disease severity was observed on *C. controversa*, *C. florida*, *C. kousa* cv 'Chinensis,' and *C. sericea*. Symptoms developing on each were similar to those observed on flowering dogwood. The other *Cornus* species tested, including the unnamed Chinese dogwood cultivar, appeared anthracnose-resistant.

A518

SIBERIAN ELM RESISTANCE TO BOTRYODIPLODIA CANKER. J. M. Krupinsky and R. A. Cunningham. USDA, Agricultural Research Service, Northern Great Plains Research Laboratory, P.O. Box 459, Mandan, ND 58554-0459.

The decline of Siberian elm (*Ulmus pumila* L.) windbreaks in the northern Great Plains has been accelerated by canker diseases, particularly Botryodiplodia canker caused by *Botryodiplodia hypodermia*. A replicated clonal test of 81 trees was screened for canker resistance. In 1989, two branches on each tree were wounded by excising a small twig. The wound was inoculated with sterile wheat kernels overgrown with *B. hypodermia*. Canker growth on the inoculated branches was measured in 1990. Six trees each of three clones with poor canker development and three clones with good canker development were reinoculated with six isolates of *B. hypodermia* in 1990. Measurements of canker development in 1991 confirmed the differences among clones observed in 1990. These studies indicate resistance to Botryodiplodia canker can be identified and utilized in a tree improvement program.

A519

EFFECTS OF PARAFORMALDEHYDE ON SUGAR MAPLES TAPPED FOR SAP. I. SURVEY OF DAMAGE IN SOME WISCONSIN SUGARBUSHES. David R. Houston, USDA Forest Service, 51 Mill Pond Rd, Hamden, CT 06514.

Tablets containing paraformaldehyde (PF) are sometimes placed in tapholes (TH's) to increase sap yields. Use of PF is controversial as xylem and cambium around treated TH's can be injured. As part of a larger study of practices to reduce the adverse effects of PF, a survey was made in 1991 of 16 sugarbushes in Wisconsin where PF either had (9) or had not (7) been used in the past 5 years. On 50 trees in each bush, the number of TH's that were a) open, b) oozing, or c) had cambial dieback was noted. The area of cambial dieback around TH's was measured. Analyses showed no apparent relationship between PF use and these taphole attributes, but the amount of cambial dieback was significantly correlated ($p=.02$, $R^2=.28$) with tree crown condition. Other factors, often unique to individual sugarbushes, that did appear to influence these attributes included tapping practices, tree age, stand density and drought stress.

A520

EFFECTS OF INOCULUM SOURCE ON PROGRESSION OF THE OAK WILT DISEASE. F. H. Tainter and S. K. McElreath, Dept. of Forest Resources, Clemson University, Clemson, SC 29634-1003.

Pole to small sawtimber-sized red oak trees at two locations were inoculated with spore suspensions of isolates of Ceratocystis fagacearum from Texas, South Carolina, Wisconsin and West Virginia. Wilting progression and biological deterioration of the trees were assessed at weekly intervals through the following spring. The trees formed two different response groups. In one group, defoliation stopped, trees recovered, and they remained free from attack by secondary organisms. In the second group, trees had largely defoliated within two months after inoculation and were subsequently degraded by twolined chestnut borer, Hypoxylon atropunctatum, Armillaria spp., termites, decay fungi, and woodpeckers. C. fagacearum was not reisolated from roots of these trees one year after inoculation. Wilting patterns were somewhat different for some isolates.

A521

ALLOZYME VARIATION AMONG GEOGRAPHIC ISOLATES OF SPHAEROPSIS SAPINEA. W. J. Swart[1], W.S. Grant[2], and M.J. Wingfield[3], [1]Dept of Plant Pathology, Univ. of the Orange Free State, Bloemfontein 9300, [2]Dept of Genetics, Univ. of the Witwatersrand, Johannesburg 2050, and [3]Dept of Microbiology and Biochemistry, Univ. of the Orange Free State, Bloemfontein 9300, South Africa.

The range of isozymic diversity among isolates of Sphaeropsis sapinea from various parts of the world was investigated by means of starch-gel electrophoresis. Gene products of 14 enzyme-encoding loci were detected of which three loci were monomorphic for all isolates. From the resultant genotypic information, allele frequencies and genetic distances were calculated. Only two loci, Acp-1 and Pep-GL, differentiated clearly between type A and B isolates of S. sapinea from the north central USA. No type B isolates were, however, found from other parts of the world. The general pattern of isozymic diversity reflected relatively high levels of genetic variation within local populations but a lack of sharp dissimilarity between geographic populations. Isolates of S. sapinea from Pinus spp. could not be differentiated from one another on the basis of host species.

A522

POPULATION DYNAMICS OF *HETEROBASIDION ANNOSUM* IN TRUE FIR AS DETERMINED BY CLONAL ANALYSIS. M. Garbelotto, F. Cobb, T. Bruns, W. Otrosina, G. Slaughter, and T. Popenuck. Department of Plant Pathology, University of California, Berkeley, California 94720.

Clonal spread of *Heterobasidion annosum* was studied in three mixed conifer stands dominated by white fir (*Abies concolor*) in the central Sierra Nevada. A total of 210 isolates was collected from stumps as well as from roots and boles of dead and live trees. *H. annosum* was found only on true firs and presumably all of the isolates belong to the "S" intersterility group. Vegetative compatibility (v-c) and molecular fingerprinting techniques were used to determine clonal identity and distribution of the pathogen. Initial results indicate a rather complex pattern of distribution of *H. annosum*. In some cases a single clone was found in a tree or in a group of neighboring trees; in other cases trees growing in close interconnection were colonized by different clones. Multiple clones were often obtained from the same tree, and a direct correlation between isolates from the roots and from the trunk of the same tree was not always detectable. The PCR-based RAPD's (Random Amplified Polymorphic DNA) were useful for clonal identification and for distinguishing among intersterility groups of *H. annosum*.

A523

IMPACTS FROM 1960 THROUGH 1989 OF *PHELLINUS WEIRII* IN TWO DOUGLAS-FIR STANDS IN WESTERN OREGON. T.T. Lawson, Lawson-Rasor Associates, 1224 Navellier Street, El Cerrito, CA 94530.

In 1960, two areas (1.1 ha and 0.9 ha) on the George T. Gerlinger Experimental Forest in western Oregon were set aside to intensively study the expansion of *Phellinus weirii* infection centers and the resulting mortality in Douglas-fir stands. On each plot, all trees were inventoried as to species, diameter, crown class and condition, and presence of *P. weirii*. Both plots were reinventoried in the summers of 1967, 1974, 1978 and 1989. Between 1960 and 1989, mortality caused by *P. weirii* accounted for 45 percent of all mortality on the two plots, causing a 29 percent decrease in the number of standing Douglas-fir trees and an 18 percent reduction in the standing volume. The total land area of the stands containing dead or dying Douglas-fir on the two plots increased from an average of 8 percent to an average of 32 percent between 1960 and 1989. The margins of these infection centers expanded into the uninfested portions of the two plots unevenly, and at an average rate of 28.8 cm per year.

A524

PHYSIOLOGICAL EFFECTS OF PROCERUM ROOT DISEASE OF EASTERN WHITE PINE CHRISTMAS TREES. J.A. Carlson and S.A. Alexander, Dept. of Plant Pathology, Physiology and Weed Science, VPI&SU, Blacksburg, VA. 24061-0330.

Procerum root disease, caused by the fungus *Leptographium procerum* (Kendr.) Wingf., causes extensive mortality in eastern white pine (*Pinus strobus* L.) Christmas tree plantings in southwest Virginia. A field study was conducted to determine the effects of the disease on several host variables including photosynthetic rate. A prominent symptom of this disease is resin production which occludes xylem in roots and lower stem. Thus, measurements were made of water potential and transpiration rates. Disease severity was estimated by measuring the resin-soaked proportion of stem cross-sectional area at the root collar. Photosynthetic rate decreased significantly with increased disease severity, and was highly correlated with stomatal conductance. Pre-dawn water potential became increasingly negative with increased disease severity, while the daily pre-dawn to mid-day change in water potential decreased significantly. The correlation of these findings with health and survival of the trees will be discussed.

A525

ENDOCRONARTIUM HARKNESSII IN THINNED PINUS CONTORTA VAR. LATIFOLIA STANDS. Blenis, P. V. and Duncan, I. Department of Plant Science, University of Alberta. Edmonton, Alberta, T6G 2P5.

Ten plots were established along a transect through each of twelve thinned P. contorta stands, at least 12 ha in size and at least 25 yr old. The five trees closest to plot center were selected. The numbers, ages, heights, and degrees of encirclement of all main stem galls were determined, as were the ages of all galls on an additional five heavily infected trees. There was no correlation between the height of main stem galls and degree of encirclement. The multiple regression equation for infection after thinning was: $Y = 135.9 + 1.72 X_1 - 6.7 X_2$; (Y = estimated percentage of trees on which galls would form after thinning, X_1 = average number of visible branch and main stem galls per tree at the time of thinning, X_2 is the age of the stand at thinning; $R^2 = 0.56$). In almost all stands, the likelihood of main stem infections decreased as the trees became older, although branch infections continued to increase over time. It thus may be feasible to predict, at the time of thinning, levels of subsequent lethal infection.

A526

EFFECT OF DROUGHT AND PITCH CANKER DEVELOPMENT ON GROWTH OF LOBLOLLY PINE SEEDLINGS. W.A. Carey, W.D. Kelley, and A.D. Barnes. School of Forestry, Auburn University, AL 36849-5418.

Ten-month-old, nursery-grown loblolly pine (*Pinus taeda*) seedlings (4.0 to 5.0 mm diam.) from four half-sib families (two inland and two coastal provenances) were transplanted randomly (50 X 50 cm spacing) in March to six, plastic-lined, sand-filled pits. Three pits (drought-stressed) were covered to exclude rain and were watered only when pre-dawn xylem potential reached -0.8 MPa. The other three pits (non-drought) were open to ambient rain and were watered if pre-dawn potential reached -0.45 MPa. Seven months after transplanting, randomly-selected seedlings were inoculated with *F. subglutinans*. At inoculation, survival and growth were both significantly greater for non-stressed seedlings. Five months after inoculation, seedling diameters and canker dimensions were analyzed for differences attributable to inoculation, family, and/or drought treatment. Significant difference occurred only between inoculated and non-inoculated seedlings in the drought-stressed treatment where inoculated seedlings were smaller.

A527

CHANGES IN WATER ABSORPTION AND EFFECTIVE TRANSFER COEFFICIENTS ASSOCIATED WITH POSTIA PLACENTA AND PHANAEROCHAETE CHRYSOSPORIUM COLONIZATION OF SPRUCE WOOD. J. Jellison, Dept. of Plant Biology and Pathology, Univ. of Maine, Orono, ME 04469 and J. Liu, Dept. of Forestry, Univ. of British Columbia, Vancouver, BC Z6T1Z4.

Permeability, expansion and weight changes over time were examined for fungally degraded and control wood samples. Even early stages of fungal attack significantly modified the wood's ability to take up water. Water-infiltrated wood samples that had been decayed by P. placenta for 2-8 weeks achieved moisture contents up to 50%. Water permeation into decayed wood samples could be expressed as a linear function of the square root of time. Effective transfer coefficients of water into wood increased as a function of weight loss in wood attacked by P. chrysosporium.

A528

RESISTANCE TO DOTHISTROMA PINI IN HALF-SIB FAMILIES FROM RESISTANT AND SUSCEPTIBLE AUSTRIAN PINES. G. W. Peterson and D. F. Van Haverbeke. Rocky Mountain Forest and Range Exp. Stn., For. Sci. Lab., University of Nebraska, Lincoln 68583.

Seedlings of half-sib families of Pinus nigra Arn., which were resistant (8 trees), moderately resistant (9 trees), or highly susceptible (12 trees), to Dothistroma pini, were planted in eastern Nebraska in 1979 and inoculated (in 1980 & 1982) to determine extent of resistance to D. pini in the families. In 1984 infection on 1st- and 2nd-year needles was rated 0 to 3 based on increasing numbers of lesions. The 29 families were segregated into 4 groups by cluster analysis (ISODATA) of infection data from 48 trees (6 reps x 8 trees) of each family. Seven of 8 families from resistant parent trees were in the group with the least infection. The results increase knowledge on resistance of P. nigra to D. pini and have implication for establishment of P. nigra seed orchards.

A531

VEGETATIVE COMPATIBILITY AND VIRULENCE TO COTTON (*GOSSYPIUM HIRSUTUM* L) AMONG ISOLATES OF *VERTICILLIUM DAHLIAE* KLEB. A.A. Bell, USDA-ARS, Southern Crops Research Laboratory, Route 5, Box 805, College Station, TX, 77845.

Mutants deficient for assimilation of nitrate nitrogen were used to determine the vegetative compatibility of 90 isolates of *Verticillium dahliae* obtained from 31 different states and countries and from 35 different crops. All, except five isolates from the USSR, could be readily assigned to one of three vegetative compatibility groups designated VCG-1, -2, and -4 to agree with other recent studies. Each of these three VCG groups could be subdivided into A and B subgroups based on the vigor of complementation among isolates within a VCG. Only isolates from the VCG -1A subgroup, which is indiginous to the southern USA and northern Mexico, caused severe defoliation of cotton. Isolates from VCG-4 were least virulent to cotton.

A532

RELATIONSHIP BETWEEN CYTOLOGICAL PHENOTYPE, PLANT GENOTYPE, AND RESPONSE TO CULTIVAR-SPECIFIC ELICITOR IN COWPEA CULTIVARS INOCULATED WITH THE COWPEA RUST FUNGUS. Michele C. Heath and Chang-Yong Chen. Botany Department, University of Toronto, Toronto, Ont. Canada M5S 1A1.

F_1 and F_2 progeny from a cross between resistant and susceptible cowpea cultivars were examined for cytological responses to the cowpea rust fungus and the degree of necrosis elicited by a cultivar-specific elicitor produced by the monokaryotic stage of the fungus. F_1 plants were resistant to infection, but allowed more growth of both monokaryon and dikaryon than the resistant parent. F_2 ratios of susceptible to resistant plants suggested that resistance to both stages of the fungus is controlled by two genes. At least five of the possible nine plant genotypes could be distinguished by the cytological characteristics of the plant-fungal interaction. The relationship between genotype and response to elicitor will be discussed.

A533

Hans R. Hohl, Chantal Guggenbühl, and Sylvia Balsiger. Antibodies against animal substrate adhesion molecules (SAM) inhibit adhesion of Phytophthora megasperma f.sp. glycinea to the host cell wall. Institute of Plant Biology, University of Zürich, Switzerland

Adhesion in animal cell systems is mediated by several families of adhesion molecules. Some bind to components of the extracellular matrix (substrate adhesion molecules or SAMs). We present evidence that cell walls of both the fungal pathogen P. megasperma f.sp. glycinea and soybean mesophyll cells contain adhesion complexes related to those of animal systems. Using an in vitro system developed in our laboratory, we demonstrate that adhesion between host cells and pathogen is inhibited, (1) almost totally by antibodies against collagen, fibronectin, laminin, and vitronectin, and (2) partially by the SAMs collagen, fibronectin, and laminin. These inhibitory antibodies label a 65 kD band on immunoblots from SDS-PAGE gels of the germination fluid of the pathogen, and several major bands from gels of the intercellular washing fluid of soybean leaves. Fluorescent antibodies against collagen, vitronectin and laminin label the surface of the pathogen homogenously, while immunolabeled soybean mesophyll cells display a number of small, fluorescing surface spots.

A534

STRUCTURAL ELEMENTS WITHIN THE TOBACCO MOSAIC VIRUS COAT PROTEIN REQUIRED TO ELICIT THE N' GENE HYPERSENSITIVE RESPONSE. J. N. Culver[1], R. Pattanayek[2], G. J. Stubbs[2], and W. O. Dawson[1]. Dept. of Plant Pathology[1], Univ. of California, Riverside, CA 92521, and Dept. of Molecular Biology[2], Vanderbilt Univ., Nashville, TN 37235.

Amino acid substitutions that interfere with the ability of the tobacco mosaic virus (TMV) (CP) to aggregate normally were found to induce the N' gene hypersensitive response (HR). Substitutions that enhanced or had no affect on CP aggregation did not result in the induction of the HR. Amino acid substitutions designed to alter the structure of the CP at various locations revealed that changes affecting the stability of the four core α-helices resulted in a loss of HR. Substitutions that affected the structures of the molecule in other regions did not affect induction of the HR. This suggests that the tertiary structure of these α-helices are important for host recognition and induction of the N' gene HR. We hypothesize that amino acid substitutions that reduce CP aggregation expose a domain that is buried within normal CP aggregates. This domain is then recognized by the N' gene plant, resulting in the induction of the HR.

A535

CONSERVATION OF *SYR* GENE SEQUENCES IN *PSEUDOMONAS SYRINGAE* PV. *SYRINGAE*, AND THE IMPORTANCE OF PLANT SIGNAL MOLECULES IN INDUCING SYRINGOMYCIN PRODUCTION. N. B. Quigley and D. C. Gross, Plant Pathology Department, Washington State University, Pullman, WA 99164-6430.

Internal sequences from the *syrB* and *syrD* genes were used as DNA probes to determine if genes required for syringomycin production are conserved among strains of *Pseudomonas syringae* pv. *syringae*. The *syr* gene sequences were found to be conserved in toxigenic strains of *P. s. syringae*, but were absent in related pathovars such as *P. s. morsprunorum* and *P. s. pisi*. Because both the *syrB* gene and syringomycin production can be induced in response to plant signal molecules, representative strains of *P. s. syringae* were tested for signal-mediated induction of toxin production. Results show that >80% of the toxigenic strains produced significantly higher levels of syringomycin when plant signals (i.e. arbutin and D-fructose) were present; a few strains produced toxin only in the presence of these signals. The importance of syringomycin production to the virulence of *P. s. syringae* will be discussed.

A536

ISOLATION AND CLONING OF A POTENTIAL SYSTEMIC MOVEMENT FACTOR FROM *XANTHOMONAS CAMPESTRIS* PV. CAMPESTRIS. J. Chen, P. D. Roberts, and D. W. Gabriel. Department of Plant Pathology, University of Florida, Gainesville, Florida 32611.

Genomic libraries of the systemic *Brassica* pathogen, *Xanthomonas campestris* pv. campestris (Xcc), and the leaf spotting pathogen, *X. c.* pv. amoraciae (Xca), were constructed. Cosmid size DNA fragments of Xcc were transferred into Xca by conjugation, and vice versa. The transconjugants were inoculated on the common host *Brassica oleracea* var. capitata (cabbage) seedlings and screened for changes in virulence. No Xca clones were identified that affected symptom expression of Xcc on cabbage, but an Xcc cosmid clone (pJC6.4) carrying a 42 kb DNA fragment altered symptom expression of Xca on cabbage. The symptom-altering activity was localized to a 5.3 kb *Hind*III fragment (pJC64.H41) by subcloning and seedling assays. Adult plants inoculated with Xca/pJC64.H41 exhibited black vein symptoms typical of Xcc, indicating that systemic virulence ability of Xcc was transferred to Xca. Tests confirming systemic movement of Xca/pJC64.H41 are in progress.

A537

TRANSCRIPTIONAL REGULATION OF HOST mRNA EXPRESSION BY A dsRNA VIRUS IN THE FILAMENTOUS FUNGUS *CRYPHONECTRIA PARASITICA*. P. Kazmierczak, L. Zhang, P. Pfeiffer, and N.K. Van Alfen. Department of Plant Pathology and Microbiology, Texas A&M University, College Station, TX 77843.

Biological control of the chestnut blight pathogen, *Cryphonectria parasitica*, by a double-stranded (ds)RNA virus appears to be the result of down-regulation of expression of a number of fungal genes important to virulence and sporulation. Our previous work has shown that the virus affects the mRNA accumulation of a number genes. It is not known if the virus affects mRNA turn-over, processing, or transcription. To determine if the virus affects transcription of these genes, a nuclear run-on assay was developed for this fungus. Isogenic strains of the fungus, which differ only in the presence or absence of the virus, were used in these studies. We found that the genes for extracellular laccase, a cell-surface hydrophobin and lectin (cryparin), a sporulation gene (vir2), and a gene with an identical open reading frame to that of vir2 (vir1) are each down-regulated by the dsRNA at the transcriptional level. Evidence was also obtained that each gene was separately regulated in the normal developmental cycle of the fungus. Transcription of ribosomal RNA and the glyceraldehyde-3-phosphate dehydrogenase gene of the fungus was used as a control for comparisons between the two isogenic strains.

A538

GENE DELETION OF LACCASE A YIELDS EVIDENCE OF AN INDUCIBLE EXTRACELLULAR LACCASE OF *CRYPHONECTRIA PARASITICA*. D.H. Kim, D. Rigling, and N.K. Van Alfen. Department of Plant Pathology and Microbiology, Texas A&M University, College Station, TX 77843-2132.

Extracellular laccase (laccase A) of *Cryphonectria parasitica*, causal agent of chestnut blight, is one of several genes or gene products that are down-regulated by a dsRNA virus. The biological function of laccase A was investigated by replacing the laccase A gene with the *E. coli* hygromycin B phosphotransferase

gene (*hph*). Restriction maps and Northern blots revealed that the laccase gene was replaced with the *hph* gene. The *lac*⁻ mutant was examined for changes in virulence, pigmentation, conidiation, conidial germination, and sexual mating capability, all of which are thought to be affected by laccases in various filamentous fungi. No significant changes in these phenotypes were detected in the *lac*⁻ mutant as compared with the wild type. However, another inducible extracellular laccase was detected when the mutant was grown in the presence of high concentrations of tannic acid. Thus, it is possible that the fungus could compensate for the mutation of laccase A activity by that of a previously unknown extracellular laccase.

A539

THE ISOLATION OF FLAX GENES INDUCED BY INFECTION WITH FLAX RUST *MELAMPSORA LINI*. J. K. Roberts and A. J. Pryor. CSIRO, Division of Plant Industry, GPO Box 1600, CANBERRA, A.C.T. 2601 Australia.

The interaction between flax and it's obligate rust pathogen, *Melampsora lini*, is the classical "gene for gene" model system first described by Flor. In order to better understand the processes involved with the fully compatible interaction we have attempted to isolate host genes which are induced during a successful infection. We have used subtraction hybridization to isolate flax cDNAs which hybridize to RNAs that increase during infection with *Melampsora lini*. The level of two different flax RNAs has been studied. Although these RNAs are present in uninfected leaves they are several fold more abundant in leaves infected with rust. The amounts of these RNA species is not changed during an incompatible reaction that induces the hypersensitive response. We have characterized the expression of these genes in uninfected and infected tissues. Sequence analysis is also currently under way.

A540

MOLECULAR CHARACTERIZATION OF CRYPARIN, A dsRNA DOWN-REGULATED PROTEIN OF *CRYPHONECTRIA PARASITICA*. D.K. Villalon, L. Zhang, and N.K. Van Alfen. Department of Plant Pathology and Microbiology, Texas A&M University, College Station, TX 77843-2132.

Specific proteins and polyA + RNAs associated with virulence and sporulation in the chestnut blight pathogen *Cryphonectria parasitica* are down-regulated by dsRNA. One of the affected proteins (Cryparin) has a glycine-serine- repeating sequence near the amino terminal end that is typical of structural proteins, and it has properties of a lectin. Antibody staining showed that this 18.6 kDa polypeptide is specific to aerial hyphae and fruiting bodies and accumulates in large amounts on hyphal cell surfaces. The gene has been isolated and structurally characterized. It is developmentally regulated; mRNA being highly expressed in log phase of liquid cultures. The physical properties of cryparin are similar to the phytotoxin cerato-ulmin which is produced by the Dutch elm disease fungus, *Ceratocystis ulmi*. It is also similar to a class of cysteine-rich proteins called "hydrophobins" from other filamentous fungi. By Southern blot analysis, a cryparin genomic DNA probe hybridizes to DNA of other genera of filamentous fungi that produce hydrophobins.

A541

GENES SPECIFICALLY EXPRESSED DURING INFECTION STRUCTURE FORMATION OF *MAGNAPORTHE GRISEA*. Y.H. Lee and R.A. Dean. Department of Plant Pathology and Physiology, Clemson University, Clemson, SC 29634.

Magnaporthe grisea, the rice blast pathogen, develops specialized infection structures to infect plants. The formation of an appressorium, a darkly pigmented adhesion structure, is the essential first step. Subsequently, an infection hyphae develops that penetrates the plant epidermal cells. To isolate infection structure specific genes, a genomic library of strain 70-15 was constructed in a cosmid vector and differentially screened. ^{32}P labelled cDNA probes were prepared from poly A+ RNA isolated from *M. grisea* induced to form appressoria and from vegetative mycelia. Several clones were found to contain genes that were highly expressed during appressoria formation. Southern and northern blot analyses of two clones, 23T and 29T, revealed both contained genes within *Eco*R1 fragments (4.5 and 7.5 Kb, respectively) specifically expressed during appressoria formation, but not during vegetative growth. Transcript sizes were approx. 1.2 and 3.0 Kb, respectively. These DNA fragments appear to be present as single copies in the haploid genome of *M. grisea*. The temporal expression of these genes *in vitro* and *in planta* and strategies to evaluate their function will be presented.

A543

CYTOCHEMICAL STUDY OF THE MYCOPARASITIC INTERACTION OF *STACHYBOTRYS ELEGANS* WITH *RHIZOCTONIA SOLANI*. M. Benyagoub, H. Chamberland & S. H. Jabaji-Hare. Dept. of Phytology, Université Laval, Ste. Foy, Québec, Canada, G1K 7P4.

Hyphal and sclerotial cells of *Rhizoctonia solani*, parasitized or not by *Stachybotrys elegans*, were ultrastructurally examined for different sugars using lectin-gold complexes. N-acetyl-D-glucosamine (GlcNAc; a basic unit of chitin) and D-galactose (D-Gal) were abundantly present in the cell walls of the mycoparasite and its host. D-mannose (D-Man) was detected only in the cell walls of *S. elegans*. In the parasitized cells of *R. solani*, GlcNAc residues were absent from regions where the cell walls have been completely lysed, suggesting that enzymatic activity had occurred. In response to invasion, papilla-like bodies were formed in host cells and appeared to be rich in GlcNAc and D-Gal sugar residues. Furthermore,ultrastructural observations showed that *S. elegans* produces an extracellular fibrillar matrix which envelops the host cells. Immunolabeling of the matrix revealed that it is rich in fimbrial proteins.

A544

SURVIVAL OF *AMPELOMYCES QUISQUALIS* IN PARASITIZED CLEISTOTHECIA OF *UNCINULA NECATOR*. S.P. Falk, P. Cortesi, D.M. Gadoury, and R.C. Pearson. Dept. Plant Pathology, Cornell University, NYSAES, Geneva, NY 14456-0462.

Ampelomyces quisqualis parasitizes the grape powdery mildew pathogen, *Uncinula necator*, forming pycnidia in hyphae, conidiophores, and immature cleistothecia. Overwinter survival of *A. quisqualis* in northeastern vineyards is poorly understood. We collected leaves of *Vitis* interspecific hybrid cv Chancellor, bearing mildew colonies parasitized by *A. quisqualis*, from vines in the fall and overwintered them in the vineyard trellis. The number of parasitized cleistothecia on leaves declined from 15.6 cm^{-2} in the fall (22% of cleistothecia) to 2.0 cm^{-2} in mid-winter (6% of cleistothecia), presumably due to removal by weathering. From 51% to 84% of parasitized cleistothecia contained viable *A. quisqualis* conidia. On leaves, the density of pycnidia in hyphae and conidiophores varied from 2.8 cm^{-2} to 1.6 cm^{-2}. The amount of vineyard floor covered by leaf litter declined from approximately 75% to 4% during this period, indicating that leaves were a poor site for overwintering. However, parasitized cleistothecia at a density of 8,250 kg^{-1} bark (3% of cleistothecia) occurred on the cordons of these Chancellor vines in late-winter. Over 90% of parasitized cleistothecia from bark contained viable *A. quisqualis* conidia. Survival of *A. quisqualis* in parasitized cleistothecia of *U. necator* on bark may be significant, as the hyperparasite may be splash dispersed to nearby developing mildew colonies.

A545

EFFECT OF DROUGHT STRESS ON RUST INFECTED YELLOW STARTHISTLE. N. Shishkoff and W. L. Bruckart, USDA/ARS, FDWSRU, Bldg. 1301, Ft. Detrick, Frederick, MD 21702

When leaves of yellow starthistle (*Centaurea solstitialis* L.) were infected with the rust *Puccinia jaceae* Otth, they often died immediately after pustule eruption. This could, therefore, be due to increased transpiration caused by rupture of the cuticle, so the effect of rust infection on leaf water status was investigated. Inoculated or uninoculated plants were watered lightly (so that wilting occurred daily) or normally. After 4 wk, dry root and shoot weight were measured. Both drought stress and rust infection caused significant decrease in root biomass, but there was no significant interaction. When single leaves were inoculated and leaf lifespan was plotted against pustule number per leaf, there was a difference in slope for drought stressed vs unstressed plants. Leaves with >300 pustules died rapidly whether drought stressed or not, but slightly infected (<100 pustules) lived significantly longer on well-watered plants. Our results indicate that we can effectively evaluate rusts as biocontrol agents of dry-adapted weeds under greenhouse environments.

A546

MECHANISMS OF BIOCONTROL OF PHYTOPHTHORA ROOT ROT BY PENICILLIUM FUNICULOSUM. J. G. Fang and P. H. Tsao, Plant Pathology Dept., University of California, Riverside 92521.

Penicillium funiculosum (PF) is effective in the biocontrol of azalea and citrus root rots caused by Phytophthora cinnamomi, P. citrophthora and/or P. parasitica. Mycoparasitism, lysis, and antibiosis are probable mechanisms of Phytophthora suppression by PF. With light and scanning electron microscopy, PF was observed to coil around, penetrate, and lyse mycelia, hyphal swellings, and/or sporangia of these Phytophthora spp. When grown in water with washed living colonies of mycelia of these Phytophthora spp. as the only nutrient source, PF completely lysed the mycelia within 2 wk. PF produced diffusible antibiotic substances in agar media inhibitory to the Phytophthora spp. It possessed a wide antifungal spectrum; the culture filtrate and ethyl acetate extract of PF liquid cultures strongly inhibited the growth of other fungal pathogens (species of Cylindrocladium, Fusarium, Pythium, Rhizoctonia, Thielaviopsis, and Verticillium) as well as the three Phytophthora spp. This might explain the increased growth response of many plant species grown in PF-treated nonsterile planting mixes.

A547

RELATIONSHIP BETWEEN THE BIOLOGICAL CONTROL OF PYTHIUM SEED AND SEEDLING DISEASE AND ADHERENCE TO FUNGAL STRUCTURES BY THE BACTERIUM *ENTEROBACTER CLOACAE*. A. P. Maloney and E. B. Nelson, Dept. Plant Pathology, Cornell University, Ithaca, NY 14853.

Our inquiry into bacterial traits important in the biological control of Pythium diseases is part of a long-term study of the ecological basis of biological control of soilborne pathogens. In previous work, adherence of *Enterobacter cloacae* to *Pythium ultimum* mycelium was suggested as a critical step in biological control of Pythium seed and seedling rot (Nelson et al., 1986. *Phytopath.* 76:327). The genetic relationship between adherence and biocontrol expression has been examined. A mini-Tn*51 PhoA* mutant

library of *E. cloacae* isolate EcCT-501 was screened for mutations in genes that encode cell-surface proteins. Assays for agglutination of carboxy-methylcellulose and aggregation of fungal cell wall fragments by isolate EcCT-501 were conducted to examine mutants for altered adherence capability. A cucumber seedling bioassay was used to examine mutants for alterations in biocontrol expression. Several mutants exhibited enhanced alkaline phosphatase expression, decreased adherence, and reduced biocontrol capability. Results of the experiments strongly suggest a genetic basis for the previously established correlation between bacterial adherence and biological control of Pythium seed rot. The mechanism of adherence and the specific role adherence plays in biocontrol of Pythium seed rot are under investigation.

A548

PRODUCTION OF NON-VOLATILE AND VOLATILE INHIBITORS OF *PYTHIUM ULTIMUM* SPORANGIUM GERMINATION AND MYCELIAL GROWTH BY STRAINS OF *ENTEROBACTER CLOACAE*. Peter Trutmann and Eric Nelson, Department of Plant Pathology, Cornell University, Ithaca, NY 14853.

Enterobacter cloacae is an effective biocontrol agent of *Pythium*-incited seed and seedling rots. However, the mechanisms involved in these biocontrol processes are not well understood. Recently both non-volatile and volatile metabolites from several *E. cloacae* strains grown for 5 days on a glucose asparagine medium were shown to inhibit both sporangium germination and mycelial growth. Inhibitory properties of non-volatile components were retained in low but not in high molecular weight fractions. Siderophores were produced by all *E. cloacae* strains, but the addition of Fe^{3+} into filtrates from low Fe^{3+} culture, or growth of strains on an iron rich medium did not reduce the inhibitory effect. On trypticase soy agar, volatile metabolites from *E. cloacae* strains reduced mycelial growth of *P. ultimum*, but metabolites from only 2 strains (Ecct-501 and E6) reduced sporangium germination. These results suggest that volatile and non-volatile inhibitors of both sporangium germination and mycelial growth could play a role in the biocontrol of *P. ultimum* by strains of *E. cloacae*. Siderophores do not appear to be involved in the inhibition of *P. ultimum* by *E. cloacae*.

A549

SOIL FACTORS ASSOCIATED WITH SUPPRESSION OF TAKE-ALL BY TRICHODERMA KONINGII. B.H. Ownley, B.K. Duffy[1], and D.M. Weller. USDA-ARS, [1]Plant Path. Dept., Wash. State Univ., Pullman 99164.

Trichoderma koningii (Tk), isolated in Australia, suppressed take-all of wheat caused by Gaeumannomyces graminis var. tritici (Ggt). To determine the relative influence of soil factors to take-all suppression by Tk, wheat seeds were sown in eight different silt loams from the Pacific Northwest. The soils were amended with Tk and infested with Ggt. Tk significantly reduced disease ratings and increased shoot heights compared to the check. Although Tk suppressed take-all in all soils tested, the level of disease suppression was variable among soils. Disease suppression by Tk was positively correlated with Fe, NO_3-N, B, Cu, Mg, and clay. Suppression was negatively correlated with pH and P. Principal component factor analysis using these 8 variables resulted in a 3 factor solution. A regression model ($R^2 > 0.99$), to describe the variance in take-all suppression by Tk, selected with step-wise regression, included the soil variables: Fe, NO_3-N, B, and Cu.

A550

Rhizobacterial suppression of common root rot of spring wheat caused by Cochliobolus sativus. M.S.Reddy, S.E.Young,R.Rennie, Esso Chemical Canada, 402-15 Innovation Blvd. Saskatoon, SK S7N 2X8; L.J. Duczek, Ag Canada, Saskatoon; K.Mortensen, Ag Canada, Regina and R. Horton, Ag Canada, Melfort.

Common root rot (CRR) is a problem on spring wheat and barley world wide. Field plots were estabiished in Saskatoon, Regina and Melfort, Saskatchewan, Canada in 1990 and 1991 to evaluate the potential of rhizobacterial strains as seed treatments to reduce the severity of CRR of wheat. The bacteria were formulated either in sterile peat or in a liquid carrier and applied to seed just before planting. Bacterized seed were planted in replicated field plots artificially infested with C. sativus. CRR was scored using the subcrown internode index expressed as a disease rating on a scale of 0-10 at the time of harvest. Grain yields were determined by harvesting the plots. Seed bacterization significantly reduced the severity of CRR compared to nonbacterized controls. Strains reducing the severity of CRR enhanced the grain yield in the range of 5-11% above that of controls. Studies such as antibiosis, production of plant growth regulators (PGR), cyanide and induction of root elongation were examined as possible biocontrol mechanisms. Strains active in suppression of CRR showed in vitro antagonistic activity to several fungal pathogens such as C. sativus, Pythium ultimum, Rhizoctonia solani, Fusarium solani and F. oxysporum. Some of these strains that induced root elongation on wheat, cucumber, tomato, canola and also produced the PGR's dihydrozeatin riboside and isopentenyl adenosine. The results suggest that the disease suppressing activity of bacterial strains is due to a variety of activities which may include the production of PGR's and antagonism to various indigenous plant-pathogenic microorganisms.

A551

BACTERIAL ANTAGONISM OF SUGARBEET SEEDLING DISEASES. A. J. Anderson, Utah State University, Logan, UT 84322-5305, C. D. Strausbaugh and J. J. Gallian, University of Idaho, P. O. Box 1827, Twin Falls, ID 83303-1827.

Fluorescent pseudomonads and strains of *Serratia*, *Enterobacter*, and *Bacillus* spp. isolated from sugarbeet (*Beta vulgaris*) seeds and roots were antagonistic to *Phoma betae* and *Rhizoctonia solani in vitro*. Antagonism of both pathogens by fluorescent pseudomonads *in vitro* under both iron deficient and sufficient conditions suggests siderophore production may not be the primary factor in antagonism. Some of the pseudomonads produced a yellow pigment with the same spectral characteristics and thin layer chromatography Rf values as 1-3 phenazine carboxylic acid, an antibiotic implicated in the control of take-all. Some of the *Serratia*, *Enterobacter* and pseudomonad strains, including the phenazine producers, that showed strong antagonism *in vitro* controlled both diseases in greenhouse tests.

A553

Biocontrol of Rhizoctonia stem rot of poinsettia (*Euphorbia pulcherrima*) in polyfoam rooting cubes. D. Kelly Cartwright and D. M. Benson. Dept. of Plant Pathology, N. C. State University, Raleigh.

A *Pseudomonas* species isolated from natural soil was efficacious in control of Rhizoctonia stem rot of poinsettia in polyfoam rooting cubes. No stem rot was observed on poinsettia cuttings rooted in cubes treated with the isolate of *Pseudomonas* compared to 73 to 100% infection and mortality of cuttings in untreated cubes infested with rice grains colonized by *Rhizoctonia solani*. No infection or mortality was observed on cuttings in benomyl treated cubes. *Gliocladium virens* (strain GL21), a known biocontrol agent of *R. solani*, gave no control (100% mortality) of stem rot. Root development was not adversely affected on cuttings in cubes treated with the isolate of *Pseudomonas*. The *Pseudomonas* species was more inhibitory to *R. solani* on dilute (0.13%) potato dextrose agar than water agar or full strength PDA. This isolate of *Pseudomonas* species has potential as a biocontrol agent of stem rot of poinsettia.

A554

ENRICHMENT AND SELECTION OF ANTAGONISTS OF FUSARIUM SAMBUCINUM BASED ON EFFICACY AND PERFORMANCE IN LIQUID CULTURE. D. A. Schisler, P. J. Slininger, and R. E. Hanneman*. USDA-ARS, NCAUR, Peoria, IL 61604 and *Univ. WI, Madison, WI 53706.

Fusarium sambucinum (Gibberella pulicaris), a causal agent of dry rot of potato tubers, destroys vegetative tissues and can produce trichothecene toxins. The prevalence of thiabendazole-resistant strains of F. sambucinum warrants the development of biological control measures. Soils from areas with low F. sambucinum incidence, potato periderm, and gamma-sterilized field soil were combined (5:2:93, wt:wt:wt, respectively) and incubated to allow microbes to proliferate in an enriched, standardized soil background. Twenty-nine different enriched soils were then assayed for suppressiveness to F. sambucinum using a whole potato bioassay. Selective media were used to isolate over 350 putative antagonists from puncture wounds and soil adhering to potatoes harvested from suppressive, enriched soils. Putative antagonists were evaluated individually on whole potatoes and superior performers further selected based on their amenability to mass production in liquid culture.

A555

CONDITIONS FAVORABLE FOR DIFFERENT STAGES IN THE LIFE CYCLE OF SPORIDESMIUM SCLEROTIVORUM. A. S. Mintz, C. P. Baker, and P. Adams*, W. R. Grace & Co.-Conn., Washington Research Center, Columbia, MD 21044 and *USDA-ARS, Beltsville, MD 20705.

Sporidesmum sclerotivorum, a potential biocontrol for Sclerotinia diseases, concurrently produces two types of conidia; the infective propagule or macroconidia, and the microconidia of the Selenosporella state. Observations suggest that the Selenosporella state is produced much more frequently and abundantly than macroconidia. Favorable conditions for macroconidial production have been previously described. Studies were conducted to determine the conditions required for the Selenosporella state. In understanding these requirements, it may be possible to increase macroconidial production by eliminating, or at least reducing the frequency of the Selenosporella state.

A556

EFFECT OF GLIOTOXIN ON GROWTH, SPORULATION, AND ZOOSPORE MOTILITY OF SEVEN *PHYTOPHTHORA* SPP. *IN VITRO*. W. F. Wilcox, G. E. Harman, and A. Di Pietro. Cornell University, NY State Agric. Expt. Sta., Geneva 14456.

An isolate of *Gliocladium virens* previously shown to provide biocontrol of root rots caused by *Phytophthora* spp. on apple (PHYTOPATHOLOGY 80: 880-885), raspberry, and soybean in pot tests was found to produce the antibiotic, gliotoxin. Subsequently, the effect of gliotoxin on mycelial growth, sporangium formation, and zoospore motility of *P. cactorum, P. cambivora, P. cryptogea, P. fragariae* var. *rubi* (Pfr), *P. medicaginis, P. megasperma*, and *P. sojae* was tested *in vitro* at concentrations of 0.06-2.0 µg/ml. The ED(50) value for mycelial growth ranged from < 0.13 µg/ml for *P. sojae* and *Pfr* to ca. 1.0 µg/ml for *P. cryptogea* and *P. medicaginis*. All species produced virtually no sporangia in soil extract including ≥0.13 µg/ml gliotoxin and relatively few at 0.06 µg/ml, where differences among species correlated with mycelial growth sensitivities. Zoospore motility generally ceased within 30 min of exposure to concentrations ≥1.0 µg/ml and was also significantly affected at lower values. These data suggest a possible role for gliotoxin in the biocontrol of Phytophthora root rots by *G. virens*.

A557

SELECTION OF GEOCARPOSPHERE BACTERIA AS CANDIDATE BIOLOGICAL CONTROL AGENTS FOR AFLATOXIGENIC FUNGI AND REDUCING AFLATOXIN CONTAMINATION IN PEANUT. K. L. Bowen, J. W. Kloepper, H. Chourasia, and C. J. Mickler, Department of Plant Pathology, Auburn University, AL 36849-5409.

Based on a previous study demonstrating that peanut geocarposphere (zone around the subterranean pod) bacteria differed from rhizosphere bacteria, it was hypothesized that geocarposphere bacteria would be good candidates for protecting developing peanut seed against aflatoxigenic fungi. Geocarposphere bacteria were collected during the 1991 growing season, and 159 strains were screened against *Aspergillus flavus*-type fungi in separately developed seed and root-radicle assays. In these assays, ungerminated seeds or germinated seeds and radicles were dipped in bacterial suspensions, incubated for 24 hr, and inoculated with *A. flavus*. Fungal colonization and conidiation, root growth and no. root branches were recorded over 6 d. Strains (19) were selected that significantly suppressed colonization (ave. 41%) and conidiation (>37%), and increased root growth (ave. 44%) and branching (78%) compared to the *A. flavus* control in repeated trials. Selected strains represented a variety of genera and species and exhibited differential physiological traits, suggesting that multiple mechanisms of fungal inhibition and root growth promotion were involved. The candidate biocontrol bacteria are being evaluated in greenhouse and field trials.

A558

OUT-MINUS MUTANTS OF *ERWINIA CAROTOVORA* SUBSP. *BETAVASCULORUM* WITH POTENTIAL FOR BIOLOGICAL CONTROL OF POTATO SOFT ROT. J. M. Costa and J. E. Loper. USDA-ARS, HCRL, 3420 N.W. Orchard Ave., Corvallis, OR 97330.

Two *Hin*dIII fragments (8.5 kb and 10.5 kb) of genomic DNA of *E. c. betavasculorum* strain Ecb168 hybridized to the cloned *out* region of *E. c. carotovora* and complemented different Out⁻ mutants of *E. c. carotovora*. Out⁻ mutants of Ecb168, which did not secrete pectolytic enzymes into the medium, were obtained when deletions internal to either fragment were introduced into the genome of Ecb168. Out⁻ mutants of Ecb168 were complemented to the Out⁺ phenotype by the corresponding cloned fragment. Due to the production of an antibiotic, strain Ecb168 inhibits the growth of *E. c. carotovora* in culture and in wounds of potato tubers. Out⁻ mutants of Ecb168 inhibited *E. c. carotovora* in culture at a level comparable to the parental strain and may have potential for the biological control of potato soft rot caused by *E. c. carotovora*.

A559

CPMAS ^{13}C-NMR SPECTROSCOPIC ANALYSIS OF SPHAGNUM PEAT IN RELATIONSHIP TO PYTHIUM ROOT ROT SUPPRESSION. M. J. Boehm[1], D. I. Frost[1], G. E. Wilson[2] and H. A. J. Hoitink[1]. [1]Dept of Plant Pathology, Ohio State Univ., Wooster, OH 44691 and [2]Dept of Chemistry, Univ. of Akron, Akron, OH 44325.

Solid-state cross polarization magic-angle spinning ^{13}C-nuclear magnetic resonance (CPMAS ^{13}C-NMR) subtraction spectra revealed that suppressive light peat differed significantly (P=0.05) from conducive dark peat in polysaccharide content. In long-term poinsettia bioassays, in which CPMAS ^{13}C-NMR spectroscopy was used to assay carbon content over time within slightly decomposed light peat, a decrease in the polysaccharide content of the fine particle fraction of the peat corresponded to a decline in microbial activity and an increase in *Pythium* populations and root rot. CPMAS ^{13}C-NMR spectroscopy shows promise as a technique for predicting the microbial carrying capacity of soil organic matter, or its ability to support microbiostasis and suppression of Pythium root rot.

A560

AGAR PLATE AND FIELD EVALUATION OF FUNGICIDES FOR ACTIVITY AGAINST *DISCULA DESTRUCTIVA*, CAUSAL AGENT OF DOGWOOD ANTHRACNOSE. F.D. Smith, USDA Forest Service, Coweeta Hydrologic Laboratory, Otto, NC 28763.

The sensitivity of three isolates of *Discula destructiva* to 10 fungicides was tested on fungicide-amended, potato-dextrose-yeast agar. Triazole fungicides were extremely active *in vitro*. Average ED_{50} values were 0.00065, 0.0021, 0.011, and 0.054 µg/ml for propiconazole, fenbuconazole, tebuconazole, and myclobutanil, respectively. ED_{50} values for other fungicides were 0.014, 0.019, 0.028, 0.36, 1.4, and 3.1 µg/ml for benomyl, CGA-173506, fluazinam, chlorothalonil, iprodione, and triforine, respectively. A field trial using potted, 2½-yr-old flowering dogwood (*Cornus florida*) was conducted in western NC during 1991. Healthy seedlings were placed in the forest and exposed to naturally occurring inoculum, beginning 2 Jul. Four applications of fungicides were applied at 3-wk intervals prior to the final disease rating of leaf area infection on 10 Sep. Fluazinam (300 mg/l), tebuconazole (56 mg/l), chlorothalonil (1,250 mg/l), and propiconazole (83 mg/l) were the most effective treatments as they significantly ($P \leq 0.05$) suppressed dogwood anthracnose by 75, 71, 57, and 53%, respectively.

A561

COMPARISON OF NEEM OIL, SUNSPRAY 6E PLUS HORTICULTURAL OIL AND BENLATE FOR CONTROL OF POWDERY MILDEW ON GREENHOUSE GROWN HYDRANGEA. J. C. Locke, USDA, ARS, Florist and Nursery Crops Laboratory, Beltsville, Maryland 20705-2350.

Florist hydrangea, Hydrangea macrophylla, is commonly attacked by powdery mildew, Erysiphe polygoni, when grown under greenhouse conditions. The recommended control measure is repeated applications of fungicides such as Benlate. As part of an ongoing program to find alternative materials for synthetic fungicides, various plant and mineral oils have been evaluated for powdery mildew control. Neem oil, a hydrophobic, organic solvent-extracted material from neem seeds, and a horticultural spray oil, Sunspray 6E Plus, had shown efficacy against powdery mildew on some herbaceous plants and woody nursery plants in outdoor plots. When applied on a 14-day schedule, a 1.0% aqueous emulsion of either neem oil or Sunspray provided complete protection against powdery mildew. In addition to disease prevention, these oil materials are also effective insecticides, thus providing dual protection.

A562

EFFICACY OF BICARBONATES ON POWDERY MILDEW OF ROSES. Porter, L.L., R.K. Horst, S.J. Ingalls, and H.W. Israel. Dept. of Plant Pathology, Cornell University, Ithaca, NY 14853.

Sodium and potassium bicarbonate are effective biocompatible fungicides for control of powdery mildew of Rosa spp., caused by *Sphaerotheca pannosa* var. *rosae*. Application rate and efficacy of three bicarbonates and a spreader-sticker were determined. Disease severity of powdery mildew on cultivars Royalty, Sonia and Mary DeVor were evaluated in greenhouse experiments. Treatments were water, 0.016% (v/v) Triton B-1956, a spreader-sticker, and 0.05% and 0.5% (w/v) solutions of NH_4HCO_3, $KHCO_3$ and $NaHCO_3$. Untreated rows were included in each treatment. Disease severity data were collected for six weeks and analyzed. Treatment and cultivar effects were highly significant; disease severity was less on leaves treated with 0.5% (w/v) $KHCO_3$ and $NaHCO_3$. This difference was more evident on Royalty and Sonia. Symptoms on Mary DeVor were less with $KHCO_3$ and $NaHCO_3$, and were not reduced with NH_4HCO_3. Fungicidal efficacy of bicarbonates, particularly $KHCO_3$ and $NaHCO_3$, is dependent on application rate and cultivar type.

A563

CHRYSANTHEMUM PHLOEM NECROSIS: INFLUENCE OF TEMPERATURE ON FLOWER ABNORMALITIES. J. R. Hogue, R. K. Horst, S. O. Kawamoto, and H. W. Israel, Cornell University, Ithaca, NY. 14853

The florists' chrysanthemum *Dendranthema* X *grandiflora* cv. Pink Marble is affected by a stress-related disease known as chrysanthemum phloem necrosis. Symptoms include prematurely senesced lower foliage, abnormally formed flowers that contain involucral bracts (phyllaries), and, occasionally, ray flowers among the disks. The influence of diurnal temperature fluctuations on flower morphology was tested in a series of experiments that began with plants grown for 10 weeks under short day photoinductive conditions with 9 h at 32° C and 15 h of darkness at either 10°, 16°, 21° or 27° C. All flowers were bracted at 10° C with increasingly fewer at the warmer night temperatures. Next, flower morphology was examined following nights at 10° C and varying day temperatures of either 21°, 24°, 27°, 29° or 32° C. Bracting was 100% at 32° C and diminished markedly with lowered day temperatures. A linear regression model predicts that less than 30% of the flowers are bracted at 24° C with nights of 10° C. We conclude that where night temperatures fall to 10° C during flower production, maintaining day temperatures below 24° C will reduce bracting abnormalities in 'Pink Marble' flowers to commercially acceptable levels.

A564

Rhizoctonia new foliar disease of Nerium Oleander E.A. Spillers and R.T. McMillan, Jr. University of Florida, IFAS, Tropical Research and Education Center, Homestead, FL 33031

In the summer of 1991 a diseased plant of Nerium oleander L. was found to be infected with a Rhizoctonia sp. on the foliage. Previous to this report Rhizoctonia had only been found on the roots of this plant in south Florida. However, web blight, as foliar Rhizoctonia infection is known, as a serious foliar disease of ornamental plants throughout the southeastern states. The disease first appeared as light brown spots with purple-brown margins on the leaf blades which coalesce and eventually cause the leaves to become dark brown. As the disease progresses, lesions appear on petioles and young stems. A fine mycelial webbing spreads over all effected surfaces.

A565

CHRYSANTHEMUM PHLOEM NECROSIS: DNA SYNTHESIS IN ROOTS FROM AFFLICTED PLANTS. J. R. Hogue, H. W. Israel, R. K. Horst, and S. J. Ingalls. Cornell University, Ithaca, NY. 14853

The florists' chrysanthemum *Dendranthema* X *grandiflora* cv. Pink Marble is affected by chrysanthemum phloem necrosis, a disease reportedly caused by a mycoplasmalike organism (MLO). Many bodies resembling certain animal mycoplasmas with dense cores occur routinely in parenchymatous vacuoles of 'Pink Marble' root meristem. To determine if these bodies synthesize DNA, high-resolution autoradiography of ^{3}[H]-thymidine incorporation was done. Excised roots of 'Pink Marble' grown *in vitro* were incubated aseptically in liquid Linsmaier-Skoog medium, 3% sucrose, containing 25 μCi/ml ^{3}[H]-thymidine. Roots were then fixed, embedded, and processed for high-resolution autoradiography. Microscopic fields were analyzed for ratio of percent developed grains observed to percent of total area sampled for each cellular region, with ratio of one expected if the grains were uniformly distributed over the fields. Ratios exceeding one, indicating specificity, were found for three internal positive controls: nuclei, mitochondria, and plastids. All other cellular regions, including vacuolar bodies, were less than one. These results indicate that the dense core bodies observed in the root apex do not synthesize DNA and may, therefore, not be self-replicating infectious organisms.

A566

FOLIAR BLIGHT AND ROOT ROT OF GIANT REDWOOD CAUSED BY *PHYTOPHTHORA CITROPHTHORA*. C. M. Sandlin and D. M. Ferrin, Department of Plant Pathology, University of California, Riverside 92521

Phytophthora citrophthora, isolated from blighted foliage of 1.5-yr-old container-grown giant redwoods (*Sequoiadendron giganteum*), caused both foliar blight and root rot in greenhouse studies. Inoculation of foliage with zoospores resulted in foliar symptom development within 2 days. Isolates of *P. citrophthora* from *Penstemon*, *Ceanothus* and *Citrus* also caused foliar blight on redwood seedlings. Light and scanning electron microscope studies showed that zoospores are attracted to and infect via stomata. Infestation of the soil with of 1-mo-old seedlings resulted in severe root infection in the lower portions of the pots, and significant reduction ($P < 0.01$) in the mean dry weight of the foliage after 3 mo.

A567

DIFFERENTIAL VIRULENCE OF SIX SPECIES OF *PHYTOPHTHORA* TO EIGHT CULTIVARS OF *CEANOTHUS* sp. D. M. Ferrin, Dept. of Plant Pathology, University of California, Riverside, CA 92521.

The pathogenicities and relative virulences of six species of *Phytophthora* (*P. cactorum, P. cinnamomi, P. citricola, P. cryptogea, P. parasitica* and an unidentified species) were assessed on eight cultivars of various species, varieties, or hybrids of *Ceanothus*, a genus of drought-tolerant native California plants grown for use as landscape plants and in revegetation. All six species of *Phytophthora* were pathogenic on all eight cultivars, but they differed in virulence within individual cultivars. Based on reductions in root weights and visual root rot ratings averaged over all cultivars, *P. parasitica* and the unidentified *Phytophthora* sp. were the most virulent. Mean reductions in root weights were 42-74 and 36-62% for these two species, respectively. *Phytophthora parasitica* also exhibited a more pronounced crown rot phase than the other species. Susceptibility or tolerance of the cultivars was not expressed equally to all species of *Phytophthora*.

A568

INACTIVATION OF *PHYTOPHTHORA* IN RECYCLED NURSERY IRRIGATION WATER WITH WIDE- (CONTINUOUS) AND NARROW-BAND (PULSED LASER) ULTRAVIOLET RADIATION. Z. Banihashemi, J. MacDonald and M. Lagunas-Solar. Department of Plant Pathology and Crocker Nuclear Laboratory, University of California, Davis, CA 95616.

Cysts or zoospores of various *Phytophthora* spp. (primarily *P. citrophthora* and *P. capsici*) were suspended in distilled water or in samples of recycled irrigation water obtained from a commercial nursery. Propagules, suspended in non-flowing systems, were exposed to wide-band (continuous) UV radiation emitted by a standard UV lamp in a commercial water purifier (Model 250, Ultra Dynamics Corp., Santa Monica, CA) or to a monochromatic (248 nm), pulsed (20 ns) KrF laser (Lamda-Physik 150 EMG Eximer Laser). The UV lamp intensity was approximately 8 mJ/cm^2·s (8 mW/cm^2) while the laser-UV source ranged from 1-2 mJ/cm^2·20-ns pulse (50-100 kW/cm^2). Survival was assessed by culturing aliquots of the treated suspensions onto corn meal agar amended with antibiotics and counting numbers of developing colonies. The UV dose (energy per unit area) required to kill propagules was smaller in distilled water than in recycled nursery water. The reduced kill effectiveness in recycled water appears to be related to soluble (probably organic) materials and to the presence of suspended solids. Pulsed-UV laser sources appear to have greater kill effectiveness than UV lamps and some differences in species sensitivity to UV have been observed.

A569

INTERACTION OF *TYLENCHORHYNCHUS NUDUS* AND *MAGNAPORTHE POAE* ON BENTGRASS AND ANNUAL BLUEGRASS. R. F. Davis[1], H. T. Wilkinson[1], and G. R. Noel[2]. [1]Dept. of Plant Pathology, University of Illinois, Urbana, IL 61801, [2]USDA-ARS, University of Illinois, Dept. of Plant Pathology, Urbana, IL 61801.

A study was conducted in growth chambers to examine the effect and interaction of *Tylenchorhynchus nudus* and *Magnaporthe poae* on creeping bentgrass and annual bluegrass at 24C, 28C, and 30C after a 2 week period. A 2x2 factorial arrangement of treatments was employed with presence and absence of nematodes and fungus as factors. *Tylenchorhynchus nudus* decreased bentgrass and annual bluegrass root length at all three temperatures. *Magnaporthe poae* increased bentgrass root length at 28C but decreased bentgrass root length at 30C. *Magnaporthe poae* had no effect on annual bluegrass root length at 24C and 28C but decreased annual bluegrass root length at 30C. A significant interaction between *M. poae* and *T. nudus* was indicated only on bentgrass at 28C and 30C: the effect of *M. poae* in the presence of *T. nudus* was not significant, but the effect of *M. poae* in the absence of *T. nudus* was significant.

A570

INFRARED IMAGE ANALYSIS OF TURF DISEASE CONTROL EXPERIMENTS IN NORTH CAROLINA. K.J. Jones, L.T. Lucas and H.D. Shew. Department of Plant Pathology, North Carolina State University, Raleigh, NC 27695-7616.

Infrared (I.R.) imaging allows visualization of stress in plants prior to onset of visible symptoms. Usefulness of I.R. images has been limited by difficulty in interpreting color changes and patterns of disease and other stresses. Turf areas were photographed and stressed areas were determined. These areas were then examined to determine the cause of the stress. Images were obtained from fungicide and nematicide field trials. Initial images were collected with I.R. sensitive 35mm color film and video camera from an aircraft at about 1000 ft altitude and from ground level. Images were digitized into computer readable form either with a gray scale video camera and frame grabber board or by color (24 bit) scanning. Analysis of computerized images was performed on a 80386 based PC. In a preliminary experiment, infection by *Pythium spp.* affected I.R. reflectance of stressed bentgrass seedlings under controlled conditions. Early detection of plant stress by automated computerized remote sensing could be a useful tool in managing turfgrass problems.

A571

EVALUATION OF BACTERIA FOR BIOLOGICAL CONTROL OF SUMMER PATCH OF KENTUCKY BLUEGRASS CAUSED BY *MAGNAPORTHE POAE*. D.C. Thompson and B.B. Clarke, Dept of Plant Pathology, Rutgers, New Brunswick, NJ 08903.

Ectotrophic growth of *Magnaporthe poae*, the causal agent of summer patch of *Poa* spp may be reduced by rhizosphere bacteria. Eighteen bacterial strains in the genera *Pseudomonas*, *Enterobacter* and *Bacillus* from other biocontrol programs, or from turf exhibiting a decline in take-all disease, were evaluated for antifungal activity against *M. poae* on agar plates, *in vivo* in growth chambers and in the field during 1990 and 1991. Severity of summer patch in the field was reduced by 25 to 36 % by five strains during 1991. Bacterial populations were evaluated in grass rhizospheres and in the soil/thatch/root matrix. Suppressive bacteria remained viable in the turf system but, bacterial populations and *in vitro* antifungal activity were not indicative of summer patch suppression in the field.

A572

PATHOGENICITY OF FUNGI ASSOCIATED WITH A PATCH DISEASE OF ZOYSIAGRASS IN KANSAS. D. Green II, J. Fry, J. Pair, and N. Tisserat. Dept. Plant Pathology and Division of Horticulture, Kansas State University, Manhattan, 66506.

Rhizoctonia solani (AG-2-2), a binucleate *Rhizoctonia* sp., *Ophiosphaerella herpotricha*, *Gaeumannomyces incrustans*, and several unidentified ectotrophic fungi were isolated from roots and crowns of zoysiagrass afflicted with a disease that results in large, circular patches of dead turf in spring and fall. In greenhouse experiments, zoysiagrass inoculated with *G. incrustans*, *O. herpotricha*, and one unidentified ectotrophic fungal isolate, exhibited root and stolon discoloration, root dry weight reduction, but not plant death after three months. Conversely, *R. solani* (AG-2-2) caused extensive sheath and crown rot within two weeks, but not root discoloration, in similar experiments. In replicated field plots, only zoysiagrass inoculated with *R. solani* (AG-2-2) developed patch symptoms. Although ectotrophic fungi may contribute to root dysfunction and turfgrass decline, our results indicate *R. solani* (AG-2-2) is the most common cause of a cool-weather, large patch disease of zoysiagrass in Kansas.

A573

SENSITIVITY OF *PYTHIUM* SPP. FROM CREEPING BENTGRASS TO VARIOUS FUNGICIDES. H. D. Shew, L. T. Lucas and B. D. Mitchum. North Carolina State University, Raleigh, 27695-7616.

The sensitivity of 17 *Pythium* species to six fungicides was determined *in vitro*. Isolates (single or multiple) of all species were obtained from roots and stolons of creeping bentgrass. Fungicides were incorporated into corn meal agar at 0, 1, 10 or 100 ug/ml a.i. Agar plugs containing mycelium were placed on the medium and radial growth measured after 48 hr. Fungicides tested were: etridiazole, metalaxyl, mancozeb, metalaxyl + mancozeb, fosetyl-Al, chloroneb, and propamocarb. LD_{50} values varied with *Pythium* sp. and fungicide. All species were sensitive to etridiazole, with LD_{50} values typically between 1 and 2 ug/ml. None of the species were sensitive to fosetyl-Al *in vitro*. LD_{50} values for other fungicides was species dependent. For example, LD_{50} values for metalaxyl ranged from <1 to >100 ug/ml across species. The role of fungicide sensitivity in the ecology of *Pythium* spp. and *Pythium* diseases on golf greens will be discussed.

A574

CULTURAL AND CHEMICAL CONTROL OF BERMUDAGRASS DECLINE CAUSED BY GAEUMANNOMYCES GRAMINIS VAR. GRAMINIS. M. L. Elliott, University of Florida, Fort Lauderdale Research and Education Center, Fort Lauderdale, FL 33314

A golf course putting green was planted with 'Tifgreen 328' bermudagrass in August 1990. One year later symptoms of bermudagrass decline had developed. Cultural and chemical control treatments were initiated on 21 Sept. 1991 as follows: 1) mowing height increased and plots lightly topdressed; 2) mowing height increased, plots aerified and lightly topdressed, fertility increased; 3) #2 plus fenarimol; 4) #2 plus propiconazole; 5) #2 plus triadimefon; 6) #2 plus thiophanate methyl. Fungicides were applied at curative rates every 21 days. After 4 wk, treatment #1 had significantly greater quality scores over all other treatments. The DMI fungicides appeared to inhibit recovery of the bermudagrass as quality scores for these treatments were still significantly lower than those for both non-fungicide treatments after 12 wk.

A575

TAXONOMY AND PATHOLOGY OF PYTHIUM SPECIES ASSOCIATED WITH DECLINE OF BENTGRASS IN NORTH CAROLINA. **Z. G. Abad, H. D. Shew, L. T. Lucas and K. J. Jones, North Carolina State University, Raleigh, NC 27695-7616.**

Twenty five Pythium spp. were isolated over a two year period from roots and stolons of declining bentgrass in NC. The most predominant were P. torulosum (40%), P. catenulatum (20%), P. arrhenomanes(8%), P. vanterpoolii (6%) and P. graminicola (3%). Pythium complexes of 2, 3 or 4 different species also were detected. P. arrhenomanes, P. aristosporum, P. aphanidermatum, P. graminicola, P. myriotylum, P. tardicrescens and P. Group "T" were highly pathogenic. P. ultimum var. sporangiiferum, P. ultimum var. ultimum were moderately pathogenic, and P. irregulare, P. vanterpoolii and P. torulosum caused low levels of disease on bentgrass seedlings. Other species were non pathogenic. Pythium spp. may play an important role in the summer decline of bentgrass in NC.

A576

RESTRICTION FRAGMENT LENGTH POLYMORPHISM ANALYSIS OF A LOCAL POPULATION OF FUSARIUM OXYSPORUM F. SP. LYCOPERSICI. K. S. Elias and R. W. Schneider, Dept. Plant Path. & Crop Physiol., La. Agric. Exp. Sta., LSU Agric. Cen., Baton Rouge, LA 70803.

A collection of Fusarium oxysporum f. sp. lycopersici (FOL) from a tomato field in which race 3 was recently discovered was assessed for genetic diversity following restriction fragment length polymorphism (RFLP) analysis. These isolates had been previously characterized for virulence, race, and vegetative compatibility groups (VCG). Thirty eight DNA clones from a random genomic library from one isolate of FOL were used as probes for Southern hybridizations to total genomic DNAs restricted with four endonucleases. The data were subjected to cluster and principal components analyses. RFLPs occurred mainly among VCGs, rather than within VCGs or between races 2 and 3. These findings, at a local population level, support our earlier conclusion that new races arise from within existing genetically isolated clonal populations.

A577

GENETIC SIMILARITY AMONG RACES OF *USTILAGO HORDEI* AND *USTILAGO NIGRA*. D.D. Pope. Department of Plant Pathology, University of Georgia, Athens, Georgia, 30602.

The genetic similarity of 13 races of *Ustilago hordei* and nine races of *Ustilago nigra*, the causal agents of covered and false loose smut of barley, respectively, was determined using random amplified polymorphic DNAs (RAPDs) generated by the polymerase chain reaction (PCR). Seven oligomer primers, 10 bases long, amplified a total of 65 different anonymous fragments from genomic templates. Levels of intra- and interspecific variation were low. Results from unweighted pair group mean analysis suggest a high degree of genetic similarity among isolates of both species. No single fragment or simple banding pattern distinguished the species. However, race 6 isolates of both species were genetically identical and were readily distinguished from all other races. Differences among the other races were not as

A578

CORRELATION OF RANDOMLY AMPLIFIED DNA POLYMORPHISMS AND STROMATAL TYPE IN HYPOXYLON TRUNCATUM. C. S. Yoon and D. A. Glawe, Dept. of Plant Pathology, Univ. of Illinois, Urbana, IL 61801.

DNA was extracted from isolates of Hypoxylon truncatum sensu Miller from a population in southern Illinois. The polymerase chain reaction was employed using randomly chosen 10-mer primers, to amplify DNA from the different isolates. Eighteen different primers were used, and a 76 character by 27 isolate matrix was generated. Results were analyzed using the software package NTSYS-pc version 1.5. Two geneticially distinct groups were recognized. When collections were re-examined, they were found to differ in stromatal features. Past taxonomists have differed in their opinions regarding the significance of these stromatal forms. Results of the present study indicate that stromatal types in this species are correlated with genetic differences, and appear taxonomically significant.

A579

DNA FINGERPRINT (MGR) ANALYSIS OF TWO LOCAL RICE BLAST POPULATIONS 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 RFLPs were used to analyze genetic variation in the rice blast pathogen (Magnaporthe grisea) population on a microgeographic scale. One hundred and thirteen isolates were collected from two rice fields (cv. Newbonnet) in Arkansas in 1991. In addition, several reference isolates representing the predominant races in Arkansas were also examined. Total DNA of each isolate was cut with EcoRI and probed with a dispersed repeated "MGR" DNA probe (Hamer et al., PNAS, 1989; Levy et al., The Plant Cell, 1991). Isolates were scored for similarity based on the presence or absence of approximately 50 DNA fragments ranging in size from 2-20kb. Based on DNA similarities, seven distinct fingerprint groups were identified. Isolates within a group had >80% shared fragments and < 50% shared fragments between groups. Of the seven groups identified (A through G), only four (A, B, C, and D) were identified in the two field populations. Group A was the predominant group found representing 72% and 53% of the isolates collected in the two fields. Groups B and D were similar to (approx. 80% shared fragments) two of the reference strains [group B = race IG-1, lineage IG-1B; and group D = race IC-17, lineage IC-17, (Levy et al.)]. Groups C and E were similar to lineages IB-49A and IB-49B, respectively (Levy et al.). Field isolates, representing the four groups (A,B,C, and D) identified in the two fields as well as several reference isolates were compared for virulence in greenhouse pathogenicity tests.

A580

DNA FINGERPRINTING AND RAPD ANALYSIS OF POPULATION DIVERSITY OF COLLETOTRICHUM ORBICULARE. 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.

In a previous study, eleven vegetative compatibility groups (VCGs) and four mtDNA phenotypes had been identified among a collection of cucurbit and non-cucurbit isolates of Colletotrichum orbiculare. The cucurbit pathogen population was made up of four VCGs (VCGs 1, 2, 3, and 4) that all had a common mtDNA RFLP phenotype. VCG 1 and 2 represent the most predominant VCGs in the cucurbit pathogen population and can be differentiated on the basis of virulence on cucurbit hosts. The collection was further examined for molecular diversity using several oligonucleotide probes, including the human mini-satellite probe HVR 33.6, and several two- and four-base decamer RAPD primers. Overall, the oligonucleotide probes identified four DNA fingerprint groups which were the same as the four mtDNA RFLP phenotypes. Minor differences were observed among isolates within certain mtDNA phenotypes with the oligonucleotide probes; however, they did not distinguish the two predominant cucurbit pathogen VCGs (VCG 1 and 2). The RAPD primers which successfully amplified multiple DNA fragments could also be used to distinguish the four DNA fingerprint groups (and the four mtDNA phenotypes). In addition, several RAPD primers were useful in distinguishing VCG 1 and VCG 2 isolates and therefore have utility in population and epidemiological studies with this pathogen.

A581

DIFFERENTIATION OF THE ANGULAR LEAFSPOT FUNGUS WITH RAPD MARKERS: EVIDENCE FOR COEVOLUTION WITH THE COMMON BEAN. P. Guzman[1], D. Mandala[2], R. Nodari[3], W. A. B. Msuku[2], A. B. C. Mkandawire[2], P. Gepts[3], S. Temple[3], and R. L. Gilbertson[1]. [1]Dept. of Plant Pathology, University of California, Davis, CA 95616, [2]Bunda College of Agriculture, Lilongwe, Malawi, and [3]Dept. of Agronomy and Range Science, University of California, Davis, CA 95616.

Phaeoisariopsis griseola (PG) causes Angular Leafspot (ALS) disease of common bean (*Phaseolus vulgaris* L.) in more than 60 countries of the world. The fungus is extremely variable, and host plant resistance against a broad range of PG isolates is not available in bean cultivars. The genetic variability and pathogenicity of 22 monosporic PG isolates from samples collected from 9 bean-growing regions of Malawi were studied.Total genomic DNA was extracted from mycelium and analyzed by RAPD (random amplified polymorphic DNA) marker analysis. Two predominant groups of isolates were identified, one originating from the Middle American host gene pool and the other from the Andean gene pool. Representative isolates from those groups were inoculated onto bean landraces from Malawi, which represented both gene pools, to facilitate identification of sources of resistance to both PG groups.

A582

CHARACTERIZATION OF *FUSARIUM OXYSPORUM* POPULATIONS FROM AGRICULTURAL FIELDS IN MARYLAND IN TERMS OF VEGETATIVE COMPATIBILITY, VIRULENCE AND VARIABILITY IN MITOCHONDRIAL DNA. D. J. Appel and T. R. Gordon, Department of Plant Pathology, University of California, Berkeley, CA 94720.

Populations of *Fusarium oxysporum* were sampled from two agricultural fields in Maryland. Among 197 isolates, 58 vegetative compatibility groups (VCGs) were identified. Greenhouse pathogenicity tests revealed that 56 VCGs, representing 97% of the VCG diversity, were not pathogenic to muskmelon. The remaining two VCGS included isolates of *F. oxysporum* f. sp. *melonis*: VCG 0131, races 0, 1 and 2; and VCG 0134, races 1 and 1,2. This represents the first report of race 1,2 in North America. Both VCGs 0131 and 0134 included nonpathogenic isolates but in all cases nonpathogens and pathogens in the same VCG had different mitochondrial DNA haplotypes. Representative isolates from each of the 56 nonpathogenic VCGs were tested for vegetative compatibility with tester strains from each of 60 VCGs previously identified in the San Joaquin Valley of California. Only one isolate from the Maryland collection was vegetatively compatible with any of the California strains.

A583

GENETIC VARIABILITY WITHIN PYTHIUM ARRHENOMANES /GRAMINICOLA COMPLEX. Chen, W., and Hoy, J. W. Ill. Nat. Hist. Surv., 607 E. Peabody Drive, Champaign, IL 61820, and Dept. of Plant Path. and Crop Phys., La. State Univ. Ag. Center, Baton Rouge, LA 70803.

Pythium arrhenomanes and P. graminicola were compared morphologically and by variations in PCR-amplified rDNA. Ranges for oogonial size and the number of antheridia per oogonium overlap in these species. Restriction enzyme digestions of amplified rDNAs were used to assess genetic variability. No variation was detected among isolates in PCR-amplified nuclear small subunit (NS1/NS8), and a portion (ML1/ML4) of mitochondrial large subunit rDNAs. The amplified ITS (ITS1/ITS4) region had the same length. Patterns of variation were detected in the ITS that were characteristic and could distinguish most isolates of each species. However, additional isolates of each species showed mixtures of restriction patterns characteristic for both species. Results comparing variation in a portion (F63/R635) of the nuclear large subunit rDNA were similar but not identical. Based on these results, P. arrhenomanes and P. graminicola could not be unambiguously separated.

A584

POPULATIONS OF *AGROBACTERIUM* IN TUMORS OF CHERRY TREES TREATED WITH *A. RADIOBACTER* STRAIN K84. S. F. Lu, and L. W. Moore, Department of Botany and Plant Pathology, Oregon State University, Corvallis, OR 97331-2902.

One hundred and nine strains of *Agrobacterium* spp. were isolated from tumors of cherry trees in an orchard in Oregon where *A. radiobacter* strain K84 was not successful in control of crown gall disease in 1991. Homology of plasmids with regions of the Ti plasmid (tmsI-tmr, virABFG) or agrocin 84 plasmid (agrG) was determined by Southern hybridization. Six phenotypes, which varied in pathogenicity, agrocin 84 sensitivity, and antibiotic production were observed among the 109 isolates. Seventy percent of the isolates were pathogenic and contained a Ti plasmid. Eighty four percent of these pathogens were resistant to agrocin 84. Two non-pathogenic isolates contained plasmid DNA homologous to Ti plasmid DNA. Some pathogenic and nonpathogenic isolates had DNA homologous to agrocin 84 plasmid DNA, but only one of these had an identical reaction to K84 antiserum. The prevalence of pathogens resistant to agrocin 84 may have contributed to the failure of biological control in this orchard.

A585

CHARACTERIZATION OF *XANTHOMONAS CAMPESTRIS* STRAINS FROM AROIDS BY GENOMIC FINGERPRINTING. J. H. Graham and A. R. Chase, Univ. of Florida, IFAS, Lake Alfred 33850 and Apopka 32703.

Patterns of large DNA fragments produced with infrequently cutting restriction endonucleases and pulse-field gel electrophoresis of genomic DNA were used to group strains from five aroid hosts. Typical strains of *X.c.* pv. *diffenbachiae* from syngonium from Florida, Hawaii and New York were highly similar, whereas atypical strains were diverse and in one nursery were also found on dieffenbachia. Strains from 'Heartleaf' philodendron in Florida formed three highly related subgroups. One strain from another cultivar of philodendron in South Florida was very similar to a population of strains from xanthosoma in the same area. These strains were different from xanthosoma strains from Barbados. Strains isolated from *Anthurium andraeanum* in Florida, Hawaii and California were related and were distinct from another subgroup of strains associated with other anthuriums in Florida and California. Distinct clonal groups of strains occur on each aroid host that are capable of infecting other aroids.

A586

CHARACTERIZATION OF *PSEUDOMONAS SYRINGAE* PV. *SYRINGAE* MUTANTS WITH REDUCED EPIPHYTIC STRESS TOLERANCE. G.A. Beattie and S.E. Lindow. Department of Plant Pathology, University of California, Berkeley, CA 94720.

To identify phenotypes that enable bacteria to grow and survive on leaves, we are characterizing epiphytic fitness mutants of ice nucleation active *Pseudomonas syringae* pv. *syringae* strain B728a. Using a leaf freezing assay and viable counts, 74 Tn5 mutants of B728a were found that had altered epiphytic population sizes during successive exposure of plants to conditions of high moisture, high light/low moisture, and again high moisture. Fifteen of these mutants experienced a population decrease that was 10- to 1000-fold greater than that of B728a upon exposure to the low moisture conditions; four mutants were further characterized. After 48 hours of growth on leaves under moist conditions, the proportion of the epiphytic population that survived surface sterilization was smaller for the four mutants than for B728a, indicating that these mutants may be poorer at attaining and/or surviving in protected sites. These mutants also attained lower population densities than B728a after vacuum infiltration into bean leaves. Cultural studies show that two of the four mutants were auxotrophs, one for tryptophan and one for methionine, and the other two had altered colony characteristics.

A587

CLONING OF THE SIDEROPHORE SYNTHESIS AND TRANSPORT GENES OF *ERWINIA STEWARTII*. K. Huffman-Kelly, and S.M. Payne. Department of Microbiology, University of Texas at Austin; Austin, Texas 78712

It has recently been found that several members of the genus *Erwinia* produce siderophores, low molecular weight iron chelators, in response to iron deprivation, and siderophore production may be associated with virulence in some species. *E. stewartii*, which causes Stewart's wilt in corn, produces a non-hydroxamate, non-catechol siderophore when starved for iron. An outer membrane protein of approximately 75 kDa, which may act as a receptor for the ferri-siderophore complex, is also produced in response to iron deprivation. A ~35 kb fragment of *E. stewartii* DNA that allowed *Escherichia coli* to produce and to transport the *E. stewartii* siderophore has been isolated. *E. coli* carrying this clone synthesized several novel membrane proteins when iron starved. In addition, ferri-siderophore transport was found to be TonB dependent in *E. coli*. Random mutagenesis was carried out on this clone using Tn*3*::*lacZ*. One iron regulated *lacZ* fusion was isolated that disrupted both siderophore production and transport in *E. coli*. This mutation was recombined back into the *E. stewartii* chromosome, resulting in the loss of siderophore production but not transport.

A588

A COMPARISON OF POPULATION SIZES DETERMINED BY PLATE COUNTS AND DIRECT VIABLE COUNTS IN EPIPHYTIC BACTERIAL POPULATIONS. M. Wilson and S.E. Lindow. Department of Plant Pathology, University of California, Berkeley, CA 94720.

Direct viable counts of epiphytic *P. syringae* populations, determined by a modified version of the yeast-extract-nalidixic acid method, were compared with plate counts from King's medium B to determine whether plate counts are an accurate estimate of the viable population size. Plate counts accurately enumerated *P. syringae* populations multiplying on beans under humid conditions for up to 60h after inoculation. The direct viable count of cells 100h after inoculation (stationary phase) was 4 times the plate count, suggesting that up to 80% of the population was viable but non-culturable. Plate counts accurately enumerated injured cells produced by subjecting a rapidly growing epiphytic population of *P. syringae* to desiccation stress. The magnitude of the decline of the viable population size estimated both by the direct viable count and the plate count were similar. When inoculum of *P. syringae* was applied to plants and subsequently exposed to low relative humidity the rate of decline of viable population size indicated by the direct viable count was less than the rate of decline indicated by the plate count. This suggests that the cells of *P. syringae* stressed by desiccation experienced a transient viable but non-culturable phase prior to death.

A590

CONJUGAL TRANSFER OF PLASMID-ENCODED ARSENATE RESISTANCE TO *CLAVIBACTER MICHIGANENSIS* SUBSP. *SEPEDONICUS*. W. Song and C. I. Ishimaru, Department of Plant Pathology and Weed Science, Colorado State University, Fort Collins, CO 80523.

Plasmid pDG101 is a large (82 kb) conjugative plasmid encoding for resistance to arsenate, arsenite, and antimony in *Curtobacterium flaccumfaciens* pv. *oortii* and related Gram-positive coryneforms. Successful transfer of pDG101 to *Clavibacter michiganensis* subsp. *sepedonicus* was accomplished by mating *C. m.* subsp. *sepedonicus* with a strain of *C. m.* subsp. *michiganensis* that acquired pDG101 from *C. f.* pv. *oortii*. Transconjugants were selected on a nutrient broth yeast-extract medium prepared without phosphate and amended with 30-50 mM arsenate and 20 μg/ml streptomycin. Transfer frequency was low (2×10^{-8} per recipient), but reproducible. Plasmid pDG101 replicated autonomously and was compatible with a cryptic plasmid in *C. m.* subsp. *sepedonicus*. Transconjugants of *C. m.* subsp. *sepedonicus* were resistant to 50 mM arsenate, 5 mM arsenite, and 2 mM antimony.

A591

VARIABILITY OF STREPTOMYCES IPOMOEAE FOR CULTURAL CHARACTERISTICS, PATHOGENICITY, INTERACTION PHENOTYPES, AND PLASMID PROFILES. **C. A. Clark, Dept. Plant Pathol. & Crop Physiol., Louisiana State Univ. Agric. Center, Louisiana Agricultural Experiment Station, Baton Rouge, LA 70803-1720.**

Twenty-seven isolates of *Streptomyces ipomoeae* from sweetpotato roots with soil rot (pox) from Louisiana, North Carolina, California, and Texas were compared. They were identical in standard International Streptomyces Project determinative tests. All induced more fibrous root necrosis on cv. Jewel (susceptible) than on cv. Beauregard (intermediate to resistant) although they varied in aggressiveness. Most induced greater necrosis on storage root slices of Jewel but some induced similar reactions on both cvs. When isolates were grown as a lawn on agar and challenged by spotting other isolates over the lawn, five different reactions (interaction phenotypes) were observed that differentiated three groups of isolates, each from a different geographic region. All isolates shared a common DNA band that comigrated in plasmid profiles (Kieser, T. 1984. Plasmid 12:19) with the 23 kb fragment of HindIII digest of λ phage. Several isolates from Louisiana had up to one smaller and three larger additional bands.

A592

MUTANTS OF *ERWINIA CAROTOVORA* DEFICIENT IN SIDEROPHORE PRODUCTION. C. Bull, S. R. Carnegie, and J. E. Loper, Dept. Botany and Plant Pathology, Oregon State University, and USDA-ARS, 3420 N.W. Orchard Ave., Corvallis, OR 97330.

A mutant of *E. carotovora* strain W3C105, deficient in aerobactin production (luc^-), was obtained by deleting a 2 kb fragment internal to the 10 kb genomic region conferring aerobactin biosynthesis. Insertion of the transposon Tn*3*-Spice into a 5.6 kb *Kpn*I fragment of genomic DNA resulted in a mutation in catechol siderophore production (Cat^-). Strain W3C105 and luc^+Cat^- derivatives grew on an iron-limited medium, TMS containing 150 μM 2,2'dipyridyl; luc^-Cat^+ and luc^-Cat^- mutants did not grow on the medium. On TMS containing 135 μM 2,2'dipyridyl, only the luc^-Cat^- mutants failed to grow. The severity of potato tuber soft rot symptoms caused by W3C105 or luc^+Cat^-, luc^-Cat^+, and luc^-Cat^- mutants were indistinguishable. In culture, an luc^-Cat^+ mutant was less sensitive than W3C105 to siderophore-mediated antagonism by *Pseudomonas* spp.

A593

IDENTIFICATION AND PARTIAL CHARACTERIZATION OF A *HRP* GENE CLUSTER FROM *XANTHOMONAS ORYZAE* PV. *ORYZAE*. M. Mazzola, F. F. White and J. E. Leach. Dept. Plant Pathology, Kansas State Univ. Manhattan, KS 66506.

Xanthomonas oryzae pv. *oryzae* (*Xoo*) is the causal organism of bacterial blight of rice. A cosmid library of *Xoo* was probed with the plasmid pEC812 that possesses a *hrp* gene cluster from *Xanthomonas campestris* pv. *vesicatoria*. Three overlapping cosmid clones (p2-2, p23-14 and p23-44) from *Xoo* hybridized with pEC812. The plasmid p23-44 was mutagenized with Tn5 and insertions were mapped by restriction enzyme analysis. Insertions were introduced into the genome of *Xoo* by marker-exchange mutagenesis. The resulting mutants were screened on rice cultivar IR-24 (susceptible) for loss of pathogenicity. Multiplication of nonpathogenic mutants was severely restricted in rice leaves. The population size of the parental strain was 10^3 to 10^5 cfu/cm^2 larger than that of the mutant strains 16 days post-inoculation. A 27bp oligonucleotide possessing homology to the central conserved domain of prokaryotic two-component regulatory proteins hybridized to an 8.5 kb *Eco*R1 fragment from p23-44.

A594

CLONING AND CHARACTERIZATION OF COPPER-RESISTANCE GENES FROM *XANTHOMONAS CAMPESTRIS* PV. *JUGLANDIS*. Y. A. Lee, M. Hendson, and M. N. Schroth. Department of Plant Pathology, University of California, Berkeley, CA 94720.

Copper-tolerant strains of *X. c.* pv. *juglandis* were found in walnut orchards located in California. Isolation of plasmids from these copper-tolerant strains and mating of these strains with copper-sensitive strains were not successful. These indicated that the copper-resistance genes may be located on chromosomal DNA or high molecular weight nonmobilizable plasmids. A cosmid library of a copper-tolerant strain of *X. c.* pv. *juglandis* was constructed in pLAFR3. One cosmid clone, pCUXJ1 (45 kb), conferred copper tolerance to copper-sensitive strains of *X. c.* pv. *juglandis* and other xanthomonas strains. Subcloning of pCUXJ1 showed that the copper-resistance genes were located on a 6.4 kb *Eco*RI fragment. Tn*3*-Spice insertional inactivation and deletion analysis indicated that at least 5.2 kb was required for full expression of copper resistance. DNA sequence analysis revealed that there were at least four open reading frames within the 5.2 kb fragment oriented in the same direction.

A595

ISOLATION OF A COPPER-INDUCIBLE PROMOTER FROM *XANTHOMONAS CAMPESTRIS* PV. VESICATORIA STRAIN 07882.
A. E. Voloudakis, and D. A. Cooksey, Dept of Plant Pathology, University of California, Riverside 92521.

A 6.8-kb *Xba*I-*Sac*I fragment (pCOP138) that contains the Cu^R genes from *Xanthomonas campestris* pv. vesicatoria was mutagenized with Tn*1737* (Cm^R, *lacZ*) to determine the length of the DNA fragment needed for Cu^R and to localize the promoter region. The orientation of transcription was determined, and a physical map of the area around the promoter was constructed. Several DNA fragments were subcloned into the reporter vector pMP190 (Cm^R, *lacZ*), and the constructs were tested for the presence of a copper-inducible promoter. A 0.8-kb *Bam*HI-*Sph*I fragment was the smallest DNA fragment cloned in pMP190 that produced β-galactosidase in a copper-inducible manner. The promoter was induced by copper in other strains of *X. campestris* pv. vesicatoria but was not expressed in strains of *Pseudomonas syringae* pv. syringae or *P. syringae* pv. tomato.

A596

SURVIVAL OF PLANT-ASSOCIATED FLUORESCENT PSEUDOMONADS AGAINST HYDROGEN PEROXIDE. Martin G. Klotz and Anne J. Anderson, Biology Department, Utah State University, Logan, UT 84322-5305.

Survival of P. putida and strains of P. syringae against H_2O_2 was dependent on growth stage and H_2O_2 concentration. Although exponential phase cells were killed by ≥4 mM H_2O_2, stationary phase cells survived treatment for 15 min with ≥50 mM. Exponential phase cultures of root-associated P. putida produced only one catalase isozyme, KatA, while additional catalase isozymes KatB and KatC occurred during growth into stationary phase. Because exponential phase cells of a KatA-impaired mutant had ≥75% reduced survival at H_2O_2 below 4 mM but survived in stationary phase, isozyme KatA activity appears essential in resistance during early growth. Pathogenic leaf-associated P. syringae isolates maintained their complement of isozymes throughout growth but the proportion of the isozymes varied and total catalase activity increased. Thus, different strategies involving catalases may be required for H_2O_2 resistance of plant-associated pseudomonads. Unexpectedly, two inhibitors of protein synthesis affected the survival to H_2O_2 of the pseudomonads depending on their growth stage. Tetracycline enhanced the survival to H_2O_2 and stimulated catalase activity from stationary phase cultures of P. putida but not P. syringae. Survival of stationary phase cultures of P. putida and P. syringae strains was increased by chloramphenicol, and we are investigating its effects on catalase activities.

A597

EXPRESSION OF P. PUTIDA CATALASE KATA SUBUNIT IN E. COLI GENERATES A CHIMERIC CATALASE. Martin G. Klotz, Jirasak Katsuwon and Anne J. Anderson, Molecular Biology Program, Utah State Univ., Logan, UT 84322-5305.

Strains of fluorescent pseudomonads apparently have evolved different strategies for defense against H_2O_2. Their multiple catalase isozymes appear to consist of different subunits encoded by distinct genes whose expression is environmentally regulated. Escherichia coli K-12 strains synthesize two unrelated oligomeric hydroxyperoxidases, HPI and HPII, and the expression of the genes coding for the HP subunits is independently regulated. Expression of plasmid pUSU101, carrying a 4 kb fragment of genomic P. putida DNA, in catalase-deficient E. coli UM255 yielded a high-activity chimeric catalase species, KatAI. The functional chimeric catalase is approximately 590 kDa and consists of a subunit-trimer from P. putida KatA and three subunits from E. coli HPI. Enzymic properties of KatAI are similar to that of P. putida isozyme KatA, while less active degradation products lose such characteristics as sensitivity to amino-triazole. Expression of pUSU101 in P. syringae pv. pisi strain AN101 did not alter its catalase isozyme composition, but decreased its catalase activity by 80% and rendered exponential phase cultures 50% more susceptible to H_2O_2 below 4 mM. Similar results were obtained with transconjugant KatA-impaired P. putida mutant strain J1, which was not complemented for KatA activity. Characterization of catalase KatAI, plasmid pUSU101and its effects on Pseudomonas catalases is in progress.

A598

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

A large proportion of *Erwinia herbicola* strains isolated from pear in California are capable of producing significant amounts of the plant hormone indole-3-acetic acid (IAA) in cultures supplemented with the precursor tryptophan. Using TLC, HPLC and GC/MS analysis of culture supernatants, indole-3-pyruvic acid and indole-3-acetaldehyde were determined to be intermediates in the IAA biosynthetic pathway of *E. herbicola* strain 299R. Indole-3-acetamide was not detected in culture supernatants and its addition to cell lyzates did not result in the synthesis of IAA unlike in other phytopathogenic bacteria. In radish root bioassays strain 299R, which can cause pear fruit russeting, significantly reduced root elongation suggesting that the strain produces exogenous IAA on plant surfaces and thus can affect the physiology of the plant. IAA production by strain 299R was over four-fold higher in broth culture under nitrogen-limiting conditions than in nitrogen replete media. A possible role for bacterial IAA synthesis in the acquisition of nutrients from plant surfaces is considered.

A599

SACCHAROMYCES CEREVISEAE GENES INVOLVED IN THE RESPONSE TO PSEUDOMONAS SYRINGAE PHYTOTOXIN, SYRINGOMYCIN. **Y. Wang[1], N. Taguchi[2], Y. Yu[1], S. Stock[1], T. Miyakawa[2], and J. Takemoto[1]. [1]Program in Molecular Biology, Utah State University, Logan, Utah 84322 and [2] Department of Fermentation Technology, Hiroshima University, Higashi-Hiroshima, Japan.**

Saccharomyces cerevisiae is highly-sensitive to the lipodepsipeptide phytotoxin, syringomycin, which is produced by strains of Pseudomonas syringae pv. syringae. To study the mechanism of action of syringomycin, S. cerevisiae haploid mutants with single recessive mutations conferring resistance to syringomycin were isolated. Diploid analysis showed that these mutants comprised at least 12 gene complementation groups. Representatives of one group, mutants R4-3G and 3N-H1,were transformed with plasmid (Ycp) yeast libraries to permit cloning of gene Smr1 involved in the syringomycin response. Sequence analyses showed that Smr1 specifies a 40 kDa membrane-associated polypeptide. Antibodies directed against an Smr1-LacZ fusion protein located the protein to the vacuolar membrane. The findings suggest that syringomycin's toxicity involves perturbation of vacuolar function. Using similar approaches, two other syringomycin response genes, Smr2 and Smr3, have been cloned.

A600

DIFFERENTIAL PROTEIN EXPRESSION IN ROOT BORDER CELLS FROM *PISUM SATIVUM* Lindy A. Brigham and Martha C. Hawes. University of Arizona. Departments of Plant Pathology and Molecular and Cellular Biology, Tucson, Arizona 85721

Protein profiles from root border cells differ significantly from protein profiles of the root tips from which they arose. Two dimensional gel comparisons of total protein from root border cells and root tips differ qualitatively and quantitatively. *In vivo* labelling experiments demonstrate even more pronounced differences in newly synthesized proteins between the two cell populations than total protein differences. At least 5 proteins are detectable in protein preparations from border cells that are not apparent in root tip preparations. Additionally, time course experiments indicate that root border cells continue to differentiate after release from the root tip. These observations are consistent with the hypothesis that border cell populations are capable of cell-specific gene expression.

A602

INDUCTION OF IONICALLY BOUND CELL WALL PEROXIDASE AND OF PAL mRNA ACCUMULATION IN POTATO TUBER TISSUE BY NON-PATHOGEN CLADOSPORIUM CUCUMERINUM. Y. Zeng and R. Hammerschmidt. Department of Botany and Plant Pathology, Michigan State University, East Lansing, MI 48824.

Previous work demonstrated that induction of localized resistance of potato tuber discs to Fusarium sambucinum by the potato non-pathogen Cladosporium cucumerinum was correlated with accumulation of lignin-like materials and induction of PAL, CAD and soluble PO activity. The current study further examined whether the induced PAL activity was due to new transcription and whether cell wall bound peroxidase was involved in defense response. Northern analysis showed that PAL mRNA accumulated at a higher level over the control by three hr after inoculation with C. cucumerinum and continued to accumulate through the following 24 hr. At 48 hr, the activity of ionically bound cell wall peroxidase was 8-fold higher in the inoculated tissue. IEF gel analysis of this activity revealed that at least two basic isozymes were induced. This suggested that cell wall ionically bound peroxidase may also be involved in the deposition of lignin-like materials on the cell wall. Further characterization of these basic peroxidase isozymes is in progress.

A603

EFFECTS OF ALFALFA PHYTOALEXIN MEDICARPIN AND RELATED BIOSYNTHETIC INTERMEDIATES ON PHYTOPATHOGENIC FUNGAL MYCELIAL GROWTH.
Jack W. Blount, Nancy L. Paiva, and Richard A. Dixon, The Noble Foundation, P.O. Box 2180, Ardmore, OK 73402

The antimicrobial activities of the alfalfa (*Medicago sativa* L.) phytoalexin medicarpin and four biosynthetic intermediates immediately preceding medicarpin in the phenylpropanoid pathway were tested against eight phytopathogenic fungi. Initially, the five compounds (medicarpin, vestitone, 2'-hydroxyformononetin, formononetin, and daidzein) were tested at a concentration of 0.1 mM in an agar plate assay measuring inhibition of linear fungal mycelial growth. Of the four alfalfa pathogens tested, only *Phytophthora megasperma medicaginis* showed absolutely no inhibition by

medicarpin, but was significantly inhibited by vestitone and 2'-hydroxyformononetin. *Phoma medicaginis* was inhibited by medicarpin only, although the data are highly variable. Three strains of *Nectria haematococca* which vary in their phytoalexin detoxification capabilities were tested, resulting in differing amounts of inhibition among the fungal strains by some of the compounds. To investigate these results further, several of these fungi were tested with the same compounds at concentrations at 0.1 mM, 0.25 mM, and 0.5 mM. The results from these experiments will be useful in the direction of our program involving genetic manipulation of alfalfa phytoalexins.

A604

CHARACTERISTICS OF CITRUS XYLEM FLUID CONTAINING FUSARIUM SOLANI NAPHTHAZARIN PHYTOTOXINS. S. Nemec and W. Osswald, USDA, ARS, 2120 Camden Rd., Orlando, FL 32803.

Naphthazarins, but no other toxins of F. solani, were detected by ELISA in xylem fluid of citrus tree roots in Florida. Protein content in xylem fluid increased as toxin content increased, with 300 $\mu g \cdot L^{-1}$ measured in fluid containing 24.1 $\mu g \cdot L^{-1}$ toxin. Xylem fluid containing about 100 $\mu g \cdot L^{-1}$ toxin contained more amino acids (by TLC), more diverse and quantitatively distinguishable protein bands (by electrophoresis), and a slightly lower pH (5.84 vs. 6.33) than fluid with no detectable toxin. Chitinase activity was 2-fold greater ($P=.10$) in fluid containing 18 $\mu g \cdot L^{-1}$ or more toxin than in fluid with no detectable toxin. Zn and Cu were significantly higher in fluid containing toxin than in fluid not containing toxin, probably because of cation-chelation by the toxins. These data suggest naphthazarins elicit host PR proteins in xylem fluid of blight-diseased trees.

A605

RAPID QUANTIFICATION OF METABOLITES FROM GLIOCLADIUM BY VIDEO IMAGING. R. D. Lumsden, M. E. Vendemia, C. J. Ridout, and W. R. Hruschka. USDA, ARS, Beltsville, MD 20705.

The biocontrol fungus G. virens produces secondary metabolites, including gliotoxin, which are thought to be important in disease control. Since conventional quantification of gliotoxin is time consuming and laborious, a rapid video-imaging system for quantification was devised. Samples were extracted with chloroform, dried, applied to Whatman LK6DF thin-layer chromatography plates in chloroform, and developed with chloroform:acetone (7:3 v/v). Gliotoxin quenched fluorescence of plates in UV light, the intensity of which was recorded with an ITM® densitometric camera in log-corrected mode. JAVA® (Jandel Scientific) program was used on an IBM® compatible computer in intensity mode. Data were expressed as μg gliotoxin by using actual intensity values, relative to the background, converted with the formula: $(\ln(1-(y/b)/a)$ where y=intensity of spot, b=54.36, and a=0.04512. Background intensity was set at 110 on a gray-scale of 256 levels. The values compared favorably with those obtained using high pressure liquid chromatography.

A606

BIOTIN-LABELING IDENTIFIES GLYCOPROTEINS IN CELL WALLS OF NECTRIA HAEMATOCOCCA MACROCONIDIA. Y.H. Kwon and L. Epstein, Department of Plant Pathology, University of California, Berkeley, CA 94720.

Macroconidia of N. haematococca (anamorph, Fusarium solani f. sp. cucurbitae) adhere nonspecifically to plant and synthetic surfaces. Because adhesion is mediated by wall-associated compounds, we compared the macroconidial walls of two adhesive wild-type strains (NhI-2 and NhI-5) with two adhesion-reduced mutants (LE1 and LE2) derived from NhI-2. Sulfo-NHS-biotin and biotin hydrazide were used to label proteins and carbohydrates, respectively; only compounds outside the plasma membrane apparently are labeled. After extraction of biotinylated compounds in Laemmli buffer at 100 C, compounds were separated by SDS-PAGE and then analyzed by western blotting. Blots with either biotinylation reagent were similar, suggesting that the labeled compounds are glycoproteins. Both wild-type strains had bands at 62, 59, 43, 39, 33, 21, and 15 kD. In both adhesion-reduced mutants, four bands had reduced intensity. To determine if these compounds are involved in adhesion, western blots of progeny of LE1 X NhI-5 are being examined.

A607

INDUCTION OF BOTH EARLIER PAPILLA FORMATION AND RESISTANCE TO POWDERY MILDEW BY A PAPILLA-REGULATING EXTRACT FROM BARLEY. S. Inoue, J. R. Aist, and V. Macko. Department of Plant Pathology, Cornell University, Ithaca, NY 14853.

A partially purified, papilla-regulating extract (PRE) has been reported to induce both the formation of over-sized papillae and resistance to penetration by the powdery mildew fungus in inoculated coleoptiles of susceptible barley (Physiol. Mol. Plant Pathol. 39:71-78). A time-course study was conducted to determine if early initiation of papillae is associated with PRE-induced resistance. On average, PRE-enhanced papilla formation was initiated 23-27 min earlier than was papilla formation in controls incubated without the PRE. Moreover, PRE treatment of coleoptiles increased, from 23% to 66%, the incidence of encounter sites at which papilla formation was initiated at least 15 min before penetration pegs were first detected. Thus, earliness of papilla formation is associated with PRE-induced resistance.

A608

ACCUMULATION AND ^{13}C-PULSE-LABELING OF A STRESS METABOLITE IN BACTERIALLY INOCULATED INCOMPATIBLE COTTON COTYLEDONS. P. M. Górski, T. E. Vickstrom, M. L. Pierce, and M. Essenberg, Oklahoma State University, Stillwater, OK 74078-0454.

Localization of phytoalexin biosynthesis in cotton cotyledons during the hypersensitive response (HR) to an incompatible strain of *Xanthomonas campestris* pv. *malvacearum* is being investigated. Mesophyll cells closest to each bacterial colony die and become yellow-green fluorescent. Time-course observations suggest that each such cell first exhibits fluorescence in its cytoplasm and chloroplasts, next becomes uniformly and brightly fluorescent, then turns brown as the fluorescence becomes weaker. Sesquiterpenoid phytoalexins and the related metabolite 2-hydroxy-7-methoxycadalene (HMC) are mainly localized in the fluorescent cells. The peak in HMC level coincides in time with the greatest number of brightly fluorescent cells. We are interested in whether biosynthesis of these compounds takes place i) in the hypersensitively responding cells, ii) in neighboring, healthy cells, or iii) in both. Pulse-label incorporation of [1,2-$^{13}C_2$]acetate into HMC is being analyzed by GC/MS to estimate biosynthetic rates at various times during the HR. Supported by the USDA Competitive Research Grants Program and the Okla. Agric. Exp. Station.

A609

LIGHT INDEPENDENT EXPRESSION OF SPECIFIC HOST RESPONSE mRNAs IN THE BARLEY POWDERY MILDEW SYSTEM. T.A. Clark, R.J. Zeyen, W.R. Bushnell, A.G. Smith*, & T.L.W. Carver**, Departments of Plant Pathology and Horticulture*, University of Minnesota, St. Paul, MN 55108; IGER** - Welsh Plant Breeding Station, Wales, U.K.

We previously demonstrated early, bimodal expression of host response mRNA in the barley powdery mildew system (ca. 4-6 h & 10-15 h post-inoculation). To test the hypothesis that host response genes may be light activated, total RNA was extracted from plants grown and inoculated under either continuous light or continuous dark (etiolated plants) conditions. Northern analysis using known response cDNA probes from barley showed that basic expression patterns were unaffected by light or dark conditions. Minor variations in host response mRNA expression were noted, and paralleled the timing of host cytoplasmic aggregate responses to the parasite's primary germ tube (PGT) and appressorial germ tube (AGT) contact stimuli. In etiolated plants, mRNA responses to PGT contact were less intense or more prolonged (in the 2 to 8 h post inoculation period), and obscured the early peak of bimodal expression observed in light-grown plants. Response genes tested are activated by parasite contact stimuli irrespective of light or dark conditions.

A610

ELICITATION OF OXYGEN RADICAL GENERATION IN CYTOCHEMICAL DEFENSE T.J. Jacks, G.H. Davidonis, and T.E. Cleveland, USDA, ARS, Southern Regional Research Center, P.O. Box 19687, New Orleans, LA 70179

An increase in oxygen consumption occurred after a short lag period when cultured cotton cells were in contact with isolated mycelial walls of Aspergillus flavus and other substances. Using electron transport inhibitors, we ruled out mitochondrial respiration as a significant factor. However, oxygen consumption was partially inhibited by both superoxide dismutase (SOD) and catalase but not with oxygen radical scavengers. The results indicate that most of the consumed oxygen was being electronically reduced to superoxide, probably by NADPH oxidase. Hydrogen peroxide and hydroxyl radicals were then generated both enzymically and spontaneously. Consumed oxygen was partly replenished by exogenous SOD and catalase, giving the appearance of partial inhibition. Conversion of molecular oxygen to microbicidally active forms by host cells comprises part of a cytochemical defense mechanism against microbial invasion.

A611

DEGRADATION OF FUSARIUM CELL WALLS BY CELERY HYDROLASES: TESTS OF THE LIMITATION OF CHITINASE ACTIVITY BY ß-1,3-GLUCANASE. S. Krebs and R. Grumet, Michigan State University, East Lansing, MI, 48824.

The plant encoded enzymes chitinase (CHIT) and ß-1,3-glucanase (B13G) are capable of hydrolyzing fungal cell walls. Effective resistance may require the synergistic activity of both enzymes in the apoplast during the early stages of fungal infection. In a celery-Fusarium system, we hypothesize that amounts and location of B13G limit the effectiveness of these hydrolases as early resistance mechanisms. We found a single celery B13G that is weakly induced by Fusarium infection and is localized intracellularly. Although celery CHIT is strongly induced, and 5 of 6 isoforms occur in the apoplast, our data suggest that CHIT activity on F. oxysporum f.sp. apii is dependent on B13G levels. Reports on other Fusarium species indicate that CHIT cannot access cell wall chitin until exterior callose is degraded by B13G. We have purified B13G and CHIT from celery and are studying their separate and combined activities against the Fusarium yellows pathogen. A novel one step method of B13G purification was developed: affinity adsorption to an insoluble substrate (pachyman) and elution with a soluble substrate (laminarin).

A612

USE OF ISOZYME ANALYSIS FOR RAPID DETERMINATION OF THE RACES OF ***BIPOLARIS ZEICOLA***. S. Gaul and A. Balducchi, ICI Seeds, Research Dept., P.O. Box 500, Slater, Iowa 50244.

Research was undertaken to apply and expand isozyme analysis, originally developed by Drs. K. Simcox and W. Pedersen at the University of Illinois, as a means of race determination among isolates of ***Bipolaris zeicola***. Thirty isolates which were race identified in the greenhouse along with thirty isolates of unknown race were examined with starch gel electrophoresis. The gels were stained for aspartate aminotransferase (EC# 2.6.1.1), esterase (EC# 3.1.1.1), and leucine aminopeptidase (EC# 3.4.11.1). Banding patterns from these stains resulted in ten different electrophenotype (EP) groups. Nine EP's were identified with individual races. Of these, six EP's were identified with race 2, two with race 3, and one with race 1. One EP was associated with isolates of both races 1 and 2. Race determination by isozyme analysis as compared to greenhouse procedures increased isolate processing efficiency from forty-two days to three days.

A613

SUPPRESSION OF DEFENSE RESPONSES OF BEAN BY THE COMPATIBLE BACTERIUM *PSEUDOMONAS SYRINGAE PV. PHASEOLICOLA*. J. L. Jakobek and P. B. Lindgren, Dept. of Plant Pathology, North Carolina State University, Raleigh, NC 27695-7616.

We have found that transcripts for phenylalanine ammonia-lyase (PAL), chalcone synthase (CHS), chalcone isomerase (CHI), and chitinase accumulated in bean 6 hours after infiltration with *Pseudomonas syringae* pv. *tabaci, angulata, glycinea, pisi, tomato, syringae*, Hrp⁻ mutants of pv. *tabaci* and *glycinea*. *P. fluorescens*, or *Escherichia coli*. In contrast, three isolates of compatible *P. s.* pv. *phaseolicola* (NPS3121, PP134 and PP19304) did not induce transcripts for PAL, CHS, or CHI up to 120 hr after inoculation when leaf tissue was badly damaged due to symptom development. Chitinase transcript was detected 72 hr after infiltration with these strains. When bean plants were inoculated with NPS3121 8 hr prior to inoculation with glutathione, an abiotic elicitor of defense responses, transcript accumulation was significantly reduced when compared to plants inoculated with glutathione alone. This suppressor activity of NPS3121 was inactivated by protein synthesis inhibitors or heat treatment. We will present data from these and other experiments which suggest that NPS3121 may have an active mechanism to suppress the production of defense responses of bean.

A614

CONTROL OF PYTHIUM ROOT ROT OF WHEAT IN THE FIELD WITH BACTERIAL SEED TREATMENTS. Milus, E. A., Rothrock, C. S. and Rhoads, M. L., Department of Plant Pathology, University of Arkansas, Fayetteville, AR 72701.

Bacterial strains were isolated from the rhizoplane of wheat roots in Arkansas and tested for control of Pythium root rot in growth chamber experiments. Effective strains were applied to seed of Caldwell winter wheat and planted at two locations in the fall of 1991. Check treatments included *Pseudomonas fluorescens* strain 2-79, metalaxyl fungicide, alginate and nontreated seed. Wheat roots were sampled 5-7 wk after planting, and the incidence of infection was quantified on a Pythium-selective medium. Three strains from Arkansas reduced incidence of root infection compared to the nontreated and alginate-treated checks and were similar to the metalaxyl and strain 2-79 checks. The most effective strain reduced the percentage of infected seedlings from 75% (for the alginate check) to 54%. All strains effective in the field were *Pseudomonas* spp. and were antagonistic to *Pythium graminicola in vitro*. Effects of bacterial seed treatments on plant growth and yield also will be determined.

A615

ANALYSIS OF METHODS FOR REINTRODUCING ENDOPHYTIC BACTERIA INTO SWEET CORN AND COTTON. J. A. McInroy and J. W. Kloepper, Dept. of Plant Pathology, Auburn University, AL 36849-5409.

A 2-yr field study of the population dynamics of endophytic bacteria yielded a collection of 564 bacteria from sweet corn and 553 from cotton. Future investigations of possible plant-growth promotion or biological disease control potential of these endophytes are predicated upon having methods for reintroducing strains into plant stems or roots. Seven reintroduction methods were tested for sweet corn and cotton with spontaneous rifampicin-resistant mutants of 7 endophytic bacterial strains. With both crops, stem injection consistently was the most effective method for reintroduction, based on recovery of bacteria 2 wk after introduction. Bacteria were also recovered, although at lowered populations, following immersion of wounded sweet corn and cotton roots in bacterial suspensions and seed treatment of cotton. Bacterial recovery was poor following reintroduction by foliar spray, vacuum infiltration of seed, drenching soil with suspensions, and a combination of seed treatment and soil drench. These results demonstrate that bacteria isolated from inside plants may be experimentally reintroduced for microbial ecology studies, and that the method of reintroduction significantly affects establishment of the bacteria inside plants.

A616

ROLE OF COPPER RESISTANCE IN SURVIVAL OF *PSEUDOMONAS FLUORESCENS* IN A CITRUS SOIL. C.-H. Yang and D. A. Cooksey, Department of Plant Pathology, University of California, Riverside.

Citrus grove soils in California often contain high levels of copper from many years of copper fungicide applications. *Pseudomonas fluorescens* 09906, which was isolated from a citrus soil and suppressed Phytophthora root rot, was resistant to $CuSO_4$ in a minimal medium (MIC=1.6 mM). Two copper-sensitive Tn*5* mutants of this strain were obtained (MIC=0.16 mM), and both mutants had reduced survival in a citrus soil. Populations of the wild type strain declined less than ten-fold over a 30-day period, from 3.2×10^7 to 4.1×10^6 cfu/g of soil, while populations of the two copper-sensitive mutants declined more than 200-fold during the same period, from 1.3×10^7 to 6.0×10^4 and 1.4×10^7 to 2.9×10^4 cfu/g of soil, respectively. Copper resistance may therefore be an important factor in survival of soil bacteria used for biological control where copper fungicides are frequently applied.

A617

CONTROL OF EUROPEAN CORN BORER IN FIELD CORN USING A GENETICALLY ENGINEERED ENDOPHYTE. Stephen F. Tomasino, R. Mark Beach, and R. Todd Leister. Crop Genetics International, Hanover, MD 21076.

Control of the European corn borer (ECB) (Ostrinia nubilalis) in field corn using the endophyte Clavibacter xyli subsp. cynodontis, genetically engineered to produce the delta endotoxin of Bacillus thuringiensis subsp. kurstaki (Cxc/Bt), was measured in field tests near Hastings, Nebraska during 1991. ECB damage (number and length of tunnels) was assessed in Cxc/Bt colonized plants and uncolonized controls across 16 corn hybrids. Cxc/Bt was established in seedling plants via a stab-inoculation method. ECB larvae were applied during the pollen shed stage of development. Across hybrids, Cxc/Bt reduced the number of tunnels 39% and the total amount of tunneling 45%. Cxc/Bt caused significant reductions in number of tunnels and total tunneling in 12 and 11 hybrids, respectively.

A618

ESTABLISHMENT OF BACTERIAL ANTAGONISTS ON BLOSSOMS OF PEAR. V. O. Stockwell, K. B. Johnson, and J. E. Loper, Department of Plant Pathology, Oregon State University, and USDA-ARS, HCRL, 3420 N.W. Orchard Ave., Corvallis, OR 97330.

At 10-30% and 70-80% bloom, aqueous suspensions (10^8 cfu/ml) of *P. fluorescens* A506 and *E. herbicola* C9-1 were sprayed separately and in combination onto pear trees located in Corvallis and Medford, Oregon. Bacterial population sizes were estimated from stigmas and nectaries of 48 treated blossoms every two days throughout the bloom period. In both locations, mean population sizes of both strains varied from 10^3 to 10^5 cfu/blossom. The proportion of blossoms that had detectable populations of a bacterial antagonist, however, did not always correlate to mean population sizes. Detectable bacterial populations on blossoms treated with a single bacterial strain decreased from ca. 100% at application to 30-50% at full bloom. In contrast, detectable bacterial populations on blossoms treated with both strains was ca. 100% in Medford and 60% in Corvallis.

A619

INTEGRATED CONTROL OF FROST INJURY, FIRE BLIGHT, AND FRUIT RUSSET OF PEAR WITH A BLOSSOM APPLICATION OF AN ANTAGONISTIC BACTERIUM. S.E. Lindow. Department of Plant Pathology, University of California, Berkeley, CA 94720.

Pseudomonas fluorescens strain A506, which is naturally resistant to 100µg/ml streptomycin and oxytetracycline, was applied to pear trees in the field either once at 20% bloom or at 20% and 90% bloom. At two weeks following treatment the mean population of strain A506 was about 10^5 cells/flower and over 95% of pear flowers had detectable numbers of this antagonist. Over 95% of the total bacteria recovered from flowers from treated trees was strain A506. The incidence of frost injury in flowers shortly after frosts with minimum temperatures of about -4C and of fruit at harvest was reduced from 70 to 85% on trees treated both with strain A506 and streptomycin compared to trees treated with streptomycin alone. The incidence of fire blight strikes was also reduced from 60 to 80% on treated trees compared to control trees. The severity of fruit russet was reduced from 11.7% on trees treated only with streptomycin to 6.5% of the fruit surface on trees treated both with A506 and subsequent streptomycin sprays.

A620

EFFECTS OF CHLORIDE, TRIADIMENOL AND GLIOCLADIUM ROSEUM ON TAKE-ALL ROOT ROT OF WHEAT. C.C. Bernier, L. Lamari, and J.-A. Stebbing, Department of Plant Science, University of Manitoba, Winnipeg, MB. R3T 2N2 Canada.

The following treatments were evaluated at two field sites on spring wheat (SW) cv. Neepawa and winter wheat (WW) cv. Norstar over 2 years: KCl, NH_4Cl and K_2SO_4, each at 3 rates, Triadimenol at 2 rates, Triadimenol and chloride, and G. roseum as infected millet seed. Triadimenol was applied to wheat seed. Other treatments, and take-all inoculum as infected millet seed, were added to the furrow at seeding time. Plots consisted of 4 rows 5m long replicated 6 times. Sterile millet served as control and all treatments were repeated in non-inoculated plots. Treatment effects were larger in SW than in WW. Some treatments consistently increased grain yield but others only in some years and sites. KCL and NH_4Cl at the highest rates significantly increased yield of SW in take-all inoculated plots only. Triadimenol alone or with KCl or NH_4Cl, and G. roseum were also effective. Yield of SW ranged from 40 to 94% of the non-inoculated control.

A621

INTEGRATED BIOLOGICAL AND CHEMICAL CONTROL OF ALFALFA SEEDLING BLIGHT. M. Aragaki and P. S. Yahata. Department of Plant Pathology, University of Hawaii, Honolulu, 96822.

Twelve isolates of Rhizoctonia solani (AG4) were sensitive to chloroneb, triflumizole, or pencycuron. Of 66 binucleate, R solani-like (BRS) fungi, 21 isolates tolerated chloroneb. BRS isolate 1238 was selected as the biocontrol tester based on insensitivity to chloroneb, avirulence to alfalfa, and limited effectiveness to protect alfalfa against R. solani. The integration of BRS 1238 with chloroneb resulted in primarily additive effects: in a representative test, 91% seedling mortality was obtained with R. solani, 79% mortality when protected with BRS 1238, 23% mortality when protected with chloroneb, 13% mortality with both agents.

A622

PRELIMINARY SCREENING OF STEM AND ROOT ROT PATHOGENS AS POTENTIAL BIOCONTROL AGENTS FOR LEAFY SPURGE. D. R. Johnson and S. M. Yang. USDA/ARS, Foreign Disease-Weed Science Research Unit, Bldg. 1301, Ft. Detrick, Frederick, MD 21702

Two hundred fifty fungal isolates were obtained on potato-dextrose-agar (PDA) from stems or roots of leafy spurge (*Euphorbia esula* L.) collected in China and the United States (Maryland and Nebraska). Fungi were transferred to new PDA plates amended with penicillin G and streptomycin sulfate (one isolate/plate). Thirty grams of autoclaved wheat kernels were placed on each PDA plate three to four days after the fungal transfer. One week later, the wheat kernels, overgrown with mycelium, were placed on the soil near stems of leafy spurge in a single pot, covered with soil, and top watered. Plants inoculated with pathogenic *Fusarium* and *Rhizoctonia* isolates showed crown rot, wilting, and death of the entire plant four to six weeks after inoculation. Plants inoculated with nonpathogenic *Aspergillus* and *Trichoderma* isolates remained healthy. Thirty isolates have been selected for further studies.

A623

Biological control of fire blight with Pseudomonas fluorescens strain A506 and two strains of Erwinia herbicola . R. J. McLaughlin and R.G. Roberts, USDA, ARS, Tree Fruit Research Laboratory, Wenatchee, WA 98801.

Laboratory and field assays were conducted to evaluate the biological control potential of Pseudomonas fluorescens strain A506 and Erwinia herbicola strains C9-1 and CN-1. Laboratory tests included assays on d'Anjou pear using immature pear slices and a forced blossom assay. Immature pear fruit slices were pretreated with a 10^8 cfu/ml suspension of the test strains and subsequently inoculated 0 or 6 hr later with a 10^7 cfu/ml suspension of Erwinia amylovora strain 87-70. At 5 d after inoculation, infection was usually absent in slices treated with both E. herbicola strains and 100% infection in treatments with strain A506. In forced blossom assays, blossoms were forced and were pretreated with 20 ul of a suspension of the test strains in the nectary and inoculated 24 hr later with 15 ul of a 10^7 cfu/ml suspension of E. amylovora. Forced blossom assays showed C9-1 as having the most potential for controlling fire blight. A 1991 field test showed significant reduction of blight by C9-1 and Terramycin under conditions of heavy infection. The E. herbicola strains occurred on flowers at $\geq$ 5 log cfu/flower from 3-10 days after the initial spray application to flowers. Colonization by strain A506 ranged from 2.5 to 5 log cfu during this time. Combined application of C9-1 and A506 reduced flower colonization by strain A506.

A624

BIOCONTROL OF THIELAVIOPSIS BLACK ROOT ROT OF PETUNIA PLUG TRANSPLANTS. R. G. Linderman and J. L. Marlow. USDA-ARS Hort. Crops Research Laboratory, Corvallis, OR 97330

Petunia plug transplants were inoculated with bacterial antagonists, identified from in vitro assays, 4 days prior to inoculation with the black root rot pathogen, Thielaviopsis basicola. After 2 wks, seedling plugs were transplanted to larger containers, grown for 5 wks, harvested, and evaluated for plant size and disease severity. Severe stunting occurred in the pathogen-inoculated control plants. Several bacterial treatments protected the transplants, and growth was equal to the non-inoculated controls; others had no effect or increased disease. The protective microbes were Bacillus spp. from a Hypnum peat and Streptomyces griseoviridis (Mycostop) from a Finnish peat. Protection began in the plug stage, since lack of disease with effective treatments was apparent at transplant. These results suggest that plug transplants can be protected against seedling diseases if antagonists are applied soon after emergence.

A625

AN ASSAY FOR SELECTION OF YEASTS INHIBITORY TO INFECTION OF TOMATO BY ALTERNARIA SOLANI. R.D. Reeleder, S. Monette, and R.A.A. Brammall.[1] Agriculture Canada Research Station, Delhi, Ontario. N4B 2W9. [1]Horticultural Research Institute of Ontario, Simcoe, Ontario, Canada. N3Y 4N5.

A screening test was developed to evaluate yeast isolates for their ability to inhibit Alternaria blight lesion development on tomato leaf tissue. Mature leaves of growth chamber grown tomato plants were placed in a glass petri dish lined with moistened filter paper. Conidia of A. solani were recovered from two-week-old mycelial mats produced in liquid V-8 juice cultures. Yeasts were produced in potato dextrose broth shake cultures. Prior to inoculation of leaves, yeast and conidial cultures were combined. Aliquots of each mixture were deposited onto a tomato leaf. The glass petri dishes containing the tomato leaves were incubated for 7 days at 22°C with a 12 hr photoperiod. At the end of the incubation period, the lesions were examined and compared with appropriate controls. With this technique, 121 yeasts were screened. Of those, 30 isolates were capable of consistently inhibiting development of Early Blight lesions.

A626

BIOLOGICAL CONTROL OF PHYTOPHTHORA CAPSICI ON CHILE PEPPERS WITH SEVERAL FUNGAL AND BACTERIAL STRAINS. S.E. Indiogine, C.M. Liddell, C.L. Biles and J.P. McEntee, New Mexico State University, Dept. Entomology, Plant Pathology, and Weed Science, Box 30003/3BE, Las Cruces, NM 88003.

Several bacteria and fungi were evaluated as biocontrol agents of *Phytophthora capsici* on pepper (*Capsicum annuum*). A series of 5 greenhouse experiments were done with chile pepper cv. 'New Mexico 6-4' grown in pots. Plants were preinoculated with *Fusarium oxysporum* C14, *Trichoderma* sp. NM2056, *Pythium oligandrum* NM2060, *Erwinia herbicola* SR2, SR3, or *Pseudomonas putida* NIR. Disease incidence was recorded every 2-4 days and plants were rated on a 0-4 scale, 0=no symptoms, 4=dead. Protection was calculated as: (positive control - treatment) / (positive control - negative control). Among the fungal biocontrol agents, *P. oligandrum* NM2060 was the most consistent and provided a high level of control (38-84%). *F. oxysporum* C14 gave less

consistent results, but provided good control (45-92%). *Trichoderma* sp. NM2056 gave the highest levels of protection(37-100%) but was the least consistent. Data from the bacterial treatments were variable and not consistent. Overall, *Erwinia herbicola* SR2 and SR3 were more effective (45 and 41% respectively) than *Pseudomonas putida* NIR (37%). Disease incidence of the positive control decreased from May to August due to decreasing temperatures in the greenhouse.

A627

SPATIAL PATTERNS OF BROOM SNAKEWEED MORTALITY INDUCED BY *PUCCINIA GRINDELIAE*. C.M. Liddell, J.M. McEntee, C.A. Waddell. New Mexico State University Department of Entomology, Plant Pathology and Weed Science, Box 3BE, Las Cruces, NM 88003.

Puccinia grindeliae Peck is a potential biocontrol agent of broom snakeweed [*Gutierrezia sarothrae* (Pursh) Britt. & Rusby], a rangeland shrub native to the western United States. The spatial patterns of dissemination and establishment of this rust on broom snakeweed were characterized. A survey site was established in a community of rust-infected snakeweed plants. An 11 m x 11 m grid of contiguous 1 m^2 quadrats was sampled 3 times: March 7-9, 1990, June 27-July 1, 1990 and March 6-10, 1992. The area of each plant was determined. Individual telia were counted on each infected plant. The results showed that disease incidence in quadrats with disease present increased from 49% to 63% over two years. The mean number of plants for all quadrats decreased from 3.8 to 2.7, an apparent mortality rate of 14%. The average area of snakeweed per quadrat declined from 15.6% to 11.3% over this period. The number of quadrats containing snakeweed plants decreased from 113 to 108 suggesting that the disease spreads slowly from dying plants to living plants. Host mortality can be linked directly to areas of high rust incidence.

A628

EFFECT OF ANTAGONISTIC BACTERIA ON ESTABLISHMENT OF HONEY BEE-DISPERSED ERWINIA AMYLOVORA IN PEAR BLOSSOMS AND ON FIRE BLIGHT CONTROL. K.B. Johnson[1], V.O. Stockwell[2], D. Sugar[1], and J.E. Loper[2]. [1]Dept. of Botany and Plant Pathology, Oregon State University, and [2]USDA ARS Hort. Crops Research Lab., Corvallis, OR 97331.

In spring 1991, a bee hive was placed in a block of 20 pear trees enclosed with shade cloth. During bloom, bees were infested with freeze-dried E. amylovora as they exited their hive. The following treatments were spray applied to trees at 30% and full bloom: 1) Pseudomonas fluorescens strain A506 plus E. herbicola strain C9-1 (both at 10^8 cfu/L), 2) streptomycin sulfate (0.75 g/L), or 3) water. At full bloom, incidence of A506 and C9-1 exceeded 90% in treated blossoms. One week after full bloom, 43% of water-treated blossoms and 18% of blossoms treated with bacterial antagonists had detectable populations of E. amylovora. Four percent of blossoms treated with bacterial antagonists developed populations of E. amylovora that exceeded 10^5 cfu/blossom compared to 19% of those treated with water. The A506 plus C9-1, streptomycin, and water treatments averaged 4.5, 0.5, and 24 fire blight strikes/tree, respectively.

A634

PARASITISM OF HYPHAE AND SCLEROTIA OF *RHIZOCTONIA SOLANI* BY *STACHYBOTRYS ELEGANS*. M. Benyagoub and S. H. Jabaji-Hare, Dept. of Plant Pathology, Université Laval, Ste.-Foy, Québec, Canada, G1K 7P4.

The mycoparasitic interaction of *Stachybotrys elegans* with *Rhizoctonia solani* Kühn was studied in dual culture by means of light and transmission electron microscopy. Hyphae and sclerotia of *R. solani* were aggressively parasitized by *S. elegans*. Parasitism of host cells was characterized by hyphal coiling or appressorial formation, penetration at different sites of the host cell walls and proliferation of the mycoparasite within the host cells, followed by destruction of the host cytoplasm. Electron micrographs showed that during the interaction, the cells of *R. solani* are enveloped by a fibrillar matrix. At the point of contact, *S. elegans* penetrates and degrades the host cell wall. Invaded *R. solani* cells are found empty or with little cytoplasmic residue. In response to invasion, a reaction zone consisting of electron-dense bodies develops between the cell wall and the invaginated plasma membrane.

A635

COMPARISON BETWEEN GROWTH CHAMBER AND FIELD EVALUATIONS OF BACTERIAL STRIPE RESISTANCE IN SOFT RED WINTER WHEATS. **Mirlohi, A. F. and Milus, E. A., Department of Plant Pathology, University of Arkansas, Fayetteville, AR 72701.**

Primary and flag leaves of ten soft red winter wheat cultivars were inoculated under controlled conditions with 10^4 and 10^6 cfu/ml, respectively, of *Xanthomonas campestris* pv. *translucens* using a syringe tipped with a short length of rubber tubing. Inoculation sites were rated for disease reaction (0-5 or 0-4 for primary and flag leaves, respectively) 5-6 days after inoculation. The same cultivars were evaluated for bacterial stripe severity in inoculated and noninoculated field plots in 1991. Disease severity was correlated highly with disease reaction on primary ($r = 0.84$) and flag ($r = 0.77$) leaves. Disease reaction on primary leaves also was correlated highly with disease reaction on flag leaves ($r = 0.89$). Further studies using a larger number of wheat cultivars and 1992 field data to evaluate the usefulness of disease reactions under controlled conditions for selecting bacterial stripe resistance also will be presented.

A636

INFLUENCE OF LONG-TERM CROP MANAGEMENT ON WHEAT DISEASES IN EASTERN OREGON. R.W. SMILEY, H.P. COLLINS, AND W. UDDIN. Oregon State University and USDA-ARS, Columbia Basin Agricultural Research Center, P.O. Box 370, Pendleton, OR, 97801.

Quantification of wheat diseases were needed on three long-term (26- to 60-yr-old) experiments at Pendleton. Root diseases were surveyed over a 3-yr period in experiments with continuous winter wheat (W-W), wheat/fallow (W-F), and wheat/pea (W-P) rotations. Root rot caused by Rhizoctonia + Pythium spp. was greater in W-F than in A-W or W-P. Eyespot was most severe in A-W, intermediate in W-F, and least in W-P. Common root rot was most severe in A-W and least in W-F and W-P. Take-all and Cephalosporium stripe were not affected by crop sequences in these long-term experiments. Eyespot in A-W was more severe during a wet year than in drier years, and the reverse was true for the root rot complex. The root rot complex in the W-F experiment was more severe with inorganic-N than animal manure treatments, and was reduced by burning stubble before plowing. During the wet year Pythium root rot was more severe in a plowed than a non-tilled treatment in the W-P experiment.

A637

ANTIBODY INACTIVATION OF THE PTR-NECROSIS TOXIN FROM TAN SPOT INFECTED WHEAT PLANTS. L. Lamari and G.M. Ballance, Dept. of Plant Science, Univ. of Manitoba, Winnipeg, MB, R3T 2N2 Canada.

The Ptr-necrosis toxin, produced by nec+ isolates of Pyrenophora tritici-repentis is a host-specific protein toxin responsible for the induction of tan necrosis in wheat. Intercellular washing fluid (IWF) was extracted from fungus (toxin producing isolates) infected leaves 48 hours post-inoculation. Rabbit anti-Ptr necrosis toxin antiserum was mixed at concentrations of 1, 10^{-1}, 10^{-2}, 10^{-3} and 10^{-4} mg/ml to IWF samples to produce a final IWF dilution of 1:4. Similar amounts of antiserum were added to purified Ptr-necrosis toxin to achieve a final toxin concentration of 10^{-4} mg/ml. Toxic activity was normal in treatments without antiserum and with dilutions of 10^{-4}, 10^{-3}, 10^{-2} mg/ml. At 10^{-1} mg/ml, partial and total loss of activity were observed for the purified Ptr necrosis toxin and the IWF respectively. The 1 mg/ml antiserum treatment resulted in total loss of toxic activity for both. The results of the bioassay, and the antibody inactivation of toxic activity suggest the presence in the IWF of the Ptr-necrosis toxin.

A638

THE EFFECT OF TEMPERATURE ON WHEAT REACTION TO PYRENOPHORA TRITICI-REPENTIS (Ptr), AND ITS NECROSIS TOXIN. L. Lamari, C.C. Bernier and R.B. Smith., Dept. of Plant Science, University of Manitoba, Winnipeg, MB, R3T 2N2 Canada.

The effect of increasing temperature on wheat reaction to the Ptr necrosis toxin, and to Ptr isolates 86-124 and D308, respectively necrosis and chlorosis inducers, was assessed on a set of six wheat lines. The host lines were either susceptible (necrotic and/or chlorotic) or resistant. Temperature regimes of 15, 22, 25, 27 and 30°C (constant day/night) were investigated. Resistant lines remained resistant to both pathotypes under all temperature regimes. However, a clear shift toward resistance to both necrosis and chlorosis was observed in all susceptible lines at 25 and 27°C and at 30°C all lines were completely resistant (small black spots without tan necrosis and chlorosis). Wheat reaction to the Ptr necrosis toxin followed a similar trend. The results of this study indicate that temperature should be a major consideration in screening germplasm for resistance to tan spot.

A639

CULTURAL AND CHEMICAL CONTROL OF COMMON ROOT ROT CAUSED BY BIPOLARIS SOROKINIANA IN 1989-90 ON OKLAHOMA WINTER WHEAT. Singleton, L.L., and Russell, C.C. Plant Pathology Dept., Okla. State Univ., Stillwater, OK 74078-9947.

Tillage regimes (**TLL**= Plow [**P**], sweep [**SW**], and sweep + disk [**SWD**]); planting date (**PDT** = early [**EPDT**; Sept. 1] vs. late [**LPDT**; Oct. 15]), and chemical seed treatment (**SDT** = PCNB/IMAZALIL, 88.7 ml/45.4 kg vs. none) were evaluated for their effects on subcrown internode lesion (**SIL**) index, stand count (**SDCT**/m of row), and grain yield (**GYLD**)in a split plot study. **PDT**: **SIL** was significantly greater ($P \geq 0.001$) with **EPDT**, and corresponded with **GYLD** reduction ($P \geq 0.01$) and **SDCT** ($P > 0.05$). **TLL**: **SIL** was less for **P** ($P \geq 0.05$) than with **SW** or **SWD**. Among **TLL** X **PDT** means, (**EPDT** + **SW**) and (**EPDT** + **SWD**) resulted in higher **SIL** ($P \geq 0.05$) than (**LPDT** + **SW**) and (**LPDT** + **SWD**). **EPDT** and **LPDT** with **P** were n.s. **SDT**: Both **SIL** and **SDCT** were reduced ($P \geq 0.05$) with chemical seed treatment, **GYLD** was n.s. **SDT** X **PDT** interaction for **SIL** was significant ($P \geq 0.05$). **SDT** + **LPDT** resulted in the greatest reduction in **SIL** ($P \geq 0.01$). **PDT** was the predominant factor.

A640

TEMPORAL AND SPATIAL DEVELOPMENT OF SEPTORIA BLIGHT OF OAT WITH STRIP INTERCROPPING. K. M. Tubajika and C. A. Martinson, Department of Plant Pathology, Iowa State University, Ames, IA 50011.

Oat diseases were studied in three strip intercropping experiments and on two Iowa farms across Iowa in 1991 (strips were 3.0-4.6 m wide and included oat, maize, and soybean in rotation). Septoria blight, incited by Septoria avenae, was the only foliar disease developing in oat strips, that appeared related to oat debris from the prior year. Heavy rainfall occurred during the first two months after planting and was followed by dry weather. Leaf blight developed initially and linearly over time in the rows of oat adjacent to the oat debris from the prior year. Disease progress in rows in the center of the strip and farthest from the debris followed a monomolecular model. The disease gradient across the strips was linear with an initial regression coefficent (b) of -0.03x (% incidence x cm distance from the line source of potential inoculum). As time progressed, b became -0.07x and greater. Disease clustering was insignificant and did not increase with time.

A641

THE PHENOTYPIC RELATIONSHIP BETWEEN RACE QCC OF *Puccinia graminis* f. sp. *tritici* AND OTHER RACES OF THE GREAT PLAINS. B. D. McCallum, A. P. Roelfs, and J. V. Groth, Department of Plant Pathology, University of Minnesota, and Cereal Rust Lab USDA ARS, St. Paul, MN 55108.

Stem rust race QCC is apparently new in the great plains of North America. It is prevalent on susceptible wheat cultivars and is virulent to all currently cultivated barley. To investigate its origin, isozyme and virulence patterns of this race were compared with those of races of the North American Great Plains. Horizontal starch gel electrophoresis in three buffer systems was used to separate isozymes of leucine aminopeptidase, dihydrolipoamide reductase, glutamate oxalate transaminase, and phosphoglucomutase. Isozyme and virulence phenotypes were compared among races on thirty single gene differential wheat lines. Race QCC has a unique virulence and isozyme phenotype that could not have been derived by a few mutations from prevalent races of the wheat stem rust fungus.

A642

SEPTORIA TRITICI AND *S. NODORUM* ON WINTER WHEAT IN WESTERN OREGON. M.E. Schmitt, S.M. Coakley, and D.J. Royle. Dept. of Botany and Plant Path., Oregon State University, Corvallis, 97331-2902, Long Ashton Research Station, Bristol, UK.

Percent diseased flag leaf area due to infections by *Septoria tritici* and *S. nodorum* was assessed at four growth stages for five winter wheat cultivars over two crop years. Infections were due to natural inoculum. In 1992, pycnidiospore production/tiller was determined for growth stage 30 (Zadoks). There is a close agreement between the rankings of cultivars by spore production per tiller and the previous year's disease severity rankings. Relative to the four most widely grown wheat cultivars in Oregon, the newly-released cultivar 'Gene' shows significant resistance to *S. tritici*, but is susceptible to *S. nodorum*. Furthermore, *S. tritici* was routinely recovered from symptomatic glumes and awns of the two susceptible cultivars. In 1991 and 1992, Oregon cultivars were included in a similar study in the UK and vice versa. In the two geographically diverse regions, the reponses of Oregon cultivars were similar. Monitoring of natural inoculum in Oregon was done with trap plants from October 1991 through the current crop year. Data on the population dynamics of *Mycosphaerella graminicola* and *Leptosphaeria nodorum* will be presented.

A644

SOIL SOLARIZATION AS A MEANS OF DETERMINING CEREAL YIELD LOSSES DUE TO SOILBORNE DISEASES IN LOWLAND NEPAL. H.J. Dubin, CIMMYT, Box 5186, Kathmandu, Nepal & H.P. Bimb, NWRP, NARC, Bhairahawa, Nepal.

Solarization was used to control soilborne diseases and for estimating yield losses in rice and wheat. A study done in May 1990 showed a 17% loss in rice yield in the unsolarized plots. This was correlated ($r=0.80$, $P=0.05$) with levels of Hirschmanniella oryzae. Another study done in November 1990, before wheat sowing, showed a 10% loss. Untreated plots had significantly less spikes/sq m, more grains/spike and shorter plants ($P<0.05$). Root necrosis and foliar blight (Bipolaris sorokiniana) were higher without solarization ($P<0.10$). B. sorokiniana and Pythium spp. appear to be the major root pathogens. Solarization increased yield and decreased disease in the following crop.

A645

MORE*CROP, AN EXPERT SYSTEM FOR MANAGING DISEASES OF WHEAT. Ramon Cu and Roland Line. USDA-ARS, Washington State Univ., Pullman, WA 99164.

More*Crop (Managerial Options for Reasonable Economical Control of Rust and Other Pathogens) is an application for Windows 3.0 or higher developed for managing rusts and other diseases of wheat in the Pacific Northwest. More*Crop uses the following sequence of variables: 1) geographical regions; 2) agronomic zones; 3) crop management practices; 4) classes of wheat and cultivars in each class; and 5) disease managerial options. A graphical user interface (GUI) is used to efficiently instantiate the variables. Object-oriented programming (OOP), a programming concept that combines data and procedures to form a data type called object, is used to define the wheat cultivars. As objects, the resistance and agronomic characteristics of the cultivars are easily manipulated down the procedural hierarchy through *inheritance*, *encapsulation* and *polymorphism* capabilities of OOP. The inference engine, the protocol for navigating through rules and data, supports forward and backward chaining. Forward chaining tells what diseases are likely to occur. Backward chaining provides reasons for the disease outcome. More*Crop predicts diseases based on cultivars and management practices; reconstructs past management decisions to replicate previous disease conditions; assists the user in reasoning what disease control option to select; and provides disease-related information for research, education and extension.

A646

SPORULATION OF *VENTURIA INAEQUALIS* ON SOLID MEDIA, D.M. Parker and W.D. Köller, Department of Plant Pathology, Cornell University, NYSAES, Geneva, NY 14456.

Laboratory studies using *Venturia inaequalis*, the causal agent of apple scab disease, occasionally require an abundance of sterile conidia. Conidia production in wick cultures, while adequate, has space limitations when large numbers of conidia are required. Production of conidia on agar media overlayed with cellophane proves to be as efficient as wick cultures in numbers of conidia obtained while at the same time demanding minimal space. Conditions to maximize conidia production on cellophane were optimized by testing the effects of media, light exposure and type of inoculum. Production on potato dextrose agar exceeds production on V-8 juice agar or malt agar. Ultraviolet light (UV-B) was found to stimulate production by a factor of 10, while a prudent time course of one week provides maximum production. In addition when using these techniques, conidia can be produced solely from a mycelial homogenate without the necessity of obtaining conidia as inoculum. Conidia production consistently exceeds 10^7 conidia per petri plate.

A647

FUNGICIDAL CONTROL OF BERRY DISEASES OF MUSCADINE GRAPE (VITIS ROTUNDIFOLIA). B. J. Smith, USDA-ARS, Small Fruit Res. Sta., and B. Graves, MS Agr. & For. Exp. Sta., Poplarville, MS 39470.

Muscadines are resistant to many diseases indigenous to the southeastern U.S.; however, most cultivars are susceptible to fungal berry diseases. Five fungicide treatments were applied early (EAR) (beginning 5-18-91) and four late (LAT) (beginning 7-23-91) season (20 treatment combinations) to evaluate their efficacy for control of berry diseases on the muscadine cultivar Doreen. No significant interactions were found between EAR and LAT, nor were there differences due to main effects in the amount of ripe rot, Macrophoma rot, or foliar diseases. All EAR fungicide treatments reduced the amount of bitter rot, black rot and total berry disease. Yield was increased by EAR benomyl and triadimefon + captan treatments. LAT mycolbutanil, mycolbutanil + captan, and benomyl + captan treatments reduced the incidence of bitter rot. Differences attributable to fungicide treatments did not economically justify their application; however, on more susceptible cultivars fungicides applications may be necessary.

A648

THE EFFECT OF SODIUM TETRATHIOCARBONATE AND FOSETYL-AL IN CONTROLLING PHYTOPHTHORA ROOT ROT OF RED RASPBERRY IN THE PACIFIC NORTHWEST. P.R. Bristow and G.E. Windom. Washington State University, 7612 Pioneer Way E., Puyallup 98371.

Sodium tetrathiocarbonate (GY-81) and fosetyl-Al (Aliette) were compared to a standard treatment with metalaxyl (Ridomil) for control of root rot of red raspberry. Plantings (cv. Willamette) were established in 1988 and 1989 in soil naturally infested with Phytophthora erythroseptica . GY-81 had no impact on the disease when soil in the planting hole (33 L) was treated at two rates (2000 and 4000 ppm) prior to planting. GY-81 was phytotoxic to dormant plants set 3 days after treatment but not when set after 14 days. Yield of the untreated check was 28% of the Ridomil standard. Yields for Aliette applied to the foliage 3 and 4 times a year (4.48 kg ai/ha per spray) averaged 61% of the Ridomil standard. The greater yields were due to more canes per hill and larger berries. Fruiting canes in the untreated check senesced earlier than those treated with either Aliette or Ridomil. Controlling root rot delayed ripening but did not lengthen the harvest period.

A649

Influence of temperature *in vitro* and *in planta* on *Anisogramma anomala*, the cause of eastern filbert blight. J. N. Pinkerton[1], K. B. Johnson[2], S. J. Nelson[2], and J. K. Stone[2]. [1]USDA ARS Horticultural Crops Research Laboratory, Corvallis, OR 97330, and [2]Department of Botany and Plant Pathology, Oregon State University, 97331-2902

Ascospore germination, mycelial growth, and infectivity of *Anisogramma anomala* were investigated under controlled temperatures. Ascospore inoculum was prepared by crushing perithecia excised from mature stromata on field-collected branches. Drops of 1×10^4 spores/ml were plated on Murashige and Skoog medium amended with bovine serum albumin or activated charcoal. In a constant temperature study, plates were incubated at 5, 10, 15, 20, 25 or 30 C for 7, 14, or 21 days. Spore germination was 0.2, 1.0, 11.7, 31.0, 21.0 and 0 % at constant 5, 10, 15, 20, 25, and 30 C, respectively. Total hyphal growth from single spores was significantly greater at 20 C than at other temperatures. When plates incubated at 5, 10, or 30 C for 7 days were transferred to 20 C for 7 days, spore germination was less than 1%. Microscopic examination revealed that most spores had decomposed in these treatments. In 1990, infectivity was evaluated by spraying 2 month-old hazelnut seedlings with a suspension of 1×10^5 spores/ml. Plants were incubated at 5, 10, 15, or 20 C for 14 days after which they were transferred to an unheated greenhouse. After a 14 month latent period, infection was observed in 13, 15.6, 35.1 and 17.4% of seedlings in the 5, 10, 15, and 20 C treatment, respectively. This study was repeated in 1991 and will be evaluated early summer 1992.

A650

Occurrence of Phytophthora Blight of Citrus in Texas. Mani Skaria and Nora Solis-Gracia, Texas A&I University Citrus Center, P.B. 1150, Weslaco, TX 78596.

A new outbreak of Phytophthora blight occurred in the fall of 1991 on nursery and mature citrus trees in Texas. In mature orchards, the disease attacked the tender grapefruit and orange shoot close to the ground. No infection was found on shoot around 18 inches above the soil surface. Numerous sporangia were found on the lesions of infected leaves. In mature orchards, 'Rio Red' grapefruit trees showed higher incidence of leaf blight compared to orange trees. Mature trees were highly defoliated, however, no tree mortality was noticed. Newly budded 'Marrs' orange nursery trees on sour orange rootstock showed stem blight at the tip and at the base of scion shoot emerging from the rootstock. The blighted area had gummosis,

and also had windblown sand deposition. In one section of the above nursery, 15% of the trees (scions) were killed. No obvious symptoms were found on the rootstock. The morphology of the fungus resembled that of *Phytophthora parasitica*. Koch's postulates were successfully done on tender leaf tissues of 'Rio Red' and 'Ruby Red' grapefruit and 'Marrs' orange trees.

A651

REMOVAL OF VISIBLE FUNGICIDE RESIDUE FROM GRAPES. E. A. Sutton and J. K. Mitchell, Dept. of Plant Pathology, University of Arkansas, Fayetteville, AR 72701.

Solutions of citric acid (food grade) and 5% acetic acid (vinegar) were tested for efficacy in the removal of visible fungicide residue from fully ripened Concord grapes. Acid concentrations ranged from 0.001 M to 0.2 M and were applied at a dilute spray rate of 200 gal/A to 8 year-old vines. Kocide 101 (2 lbs/A) and lime (4 lbs/A) were applied (prior to acid sprays) to vines at a rate of 50 gal/A to provide fungicide residue. At temperatures above 30 C, all concentrations tested of citric and acetic acids significantly reduced visible residues; whereas below 30 C, only acid concentrations above 0.005 M significantly reduced residues. Optimal removal occurred between 0.025 M to 0.05 M acid concentrations, with citric acid exhibiting greater efficacy than acetic at comparable rates. The surfactants Kinetic and Triton CS-7 (at high and medium rates) were also tested to determine if they could affect acid efficacy of residue removal. Results suggest that at lower acid concentrations (eg. 0.005 M), both the rate and type of surfactant significantly affected visible fungicide residue removal.

A652

MYCOPLASMALIKE ORGANISMS ASSOCIATED WITH STRAWBERRY MULTIPLIER DISEASE. M. F. Heimann, R. N. Spear, S. N. Jeffers. Department of Plant Pathology. University of Wisconsin, Madison, WI 53706.

Sections of leaf petiole from a strawberry plant exhibiting typical symptoms multiplier disease were examined microscopically for mycoplasmalike organisms (MLOs). Phloem sieve tube elements flouresed intensely when tissues were stained by the DAPI technique and examined by fluorescence microscopy. When sections were examined with the electron microscope, numerous bodies resembling MLOs were present in phloem cells. Phloem tissues in petiole sections from a healthy appearing plant did not flouresce after staining with DAPI so sections were not examined by electron microscopy. This is the first report of MLOs associated with strawberry multiplier disease. Our results provide further evidence that multiplier and witches' broom diseases of strawberry are related.

A653

EFFECT OF PECAN SCAB DISEASE CAUSED BY CLADOSPORIUM CARYIGENUM ON NUT PARAMETERS OF THREE CULTIVARS. R. S. Sanderlin and R. R. Shelton, Pecan Research-Extension Station, LA Agricultural Exp. Station, LSU Agricultural Center, Shreveport, LA 71135-5519.

The effect of scab disease on nut drop, nut size and weight, and kernel weight of 3 cultivars was evaluated by using differential fungicide applications to modify scab severity. When lesions covered 31-50% of the shuck surface by the first week of July, end of season nut drop on cv. 'Schley', 'Desirable', and 'Maramec' was 100, 100, and 85%, respectively. When lesions cover 11-30% or more of the shuck tissue by Aug.1, nut size, wt., and kernel wt. were significantly reduced (DMRT, P=0.05) when compared to nuts that had less than 11-30% diseased tissue through Aug. Occassionally when 11-30% disease was not present on Aug. 1 but was reached by Aug. 15, there was a significant reduction in wt. Based on these data, collected in a year of extreme scab disease severity, an increase in scab should be prevented through July and perhaps mid-Aug. to avoid a reduction in nut size and wt.

A654

SEED TRANSMISSION OF APPLE MOSAIC VIRUS IN HAZELNUT (CORYLUS AVELLANA). J. D. Postman, USDA/ARS National Plant Germplasm Repository, 33447 Peoria Road, Corvallis, Oregon 97333, and S. A. Mehlenbacher. Department of Horticulture, Oregon State University, Corvallis, Oregon 97330.

Several hazelnut cultivars, important as parents in the Oregon State University breeding program, have only been available in the United States as apple mosaic virus (ApMV) infected plants. Progeny resulting from seed of infected cultivars Negret and Tombul (synonym = Extra Ghiaghli), and progeny from pollen of infected cultivar Mortarella were tested for ApMV by ELISA. Infected female parents produced an average of 6.5% infected progeny (range 2.5% to 11.8%) in tests of 338 three year old seedlings from several crosses. No infected progeny were detected in 89 seedlings from crosses with infected pollen parents, after testing for three consecutive years. All progeny from crosses between two non-infected parents tested negative. Seedlings testing positive for ApMV were nearly all symptomless. These results confirm reports of seed transmission of ApMV in *Corylus*, but contradict the one reported incident of pollen transmission in this host.

A655

DETECTION AND ELIMINATION OF APPLE MOSAIC VIRUS IN IMPORTED CLONAL HAZELNUT (CORYLUS SP.) GERMPLASM. J. D. Postman, USDA/ARS National Plant Germplasm Repository, 33447 Peoria Road, Corvallis, Oregon 97333, and S. A. Mehlenbacher. Department of Horticulture, Oregon State University, Corvallis, Oregon 97330.

Since 1985, 255 imported hazelnut clones have been added to the U.S. *Corylus* germplasm collection, and are providing new sources of genetic traits to the hazelnut breeding program at Oregon State University. Apple mosaic virus (ApMV) is not known to occur in commercial hazelnut plantings or in the wild in the U.S. but is common in several European countries. ELISA testing established that 37% of 60 clones imported from Spain, 24% of 34 clones from Turkey and 9% of 67 clones from Italy were infected with ApMV. No ApMV infected plants were detected among 85 clones from Australia, China, Denmark, Finland, France, Korea, The Netherlands, Norway, United Kingdom and Yugoslavia. Heat therapy of infected plants for 21 or more days at temperatures alternating every 4 hours between 30 and 38 C, followed by shoot-tip grafting onto healthy *Corylus* rootstocks resulted in the elimination of ApMV from 22 of 28 infected European hazelnut cultivars.

A656

GRAPEVINE FANLEAF VIRUS AND TOMATO RINGSPOT VIRUS DISTRIBUTION IN VINEYARDS IN THREE CALIFORNIA COUNTIES AS DETERMINED BY $F(ab')_2$ ELISA TESTING. D. A. Golino*, A. Rowhani, P. Verdegaal, R. Smith, E. Weber, and A. Walker. *USDA, University of California, Davis, CA 95616.

Grapevines were sampled in three California counties using an $F(ab')_2$ ELISA system for Grapevine Fanleaf Virus (GFLV) and Tomato Ringspot Virus (TomRSV) to provide information on sampling techniques and virus distribution in vineyards. Sampling was done in spring of 1990, 1991, and/or 1992 in Napa county, San Joaquin county and Sonoma county. In each vineyard, 0.2 gm of succulent tissue was collected from each of 27 vines from a predetermined sampling grid which was 42 vines deep and 40 rows wide. Grapevine fanleaf virus was detected in a high percentage of sites (17 of 44 sites in San Joaquin county, 11 of 27 sites in Sonoma county and 22 of 32 sites in Napa county). TomRSV was rarely detected (0 sites in San Joaquin county, 1 site in Napa county and 5 sites in Sonoma county).

A657

RELATIVE SUSCEPTIBILITY OF PEACH AND PLUM GERMPLASM TO *ARMILLARIA TABESCENS*. W.R. Okie, T.G. Beckman, A.P. Nyczepir, P.L. Pusey and C.C. Reilly. USDA-ARS, P.O. Box 87, Byron, GA 31008.

Nearly 5,000 seedling trees representing over 100 peach and plum lines were planted at a 4 X 0.6 m spacing in January, 1983, on a site with a known history of peach tree short life (PTSL) and Armillaria root rot. Trees were in a randomized complete block with eight replicates of six trees each. Beginning in the spring of 1984 and each year thereafter as trees died, cause of death was determined. At the end of nine years, 50% of trees had died due to PTSL and 35%had succumbed to root rot caused by *Armillaria tabescens*. Analysis of percent death due to Armillaria root rot showed a wide range in mortality (dataset excluded trees which had died due to PTSL). Some peach lines appeared significantly more tolerant to Armillaria than others as indicated by mortality analysis. Plum lines derived from native North American species also appear to be a potential source of improved tolerance. It is not known whether Armillaria tolerance is affected by PTSL.

A658

WIND SCAB PREDISPOSES PRUNE FRUIT TO PREHARVEST AND POSTHARVEST FUNGAL DECAY. Themis J. Michailides and D.P. Morgan, Department of Plant Pathology, University of California, Berkeley/Kearney Agricultural Center, Parlier, CA 93648.

Wind Scab (WS) of French prune (Prunus domestica 'French') was caused by developing fruit rubbing against other fruit, leaves, and shoots during strong northern and northwestern winds. It occurred only in years that had at least 10 days with such winds within 3 wk after full bloom and three of these days with winds exceeding speeds of 20km/hr. Wind-scabbed fruit areas developed several layers of suberized cells, but had deep fractures which allowed fungal spores to germinate and penetrate. The incidence of decay caused by Phomopsis cinerascens was significantly higher in fruit with WS. Although without wounds, α spores of P.

cinerascens could penetrate directly through the epidermis of ripe and overripe fruit, wounds, such as those created by WS, were necessary for infection of immature fruit. Significantly more propagules of filamentous fungi and yeasts were recovered in washings of wind-scabbed fruit than of healthy fruit. Both the incidence and severity of WS on dehydrated fruit correlated positively (r = 0.85-0.97) with the incidence and severity of WS on mature fruit. WS resulted in percentages of off-graded prunes equivalent to prune russet scab as determined by commercial inspectors in an industrial dehydrator.

A659

RELATIVE SUSCEPTIBILITY OF FOUR TABLE GRAPE VARIETIES TO FUNGAL COMPONENTS OF THE SUMMER BUNCH ROT COMPLEX IN THE SAN JOAQUIN VALLEY. H. Yunis, R. A. Duncan, and J. J. Stapleton, University of California, Kearney Agricultural Center, Parlier, CA 93648.

Four table grape varieties (Cardinal, Emperor, Flame, and Thompson) were tested for susceptibility to five fungi (Botrytis, Aspergillus, Alternaria, Penicillium, and Cladosporium spp.) associated with the summer bunch rot complex in the San Joaquin Valley. Significant differences in susceptibility among varieties were observed, as were differences in pathogenicity of the fungi. Flame was the most susceptible variety, while Emperor was the most resistant. All fungi colonized wounded berries, but only Botrytis and Alternaria were able to infect nonwounded berries when inoculum was suspended in water. Suspension of spores in grape juice increased infectivity.

A665

ALGAL POPULATION DYNAMICS IN A FORESTED WETLAND OF SOUTH ALABAMA. D.A. Brown, B.G. Lockaby, and W.D. Kelley. School of Forestry, Auburn University, AL, 36849.

The importance of algae in nitrogen fixation and the immobilization of nutrients and their internal cycling in wetlands ecosystems is well recognized. In 1991, a study was initiated on a logging site characterized as a branch-bottom wetland dominated by histosols. Treatments included up- and down-stream controls and handfell/helicopter (h/h) and feller-buncher/helicopter/skidder (f/h/s) logging plots. Algal biomass was indirectly estimated by solvent partitioning and spectrophotometry. The mean concentration of chlorophyll a and b in each sample was determined. Increases as great as 170% were observed in the f/h/s plots. Increases in the less-disturbed h/h plots were much less (31-45%). Chlorophyll concentrations in down-stream controls were 36% greater than up-stream controls. Data suggest that periphytic and planktonic algae in wetland forest sites may play important roles in nutrient cycling, and that soil disturbance due to timber harvesting may influence algal population dynamics.

A667

EFFECT OF *BEAUVERIA BASSIANA* ON MORTALITY AND FECUNDITY OF THE RUSSIAN WHEAT APHID (*DIURAPHIS NOXIA*). Z. G. Wang and G. R. Knudsen, Plant Pathology Division, University of Idaho, Moscow 83843.

Entomopathogenic fungi have potential for microbial control of aphid pests. Fecundity is an important aspect of insect population dynamics, but little is known about effects of entomopathogens on aphid reproduction. In this study, ten-day-old Russian wheat aphid apterae (25 per treatment) were inoculated with *B. bassiana* (SGBB 8601) conidial suspensions (10^6/ml) or sterile water, then caged individually on wheat seedlings. Temperature was kept at 22 C; relative humidity was $\geq$95% for 24 hr and about 85% subsequently. Each aphid was monitored daily, over 14 days, for mortality and nymph production. All newborn nymphs and dead adults were removed and plated. The experiment was performed four times. *B. bassiana* significantly increased aphid mortality (mean mortality due to mycosis= 82%; mean time to death by mycosis= 4.7 days). However, the pathogen did not affect nymph production by individual aphids (mean= 3.2 nymphs/day/aphid for both treatments). Control aphids produced on average 919 nymphs/cohort over 14 days, compared to 384 for *Beauveria*-treated aphids, due only to differential mortality of adults. Most (>99%) nymphs from infected adults were uninfected.

A668

VERTICAL DISTRIBUTION OF FUSARIUM SOLANI, CAUSAL AGENT OF SUDDEN DEATH SYNDROME OF SOYBEAN, IN TWO SOYBEAN FIELDS. J. C. Rupe and C. M. Becton, University of Arkansas, Fayetteville, 72701.

Population levels of Fusarium solani, total fungi, and soybean cyst nematode were determined to a depth of 1 m from plots of the susceptible soybean cultivar, Lee 74, at two locations in Arkansas. Soil samples were collected at planting, flowering, full seed, and harvest. Soil bulk densities were taken at planting and harvest and soil nutrient levels determined at planting. Disease ratings were taken weekly from the end of July through September. The highest populations of F. solani, total fungi and soybean cyst nematode were in the top 10 to 20 cm of the soil profile and declined rapidly below this depth. The majority of the soybean roots and the highest frequency of root infection by F. solani occurred in this zone. Differences in disease development between the two locations corresponded to differences in inoculum levels of F. solani in this upper 10 to 20 cm of soil.

A675

WASTE CORN AS AN INOCULUM SOURCE FOR ASPERGILLUS FLAVUS. G. Hoyos, D. C. McGee, and L. H. Tiffany, Depts. of Plant Pathology, Botany and the Seed Science Center, Iowa State University, Ames, IA.

In the summer of 1991, Aspergillus flavus Link was readily found to be sporulating on deposits of waste corn in the vicinity of corn cribs and storage bins throughout Iowa. High infection levels also were detected in laboratory tests on nonsporulating kernels sampled from these deposits and from kernels from ears within cribs. Airborne inoculum, measured with an Andersen sampler within 20 m of bins and cribs, was up to 20 times higher than that detected in corn fields. Nitidulid beetles (Glischrochilus and Carpophilus spp.) were observed on the waste corn and found to be heavily infested by A. flavus when retrieved from corn deposits or from insect traps close to the bins or cribs. Previous work had indicated that a low background population of A. flavus existed in air, soil and crop residues in Iowa corn fields. This is the first report of a concentrated inoculum source of the fungus in the corn ecosytem.

A676

ECOLOGY OF ASPERGILLUS FLAVUS IN IOWA CORN FIELDS. D. C. McGee, G. Hoyos and L. H. Tiffany, Depts. of Plant Pathology, Botany and the Seed Science Center, Iowa State University, Ames, IA.

In 1991, Aspergillus flavus Link was found to be widely distributed at low population levels in the soil and crop residues of 40 fields in Iowa that had experienced significant aflatoxin contamination in 1988. Isolates of the fungus from residues and soil were very similar in their capacity to produce aflatoxin and sclerotia. A. flavus also was detected at low population levels in the air, soil, crop residues and on new plant tissues during the growing season in conservation tillage plots in northern Iowa in 1979, 1980, and, after a period of 11 years, in 1991. In general, recovery of A. flavus from these sources was not related to tillage or rotational practice, except for 1991, when there was some indication of a population build-up on crop residues in continuous corn plots under minimum tillage. Further data will be required to confirm this trend.

A678

REDUCED MELAMPSORA LEAF RUST IN MULTI-CULTIVAR POPLAR WINDBREAKS. J.A.Walla, North Dakota St. Univ., Fargo, ND 58105.

Most windbreak tree rows in North Dakota are planted with single species or cultivars. Diseases tend to occur uniformly in those rows, likely due in part to the monoculture setting. Hypothetically, genetically diverse tree rows would have less disease damage. Poplar (*Populus* hybrids) windbreaks were planted in single cultivar and multi-cultivar replicated plots to examine the hypothesis. In 1991, leaf rust (*Melampsora medusae*) was severe on susceptible poplar cultivars in parts of

ND. Trees were rated for percent infected leaves and percent defoliated leaves. A susceptible cultivar (Northwest) in mixed plots (interplanted with the resistant cultivars Imperial, Norway, and Robusta) had less damage than Northwest in monoculture plots. In late July, infection on Northwest in monoculture plots was 87% vs. 28% in mixed plots. By early September, infection was similar in both plot types, while defoliation of Northwest in monoculture plots was 86% vs. 63% in mixed plots. These results support the hypothesis.

A679

TELIOSPORE UPPER WALL THICKNESS AS A CHARACTER FOR DISTINCUISHING *MELAMPSORA* SPECIES ON *POPULUS*. P. A. Mason and R. W. Stack. Dept. of Plant Pathology, North Dakota State Univ., Fargo, ND 58105.

Leaves of *Populus deltoides* (PD) and *P. tremuloides* (PT) with *Melampsora* leaf rust were collected from nine central and western states and two western Canadian provinces. The upper walls of teliospores were examined by light and scanning electron microscopy. Teliospores from 30 collections on PD had uniformly thin upper walls (0.98 ± 0.02μm). Although more variable, upper walls of teliospores from 25 collections on PT were significantly thicker (1.68 ± 0.38μm, $p<0.001$). Walls on five collections from PT were thin (0.98μm), similar to those from PD. Arthur distinguished two *Melampsora* species on these hosts: *M. albertensis*, having thicker walled teliospores, and *M. medusae*, having thinner ones. These observations suggest that, in this region, both species are present on PT while only *M. medusae* is present on PD.

A680

A COMPARISON OF OAK DECLINE RESULTS FROM DIFFERENT AREAS OF THE SOUTH. Vernon D. Ammon[1], T. Evan Nebeker[1], and James D. Solomon[2]. [1] Mississippi State University, Mississippi State, MS, 39762 and [2] Southern Forest Experiment Station, Stoneville, MS 38776.

Tree and site data from oak decline areas in bottomland stands growing in the lower midsouth (Mississippi River and Tennessee-Tombigbee River basins) were compared. Bottomland data were then compared to oak decline data collected by Starkey et al. from upland oak stands in the upper South. Variables which were higher in the Mississippi River bottomlands than the Tennessee-Tombigbee bottomlands included basal area, site index, current years growth, organic matter, pH, and soil minerals. Decline tended to occur more on bottomland ridges and flats with northern aspects in the Mississippi River basin whereas it occurred most frequently on upland sideslopes on various facing aspects in the Tennessee-Tombigbee basin. In the upper south, basal area differences between decline and control plots were similar to those in the lower south. Overall decline was greatest on shallow soils and no significant relationships with regard to aspect were detected in the upper south region.

A681

USING BACTERIA FOR BIOLOGICAL CONTROL OF WOOD-DISCOLORING FUNGI. S. C. Croan, T. L. Highley, USDA, FS, Forest Products Laboratory, One Gifford Pinchot Drive, Madison, Wi 53705-2398.

The objectives of this study were (1) to accelerate and scale up fermentation of Streptomyces rimosus for production of anti-fungal metabolites and (2) determine if boron produces a synergistic effect with the antifungal metabolites for control of sapwood-inhabiting fungi. The following sapwood-inhabiting fungi were selected: sapstain--Ceratocystis coerulescens, C. minor, C. pilifera, and Aureobasidum pullulans; mold fungi--Aspergillus niger, Penicillium spp., and Trichoderma spp. The antifungal activities were studied using aspergillus plate bio-assay, wood-block tests, and green pine log sections. Inhibition of spore germination in plate bioassay by antifungal metabolites with boron was more effective than without added boron. Treatment of Southern Pine and sweetgum blocks and green pine log sections with living bacterial cells, concentrated metabolites, or nonconcentrated metabolites with boron inhibited spore germination and, therefore, prevented discoloration of wood.

A682

PROPOSED PEST RISK ANALYSIS PROCESS FOR IMPORTATION OF LOGS AND LOG PRODUCTS INTO THE UNITED STATES. S. D. Cohen and R. L. Orr, Planning and Risk Analysis Systems, Animal and Plant Health Inspection Service, Hyattsville, Maryland 20782.

A process was developed to assist in the assessment of the pest risks associated with the importation of logs and log products into the United States. The core of the process is a pest risk model which focuses on the probability of establishment of non-indigenous pests and, if established, the magnitude of the resulting damage. The "establishment" category of the pest risk model is subdivided into the probability of the pest organisms being associated with the imported commodity, the probability of the organism becoming established, and its potential for spread in the United States. The "consequences" category is subdivided into environmental, economic, and social damage. Combined information about the pest is applied to the risk model to evaluate the individual pest risk. The level of uncertainty associated with the biological data is also indicated. An overall risk is determined for the commodity by considering all individual pest risk assessments.

A683

DO HEALTHY TREES MAKE A HEALTHY FOREST? M. E. Ostry and T. H. Nicholls. North Central Forest Experiment Station, 1992 Folwell Ave., St. Paul, MN 55108.

Forest health, depending on how it is described, can be viewed in different ways. Tree pathogens and insect pests have been widely viewed in the past as having primarily negative impacts, even when the damage they cause is not widespread. In our research we have observed that many of these interacting agents and the effects on their tree hosts enhance wildlife habitat and diversity in natural and managed ecosystems. Their beneficial values should be considered when making forest management decisions. Selected examples of these agents include dwarf mistletoe, various heart rot and canker fungi, fruit bodies of many root-inhabiting fungi, and insect outbreaks. Their beneficial effects include the alteration of habitat to increase the diversity and quantity of food and shelter available to mammals, birds, fish, and herps.

A684

EFFECT OF MOISTURE STRESS ON CRYPHONECTRIA CUBENSIS. W.J. Swart[1], E. Conradie[1], and M.J. Wingfield[2], [1]Department of Plant Pathology, and [2]Department of Microbiology and Biochemistry, University of the Orange Free State, Bloemfontein 9300, South Africa.

The restriction of Eucalyptus canker caused by Cryphonectria cubensis to high rainfall areas in South Africa has led to speculation regarding the influence of drought on the disease. A study was, therefore, conducted to determine whether a relationship exists between the growth of C. cubensis and water stress. In axenic culture, the pathogen displayed reduced growth with increasing osmotic stress. Pressure bomb assessment of the water potentials of inoculated E. grandis pot-plants indicated that drought-stressed plants developed significantly ($P < 0.01$) smaller cambial lesions than non-stressed plants. These results are in contrast with numerous reports on other canker pathogens where drought stress is associated with increased growth of the pathogen.

A685

FIRST REPORT OF ENDOTHIA GYROSA AS A CANKER PATHOGEN OF EUCALYPTUS SPP. IN SOUTH AFRICA. I.P. v.d. Westhuizen[1], M.J. Wingfield[1], G.H.J. Kemp[1] and W.J. Swart[2]. [1]Dept of Microbiology and Biochemistry, and [2]Dept of Plant Pathology, Univ. of the Orange Free State, Bloemfontein 9300, South Africa.

During countrywide surveys of Eucalyptus plantations, cankers distinctly different from those associated with Cryphonectria cubensis were observed. These cankers occurred higher up on the stem than those typical of C. cubensis. Cankers were exemplified by cracked and slightly swollen areas on the bark. Endothia gyrosa, a well known pathogen of woody plants including Eucalyptus spp., was consistently associated with these cankers. The pathogen is easily distinguished from C. cubensis by the presence of orange-brown stromata and non-septate ascospores. Inoculations on Eucalyptus grandis resulted in lesions similar to those observed on naturally infected trees. The disease associated with E. gyrosa is widespread in South Africa, and research is underway to establish control strategies.

A686

VEGETATIVE COMPATIBILITY AND CONVERSION TO HYPOVIRULENCE AMONG ISOLATES OF *CRYPHONECTRIA PARASITICA* FROM NORTHERN ITALY. M. Garbelotto[1], G. Frigimelica[2], and S. Mutto-Accordi[2]. [1]Department of Plant Pathology, University of California, Berkeley, CA 94720 and [2]Istituto di Patologia Vegetale della Facolta' di Agraria dell' Universita' degli Studi di Padova, 35100 Padova, Italy.

Twenty-two virulent and four hypovirulent isolates of *Cryphonectria parasitica* from chestnut trees in forest stands and commercial orchards were tested for virulence, presence of ds-RNA, vegetative compatibility (v-c) and conversion to hypovirulence. The isolates belonged to nine different v-c groups, and v-c diversity was higher than

that of previous reports. When paired with four local hypovirulent isolates, 77% of the virulent isolates were converted to hypovirulence. There was a strong correlation between v-c and conversion groups as determined by cluster analysis, but hypovirulent isolates could convert virulent isolates belonging to different v-c groups. Converted strains could also transmit hypovirulence to the unconverted strains, thus increasing the overall efficiency of conversion.

A687

EUCALYPTUS DISEASES OCCURRED IN TAIWAN. W. Y. Wang, Taiwan Forestry Research Institute, 53 Nan-Hai Rd., Taipei, Taiwan, ROC.

Since 1985, Eucalyptus camaldulensis, E. grandis, E. urophylla and E. tereticornis are the major economic plantation species for fiber production at lower elevation areas in Taiwan. Diseases and disorders of eucalypts found in nursery and plantations during a two year survey including crown gall, bacterial wilt, little-leaf, seedling blight, angular leaf spot, leaf spot, brown spot, leaf rust, grey mold, shoot blight, stem canker, and butt rot. Several noninfectious disorders including the leaf red spot, gumosis, herbicide injury, typhoon damage and genetic albinism and an unidentified leaf crinkle disease are also observed. Bacterial wilt, little-leaf, seedling blight, brown spot. leaf rust, shoot blight, stem canker and butt rot are new disease records in Taiwan.

A688

FACTORS AFFECTING SURVIVAL OF FALL-LIFTED LOBLOLLY PINE SEEDLINGS DURING STORAGE. R.J. Nevill and W.D. Kelley. School of Forestry, Auburn University, AL 36849-5418.

Survival of fall-lifted loblolly seedlings generally declines following cold storage. To determine if pathogenic fungi are involved, seedlings from four nurseries were lifted at 2-week intervals (October to mid-December), treated with 10 ppm metalaxyl (controls were not treated), and stored. At two-week intervals thereafter one sub-sample of seedlings from each lifting date was planted in the greenhouse and another was assayed for pathogenic fungi, root respiration, and sugar: starch ratios; after 10-weeks storage remaining seedlings from each lifting date were outplanted. Potential pathogens isolated included three species of *Fusarium* and an unidentified, fast-growing culture on a medium selective for *Phytohothora* and *Pythium*. Metalaxyl did not provide protection from infection by the *Fusarium* spp., but it did result in significantly less infection by the unidentified Phycomycete. Overall, survival was highly related to nursery source, but not to lifting date, storage time, or percentage of seedlings with pathogenic fungi.

A689

CHARACTERIZATION OF *RHIZOCTONIA* SP. CAUSING FOLIAR BLIGHT OF LOBLOLLY PINE SEEDLINGS. G.B. Runion and W.D. Kelley. School of Forestry, Auburn University, Auburn, AL 36849.

Foliar blight of loblolly pine (*Pinus taeda* L.) has been observed in forest nurseries in the southeastern U.S. Affected foliage turns gray and is covered with mycelial webbing. *Rhizoctonia* spp. are consistently isolated from symptomatic seedlings. Isolations on PDA were made from symptomatic loblolly seedlings from two nurseries. All loblolly and three longleaf pine isolates were paired with five tester isolates of *R. solani* (CAG-1 to CAG-5), and with each other. The CAG-3 tester isolate was the only one that anastomosed with the pine isolates and it anastomosed with all loblolly and longleaf isolates. All loblolly isolates were able to anastomose with each other, regardless of nursery of origin, and with the three longleaf isolates. The fungus associated with foliar blight of loblolly pine seedlings is a binucleate *Rhizoctonia* sp. in anastomosis group CAG-3 and is similar to the fungus causing longleaf pine seedling blight. This is the first report of a loblolly pine seedling blight caused by *Rhizoctonia* sp.

A690

GROWTH OF SOUTHERN PINE BEETLE ASSOCIATED FUNGI IN RELATION TO THE INDUCED WOUND RESPONSE IN LOBLOLLY PINE. D.W. Ross*, P. Fenn and F.M. Stephen. Departments of Entomology and Plant Pathology, Univ. of Arkansas, Fayetteville, AR 72701. *Dept. of Forest Science, Oregon State Univ., Corvallis, OR 97331.

Fast developing, resin soaked, discolored zones (lesions) in the phloem of healthy pines may confer resistance to invasion by beetle-vectored fungi. Loblolly pines were wound inoculated with fungi associated with the southern pine beetle; Ophiostoma minus, Ceratocystiopsis ranaculosus and an unnamed basidiomycete (SJB 122) singly and in combination. After 3,7, and 28 days, lesion lengths were measured and tissue plated to recover the fungi. O. minus, a pine pathogen, induced the longest lesions. O. minus and C. ranaculosus were recovered frequently from inside the lesions and from healthy phloem outside the lesion margins, but recovery frequencies decreased with time. The basidiomycete was rarely recovered and only inside the lesions. O. minus appeared to inhibit the other fungi in combined inoculations. The induced wound response of phloem may slow and then confine invasion by nonpathogenic fungi more than by pine pathogens.

A691

FUSIFORM RUST IN 100-YEAR-OLD LOBLOLLY PINES. C.H. Walkinshaw. USDA, Forest Service, Southern Forest Experiment Station, 2500 Shreveport Highway, Pineville, LA 71360.

Galls on mature loblolly pines were examined for remission along a one hundred mile stretch of the Natchez Trace Parkway in Mississippi. Trees were 100 to 120 ft (31 - 37 m) tall and 11 to 38 in (28 to 96 cm) in diameter at breast height. Galls were 0.5 to 12 ft (15 to 366 cm) from the ground (mean of 2 ft or 61 cm). Thirty-three percent of the 347 galls measured were active. Average length of galls was 48 in (122 cm) and width was 14 in (36 cm). This width represented a girdling of 26± 16%. One hundred twenty-nine galls girdled less than 15% of the tree. Many galls were as wide as 60 in (152 cm). Death of galls was not related to their size. It was confirmed that the stem infection came from branch galls, and it appears that these infections can remiss. Infections in the second or third growing season had little accumulative effect on growth of survivors.

A692

COMPARISON OF *IN VITRO* WOOD DECAY POTENTIALS OF MONOKARYOTIC AND DIKARYOTIC STRAINS OF *ECHINODONTIUM TINCTORIUM* ON GRAND FIR HEARTWOOD. A. D. Wilson, USDA Forest Service, Southern Hardwoods Laboratory, P. O. Box 227, Stoneville, Mississippi, 38776.

The *in vitro* wood decay potentials of 50 monokaryotic (monosporous) and 100 dikaryotic strains of *Echinodontium tinctorium* were compared in 1-year and 2-year decay studies. Decay was measured as a percentage loss in oven dry weight of grand fir heartwood. Decay by all isolates was cumulative over time. Dikaryons generally caused more rapid decay than monokaryons after 1-year and 2-year decay periods. However, monokaryons caused greater weight loss after 2 years than dikaryons caused after 1 year of decay. The range of weight loss caused by monokaryotic and dikaryotic strains was narrower the first year (0-25%) than the second year (6-40%) of decay. The first year, most monokaryons (40%) caused decay in the 6-10% weight loss category, while 52% of dikaryotic strains caused decay in the 11-15% weight loss category. Individual monokaryons caused 2-17% weight loss the first year and 7-36% the second year, while dikaryons caused 7-23% weight loss the first year and 16-36% the second year. The wood decay potentials of sib-composed dikaryons appeared to be determined by the decay potentials of monokaryotic strains composing each dikaryon. *E. tinctorium* decay was characterized as a physiological white rot (both cellulose and lignin removed) causing softening, darkening, and shrinkage of wood leading to separation of wood between growth rings (laminated rot) and eventually stringy rot.

A693

THE BIOLOGICAL SYSTEM OF A SEEDBORNE SCLEROTIAL BOTRYTIS ON BITTERBRUSH. D. L. Nelson, USDA Forest Service, Intermountain Research Station, Shrub Sciences Laboratory, Provo, UT 84606.

An indigenous sclerotial Botrytis sp. induces a severe seed and seedling disease of bitterbrush (Purshia tridentata). Airborne conidia infect the developing seed during flowering. The fungus can remain dormant in dry stored seed for long periods. In axenic culture at room temperature the fungus tends to remain mycelial, forming conidia directly; while at low temperature (1 C) sclerotia form first, germinating later to form conidia. During winter in rodent buried seed caches the fungus spreads from infected seed(s) enveloping the entire seed cache in a sclerotial mass. In spring stout conidiophores from germinating sclerotia penetrate upward to the soil surface where sporulation occurs. Conidial spore masses are extremely hydrophobic and wind dispersed. Because of the evolved rodent seed cache dispersal mechanism, a low percentage of seed infection can result in extensive seed destruction. No apothecial state of the fungus is known.

A694

INFLUENCE OF SEED SANITATION ON *FUSARIUM* ROOT INFECTION OF CONTAINER-GROWN DOUGLAS-FIR SEEDLINGS. M. J. Neumann[1], P. E. Axelrood[1] and B. J. van der Kamp[2]. [1]B.C. Research, Vancouver, B.C. V6S 2L2 and [2]University of British Columbia, Dept. of Forest Sciences, Vancouver, B.C. V6T 1W5.

Fusarium related diseases have caused losses in British Columbia conifer nurseries. Two Douglas-fir seedlots, with different levels of seedborne *Fusarium,* were stratified using a standing water imbibition or a running water imbibition combined with a post stratification treatment of 3% hydrogen peroxide. Root infection was assessed under operational conditions in a conifer nursery. The prevalence of seedlings infected with *Fusarium* and the level of root infection increased throughout the growing season for all seedlot treatments. Sanitation of the seedlot, which had a high level of seedborne *Fusarium,* resulted in a significant reduction in *Fusarium* root infection and a significant increase in seedling growth compared to non-sanitized seed.

A695

USE OF ISOZYME AND RFLP ANALYSES TO DISTINGUISH *CERATOCYSTIS COERULESCENS* AND SIMILAR SPECIES. T. C. Harrington, R. A. DeScenzo and D. L. McNew. Department of Plant Pathology, Iowa State University, Ames 50011.

Starch gel electrophoresis for seven isozymes and RFLP analysis with the oligonucleotide probe $(CAT)_5$ were used to distinguish morphologically similar species of Ceratocystis *sensu stricto.* Isolates of the conifer bluestain fungus *C. coerulescens* (Münch) Bakshi from North America and Europe and *C. laricicola* Redfern & Minter, a recently described species from larch in Scotland, had similar isozyme electromorphs and RFLP patterns, but the two species differed in ascospore morphology. Isolates of *C. coerulescens* f. *douglasii,* in contrast, were morphologically similar to the typical form of *C. coerulescens* but differed in isozyme electromorphs. Isolates of *C. virescens* (Davids.) C. Moreau, a pathogen and saprophyte on maple and other hardwoods in eastern North America, were distinct from *C. coerulescens* in anamorph morphology, isozyme electromorphs and RFLP patterns; these two species should not be considered synonyms. All isolates of Chalara *australis* Kile & Watling (a pathogen on *Nothofagus* in Australia) had a single RFLP pattern. Although *C. australis* is morphologically similar to the anamorph of *Ceratocystis virescens,* the two taxa have distinct RFLP patterns.

A696

RESPONSE OF HETEROBASIDION ANNOSUM TO MANGANESE. B.L. Illman and W. J. Otrosina, USDA/FS, Forest Products Laboratory, Madison WI 53705 and USDA/FS, PSW Research Station, Albany, CA 94710.

The in vitro response of H. annosum (Fr.) Bref. to manganese (Mn) was determined and a comparison was made between 3 isolates of the "S" and 3 isolates of the "P" intersterility groups from the western United States. On manganese-amended malt agar plates H. annosum produced a brownish-black pigment that increased with increasing concentration of Mn (1, 5, and 10 mM). The amount of pigment production varied among the isolates, but isolates of the "S" group clearly produced more pigment than isolates of the "P" group. In pigmented areas, hyphae appeared to be packed with black granules and brownish-black granules/crystals were present in the medium. The black pigment implicates the presence of MnO_2, potentially resulting from the activity of Mn-dependent peroxidase (MnP). An assay for MnP did not detect the enzyme in crude culture filtrates of a chemically defined, nitrogen limited, buffered medium of the 6 isolates. The differential response of "S" and "P" group isolates to Mn raises questions about a role for this transition metal in H. annosum physiology.

A697

RADIAL GROWTH LOSS ASSOCIATED WITH MYCOPLASMALIKE ORGANISMS IN WHITE ASH AND VELVET ASH. W. A. Sinclair, M. Treshow[1], and H. M. Griffiths; Dept. Plant Pathology, Cornell Univ., Ithaca, NY 14853; and [1]Biology Dept., Univ. of Utah, Salt Lake City, UT 84112

Mycoplasmalike organisms (MLOs) are often detected in slowly growing or declining ash (*Fraxinus*). To assess MLO impact, we measured 1980-1989 radial growth of ash in which MLOs were detected or not by DAPI (4',6-diamidino-2-phenylindole·2HCl) tests in 1990-1991. In white ash (*F. americana*) on one site in New York, 10-yr growth of infected and noninfected trees averaged 23.4 and 28.6 mm, respectively. Growth rates of the two groups diverged beginning in 1985, and growth in 1989 averaged 1.4 and 3.0 mm, respectively ($P = 0.05$). For velvet ash (*F. velutina*) in Zion National Park, Utah, data from trees repeatedly defoliated by insects (healthy trees not present) were grouped according to total radial growth in 1980-1989. Among trees that grew > 10 mm, the MLO-infected and noninfected trees averaged 15.4 and 20.0 mm, respectively ($P = 0.06$). MLO-associated growth loss was not detected in trees that grew < 10 mm, because these produced scant xylem other than springwood vessels.

A698

USE OF MAGNETIC RESONANCE IMAGING FOR NONDESTRUCTIVE ASSESSMENT OF KNOTS AND ROTS IN WOOD. J. M. Halloin,[1,2] J. H. Hart,[2] T. G. Cooper,[3] and E. J. Potchen[3]. [1]Agricultural Research Service, USDA, SBCRU, [2]Dept. of Botany and Plant Pathology, and [3]Dept. of Radiology, Michigan State University, East Lansing, MI 48824.

Noninvasive visualization of internal structures of healthy logs of Acer negundo and Salix sp., and diseased logs of Populus tremuloides was accomplished using spin-echo magnetic resonance imaging (MRI). Image intensities and contrast are determined by the protons (H nuclei) of water in the tissues and are influenced both by the abundance of water and by physical interactions of the water protons with surrounding molecules. Observed features corresponded closely to visual appearances following sectioning of the logs. Annual ring structure was apparent, with highest image intensity in the more porous spring wood. Bark and dead knots gave low image intensity. Live knots were discernible due to changes in orientation of the annual rings. Rotted tissues produced higher image intensities than surrounding healthy tissues apparentaly due to less intense binding of water in the diseased tissues. MRI should prove useful for nondestructive assessment of internal structures in wood and for sequential studies of rot development.

A699

FUSARIUM, CYLINDROCARPON AND PYTHIUM ROOT INFECTION ON CONTAINER-GROWN SEEDLINGS. H.H. Kope[1], P.E. Axelrood[2] and J.R. Sutherland[1]. [1]Forestry Canada, PFC, Victoria, V8Z 1M5; [2]B.C. Research, 3650 Wesbrook Mall, Vancouver, V6S 2L2.

Isolations from roots of commercially grown Douglas-fir and spruce indicated the occurence of Fusarium, Cylindrocarpon and Pythium increased over the growing season. To determine prevelance and intensity of root infection over time, we monitored eight seedlots of Douglas-fir and spruce, sown in geographically different container nurseries in British Columbia. Root infection levels for Fusarium and Cylindrocarpon were moderate, whereas Pythium was low for all seedlots at all nurseries. Incidence and abundance of Fusarium, Cylindrocarpon and Pythium isolated from the container mix and from within the roots were correlated with nursery cultural practices and seedling age.

A700

PHYSIOLOGY OF PONDEROSA PINE SEEDLINGS EXPOSED TO OZONE, DROUGHT, AND HETEROBASIDION ANNOSUM. T.D. Leininger and M.E. Fenn. USDA Forest Service, P. O. Box 227, Stoneville, MS 38776 and USDA Forest Service, 4955 Canyon Crest Drive, Riverside, CA 92507.

Ponderosa pine (Pinus ponderosa) seedlings (329 total) potted in mineral soil were exposed to charcoal-filtered air (CFA) or ozone (O_3) at 33 ppm-hr, integrated sum for all hours of 140 d, 1989; and 120 d, 1990). Half the seedlings in CFA and O_3 were well-watered (WW, 1200 ml H_2O/7-10 d), the other half were drought-stressed (DS, 800 ml H_2O/2-3 weeks) during both growing seasons. During the fall of 1989, half of the WW and DS seedlings were inoculated with Heterobasidion annosum, the other half served as controls. WW trees had greater height and diameter growth, longer 1990 needles, and greater rates of maximum and net photosynthesis (P_{max}) than DS trees across O_3 and disease treatments. Seedlings exposed to O_3 had greater P_{max} and greater stomatal conductance than those in CFA across drought and disease treatments suggesting a compensatory response mechanism due to O_3 stress. Total chlorophyll concentrations were greater in DS seedlings. Average length of disease lesions was unaffected by drought or O_3.

A701

UNIQUE $C_{15}H_{24}$ VOLATILE COMPOUNDS IN SYNTHETIC CULTURES OF ASPERGILLUS FLAVUS. H. J. Zeringue, Jr., USDA, ARS, Southern Regional Research Center, P.O. Box 19687, New Orleans, LA 70179.

Head space volatiles from 9 strains of Aspergillus flavus (4 toxigenic strains, 5 atoxigenic strains) were collected on Tenax GC traps from 3-, 4-, 8- and 10-day old synthetic cultures. The traps were desorbed with heat and gas purge by an external direct injector device onto a 50 m GLC capillary column that was interfaced with a mass spectrometer data acquisition system. Peaks were identified with a computer-assisted mass spectral database. All A. flavus strains produced low molecular weight alcohols and aldehydes which peaked in 4-day old cultures. Toxigenic strains of A. flavus also produce several $C_{15}H_{24}$ compounds (e.g., alpha-gurjunene, trans-caryophylene, cadinene, etc.) which peaked in 3 and 4-day old cultures and were not present in 8-day old cultures. These $C_{15}H_{24}$ compounds may be correlated with the beginnings of toxin (aflatoxin) biosynthesis during this time period.

A702

VARIATION IN AGGRESSIVENESS AMONG ISOLATES OF *COCHLIOBOLUS HETEROSTROPHUS* AND ITS RELATIONSHIP WITH THE ABILITY TO OVERWINTER IN NORTH CAROLINA. M. L. Carson, USDA-ARS, Dept. of Plant Pathology, North Carolina State University, Raleigh, NC, 27695-7616.

In hemibiotrophic plant pathogens such as *Cochliobolus heterostrophus* (anamorph=*Bipolaris maydis*), the role of selection during the saprophytic (or overwintering) phase on population composition is largely unstudied. Leaf tissue infested with twenty-two isolates of *C. heterostrophus* varying in their aggressiveness was overwintered under both ambient outdoor conditions and in a controlled indoor climate. Subjecting tissue samples to six months of overwintering conditions outdoors reduced inoculum production as assayed by the number of lesions produced on a susceptible hybrid in a 1991 field test. The percentage reduction in inoculum production of individual isolates was significantly (P=.05) positively correlated with their aggressiveness. These results suggest a negative relationship exists between overwintering ability and aggressiveness in *C. heterostrophus*. This relationship could at least partially explain the persistence of less aggressive isolates in the *C. heterostrophus* population as well as the apparent durability of partial resistance in maize. Experiments are being repeated in 1992 and updated results will be presented.

A703

IN VITRO EVALUATION OF *CALONECTRIA CROTALARIAE* ON SOYBEAN SEEDLINGS AND RELATIONSHIP BETWEEN PRODUCTION OF PERITHECIA AND PATHOGENICITY.
K. D. Kim, J. P. Snow, and C. S. Kousik. Department of Plant Pathology and Crop Physiology, Louisiana Agricultural Experiment Station, Louisiana State University Agricultural Center, Baton Rouge, LA 70803.

In vitro evaluation of pathogenicity of *Calonectria crotalariae* (Loos) Bell & Sobers was conducted in agar disc tests with 11 soybean and 14 peanut isolates on 10-day-old seedlings of six soybean cultivars. Most soybean isolates were more virulent than peanut isolates. Variation in virulence of *C. crotalariae* isolates on specific soybean cultivars was also observed. Two highly virulent isolates, one soybean and one peanut, and two less virulent soybean and peanut isolates from the previous experiment were used to inoculate seedling stems of 11 cultivars. The highly virulent isolates were uniformly virulent on all cultivars tested and the less virulent isolates were uniformly less virulent. Ability of isolates to produce perithecia on PDA under continuous fluorescent light was positively correlated with virulence and/or pathogenicity of *C. crotalariae* on soybean seedlings. These results suggest that different degrees of pathogenicity among *C. crotalariae* isolates exist and are significantly correlated with production of perithecia.

A704

EFFECT OF SOIL WATER MATRIC POTENTIAL ON POTATO PLANT GROWTH AND ROOT INFECTION IN VERTICILLIUM DAHLIAE INFESTED SOIL.
Gaudreault, S. M., Powelson, M. L., Christensen, N. W., Depts. of Botany & Plant Pathology and Crop & Soil Science, Oregon State University, Corvallis 97330

Greenhouse experiments were conducted in 1991 and 1992 to determine effect of soil water matric potential on potato (cv Russet Burbank) plant growth and root infection in soil infested with Verticillium dahliae. In 1991, soil water matric potentials of -0.03, -0.08, and -0.15 MPa were combined factorially with two inoculum concentrations, 0 and 25 cfu/g soil. Plants were sampled destructively over a five week period and weighed. By week four, plant weights in noninfested soil at -0.03 MPa were 11 and 30% higher than at -0.08 MPa and -0.15 MPa. In infested soil, however, plant weights were 5% higher at -0.03 MPa than at -0.15 MPa and 20% lower than at -0.08 MPa. After each harvest, roots were assayed for V. dahliae colonization by plating root pieces on a semi-selective medium. The fungus was first detected in roots at week two; by week five, colonization had reached a mean of 2.1 cfu/~90 cm of root. Soil water matric potential had no effect on onset or amount of root infection. Results from immunoenzymatic staining of roots and data from 1992 also will be presented.

A705

EFFECT OF SOIL MOISTURE STATUS ON SYMPTOM EXPRESSION OF POTATO EARLY DYING. M. R. Cappaert, M. L. Powelson, and N. W. Christensen. Dept. of Botany and Plant Pathology, Oregon State University, Corvallis. 97331-2902.

A field microplot study was conducted in 1991 in the Columbia Basin of Oregon to evaluate the effect of soil water status on the severity or potato early dying in Russet Burbank potatoes. Treatments consisted of nine irrigation regimes and six inoculum densities. Fumigated soil was either noninfested or infested with 1, 3, 5, 15, or 50 colony forming units of Verticillium dahliae/g of soil. Irrigation treatments were split over the season; early-season from planting to tuber initiation and late-season from tuber initiation to harvest. Early-season treatments were deficit, moderate, and excessive soil moisture designed to exceed, meet, or be less than the estimated water consumption by the crop. Late-season treatments were factorial combinations of the same three options. Disease severity was significantly (P=0.05) greater in plots with excessive compared to moderate or deficit early season moisture treatments at all inoculum densities. Disease was suppressed 17% when early season soil moisture status was equal to or less than consumptive use by the plant. Late season soil moisture status had no effect on symptom expression.

A706

OCCURRENCE AND DISTRIBUTION OF POTATO PINK ROT, CAUSED BY *PHYTOPHTHORA ERYTHROSEPTICA*. R. W. Stack, B. Salas, N. C. Gudmestad and G. A. Secor. Dept. of Plant Pathology, North Dakota State Univ., Fargo ND 58105.

Potato farmers often refer to "water rot" of potatoes in storage without distinguishing the pathogen(s) involved. In 1991 we examined tubers with water rot from 45 potato storage sites in seven states and one Canadian province. Tuber pieces were cultured on a medium selective for Oomycetes. *Phytophthora erythroseptica* (PE) predominated in 32 (71%) of the sites while *Pythium* spp. predominated in four locations. In those locations where it was present, PE was recovered from 57% of the rotted tubers. PE was recovered from samples from all regions included in the survey. All isolates of PE tested were pathogenic to potato tubers. Pink rot is widespread in stored potatoes and its management may deserve greater attention than it has generally received.

A708

SYSTEMIC INFECTION OF CORN BY FUSARIUM MONILIFORME.
C. J. Kedera, J. F. Leslie, and L. E. Claflin, Dept. of Plant Pathology, Kansas State University, Manhattan KS 66506-5502.

Seeds of two corn inbred lines were inoculated with one of four Fusarium moniliforme isolates, planted in single-row plots, and thinned to 15 plants per row, with three replications. Five mature standing stalks were harvested from each treatment. Isolations of F. moniliforme were made from the crown, nodes, cob, and seed samples of each stalk. Recovered F. moniliforme isolates were paired with the respective inoculant in a heterokaryon test. Recovered isolates from the uninoculated control treatments were not the same as the inoculants. Average percentage recovery of the inoculants was 65.2, 45.2, 10.4, and 8.4 for the crown, nodes, cob, and seed respectively. The results indicate that F. moniliforme was systemic in the corn plants and can be transmitted from seed to seed.

A709

THE EFFECTS OF POSTHARVEST CALCIUM AND HEAT TREATMENT ON FIRMNESS AND DECAY OF APPLES.
W. S. Conway, Hort. Crops Quality Lab., Beltsville, MD 20705, C. E. Sams, Univ. of Tennessee, Knoxville, TN 37996, J. A. Abbott, Instr. Sensing Lab, Beltsville, MD 20705, and N. Ben-Shalom, Dept. of Food Sciences, The Volcani Center, Bet-Dagan 50250 Israel.

Heat treatment of Golden Delicious apples for 4 days at 38 C or pressure infiltration with a 4% solution of calcium chloride at harvest maintained firmness and reduced decay of fruit inoculated with Penicillium expansum after 6 months storage at 0 C. Heat treatment reduced decay by 30%, while pressure infiltration with a calcium chloride solution reduced decay by over 60%. Pressure infiltration with a calcium chloride solution following heat treatment reduced decay by 40%. Calcium chloride infiltration best maintained firmness (84 N) followed by heat and calcium chloride (76 N), then heat alone (71 N) and the controls (60 N).

A710

CHARACTERIZATION OF *RHIZOCTONIA* SPP. CAUSING DISEASE OF LEAFY SPURGE IN THE NORTHERN PLAINS. Caesar[1], A. J., Rees[1], N. E., Spencer[2], N. R., and Quimby[1], P. C. USDA/ARS Biological Control of Weeds Research Unit, Montana State University, Bozeman[1] and Sidney[2] MT 59717.

A number of disease syndromes of the noxious rangeland weed leafy spurge, *Euphorbia esula*, occurred extensively in Montana, and were additionally detected in Colorado and North Dakota. Symptoms observed were stem cankers, crown rots and necrotic root buds. Isolations from above-ground portions of diseased plants typically yielded binucleate *Rhizoctonia*-like fungi, whereas isolations from crown rots, root stunting and necrotic root buds yielded multinucleate strains of *Rhizoctonia solani*. Anastomosis tests using fourteen tester strains representing nine anastomosis groups indicated that all the multinucleate strains are of anastomosis group four. Pathogenicity tests on stems, seedlings and roots of leafy spurge confirmed differences in modes of pathogenicity apparent in the field and revealed differences in virulence among the strains that were not readily apparent in the field. Relationships among the *Rhizoctonia* strains from leafy spurge will be discussed as well as observations on biological interactions that affect the apparent virulence of various strains in the field. The potential of *Rhizoctonia* spp. as biological control agents of leafy spurge will be discussed.

A711

ISOLATES OF *Rhizoctonia solani* CAUSING LEAF SPOT OF TOBACCO ARE CHARACTERIZED AS AG-3. J. Stevens Johnk and R. K. Jones, Dept. of Plant Pathology, University of Minnesota, St. Paul, 55108.

Isolates of *Rhizoctonia solani* causing leaf spot of tobacco have been reported in the USA as AG-2-2 and in South Africa as AG-3. Anastomosis tests paired three tobacco isolates against twelve isolates of *R. solani* AG-2-2 from mat rush (*Juncus effusus*) (AG-2-2 IIIB), sugar beet (AG-2-2 IV), turf, corn and soybean, and three isolates of *R. solani* AG-3 from potato. Tester isolates were picked from widely different geographic locations to increase diversity. Anastomosis reactions of tobacco isolates indicated identification as AG-3. Tobacco isolates tested were thiamine autotrophic while isolates of AG-2-2 tested were thiamine auxotrophic. Tobacco isolates had a unique and uniform cultural appearance on potato dextrose agar.

A712

VIRULENCE PATTERNS IN BEAN RUST FROM AFRICA AND THE AMERICAS. M. T. Mmbaga, J. R. Steadman, D. O'Keefe, M. Meskine. Department of Plant Pathology, University of Nebraska, Lincoln, NE 68583-0722.

Single uredinia cultures of Uromyces appendiculatus from Honduras, Dominican Republic, Puerto Rico, Tanzania and USA were evaluated for virulence on 19 standard bean differentials using a rating scale for uredinia diameter. Virulence was computed as a mean disease score (mds) on all 19 differentials and a FASTCLUS program was used to cluster the cultures according to virulence similarity. A total of 343 different virulence patterns, forming 5 clusters, were identified from more than 2000 cultures. None of the cultures produced a susceptible reaction on all the 19 differentials. Cultures that produced uredinia >500 μm on 15 differentials were considered to have high virulence. Honduran cultures had the greatest frequency, 68%, of high virulence with Dominican, Puerto Rican, American and Tanzanian cultures having 30, 21, 16 and 12% respectively. Early Gallatin, Pinto Olathe, Compuesto Negro Chimaltenago, Mexico 309, Ecuador 299 and Mexico 235 gave a high degree of resistance to most cultures.

A714

ARE ASEXUAL POPULATIONS OF THE BEAN RUST FUNGUS LESS DIVERSE THAN SEXUAL ONES? J. W. McCain, J. V. Groth, Dept. of Plant Pathology and A. P. Roelfs, USDA-ARS Cereal Rust Lab, Univ. of Minnesota, St. Paul, MN 55108.

Sexual populations are expected to include many, equally frequent phenotypes, but a few, abundant phenotypes may dominate asexual populations. This was tested in the bean rust fungus (Uromyces appendiculatus) with 20 isolates from each of two sexual (producing all spore stages) and two asexual (only uredinial) collections. We tested all 80 isolates for virulence on ten differential lines of beans and obtained isozyme phenotypes for five polymorphic enzymes on polyacrylamide gels for many of the isolates. Samples from one asexual and both sexual collections had similar virulence combinations. The other asexual collection had half as many virulence combinations. The lowest ratio of isozyme phenotypes to isolates was in one of the sexual populations, which had one predominant phenotype. Asexual collections had more of the complex, probably heterozygous isozyme patterns. Thus, our results show that asexual populations are not always genetically less diverse.

A715

HOST SPECIFICITY AS A PRE- AND POST-REPRODUCTIVE ISOLATING MECHANISM IN *PHYTOPHTHORA*. S. B. Goodwin and W. E. Fry, Department of Plant Pathology, 334 Plant Science, Cornell University, Ithaca, NY 14853.

Interspecific crosses were made between isolates of two host-specific *Phytophthoras*, *P. infestans* and *P. mirabilis*. Among 86 putative hybrid progeny, allozyme analysis revealed that 79 were hybrids, 3 were selfs and 4 were either selfs or nonrecombinant parental types. These represent the first unambiguously confirmed interspecific hybrids in the genus *Phytophthora*. The hybrids were scored for mating type, glucose-6-phosphate isomerase (GPI) genotype, DNA fingerprinting pattern, mitochondrial DNA (mtDNA) haplotype, growth rate, and pathogenicity to potato, tomato and *Mirabilis jalapa*. Mating type and GPI each segregated 1:1 and were independent of each other. DNA fingerprinting bands segregated independently in both species, and confirmed that the progeny were interspecific hybrids. There was transgressive segregation for growth rate. Most (> 80%) of the progeny inherited their mtDNA from the *P. infestans* parent. None of the hybrids infected *Mirabilis* and only three infected potatoes. Four isolates were weakly pathogenic to tomato. Although interfertile, host specificity provides a pre- and post-reproductive isolating mechanism for these two *Phytophthora* species.

A716

INHERITANCE OF AVIRULENCE/VIRULENCE ON FIVE U.S. RICE CULTIVARS IN A CROSS BETWEEN TWO ISOLATES OF *MAGNAPORTHE GRISEA*. C. T. Chao and A. H. Ellingboe, Department of Plant Pathology, University of Wisconsin-Madison, Madison, WI 53706.

A cross was made between two isolates of *Magnaporthe grisea* that were both pathogenic on cultivars Bluebelle, L202, Maybelle, M201, M301, and S201, but differed in virulence on five other cultivars. Isolate P4 (MAT1-1), a derivative of an isolate from Texas, was avirulent on cultivars Newbonnet, Lemont, Lebonnet, Leah, Katy, and IR36. Isolate 70-6 (MAT1-2) was selected because it was highly competent in mating and was virulent on all cultivars except IR36. The progenies segregated for avirulence/virulence on Newbonnet, Lemont, Lebonnet, Leah, and Katy, but not on IR36. There was cosegregation for avirulence/virulence on Newbonnet, Lemont, and Lebonnet. The data suggested that two genes from P4 control avirulence on these three cultivars. Two genes from P4 control avirulence on Leah. At least one gene from P4 controls avirulence on Katy. Populations segregating for single avirulence genes are being developed by backcrossing to isoaltes 70-6 or its sibling 70-15 (MAT1-1). In another population, cross T7, there were at least four avirulence genes segregating independently on Newbonnet, Lemont, Lebonnet, and Leah. Backcross progenies from P4 were crossed with T7 isolates for studying the relationship between avirulence genes from P4 and avirulence genes segregating in T7 isolates.

A717

GENETIC VARIATIONS IN *PUCCINIA STRIIFORMIS* ASSESSED BY VIRULENCE AND DNA POLYMORPHISMS. Xianming Chen, Hei Leung, and Roland F. Line. USDA/ARS, Dept. Plant Pathology, Washington State University, Pullman WA 99164-6430

To study the evolution of races of *P. striiformis*, 115 single-urediospore isolates from 23 North American races were subjected to virulence and random amplified polymorphic DNA (RAPD) analysis. Virulence was tested on 15 wheat differential cultivars. Random primers were used to detect DNA polymorphisms with genomic DNA extracted from urediospores in a polymerase chain reaction. Most virulence patterns of single-spore isolates were identical to those of the parental races, but pathogenic variations were detected among single-spore isolates derived from 3 races. DNA polymorphisms were detected between races and among isolates within a single race in 21 of the 23 races tested. Race CDL-21, originally collected from triticale in California, differed from the other races at 11 of the 32 RAPD loci assayed. The phylogenetic relationships of *P. striiformis* races will be discussed based on virulence and molecular data.

A718

EVOLUTION AND REGULATION OF THE *DRR49* MULTIGENE FAMILY. M. De Pauw, T. Russnak, S. Tewari, S. Brown and B. Fristensky. Department of Plant Science, University of Manitoba, Winnipeg, MB R3T 2N2 Canada.

The *drr49* multigene family is induced in pea (*Pisum sativum* L.) within the first few hours after inoculation with fungi, bacteria or the elicitor chitosan. Other workers have reported homologous gene families in potato, parsley and soybean that are inducible by pathogens, elicitors and other stresses. We have also detected *drr49* homologues in wild relatives of the pea, *P. fulvum*, *P. humile* and *P. elatius* as well as *Lathyrus sativa*, which express tolerance or resistance to fungal pathogens of *P. sativum*. We are investigating the hypothesis that divergence in host/pathogen compatibility is accompanied by a divergence in *drr49* gene expression. *P. sativum* has approx. 5 copies of *drr49*/haploid genome, of which 3 genes have been sequenced. We are now isolating the remaining genes in order to explore the role of differential expression of *drr49* genes with respect to: a) specific pathogens b) the infection timecourse c) resistance or susceptibility.

A719

MUTATIONAL ANALYSIS OF SPORULATION IN *MAGNAPORTHE GRISEA*. Zhixin Shi and Hei Leung. Dept. Plant Pathology, Washington State University, Pullman, WA 99164

Diepoxyoctane (DEO) was used to generate mutations in the sporulation pathway of *M. grisea*. Conidia of strain Guy11 were treated with DEO to yield 0.01% surviving colonies. Although reduced sporulation was common among survivors, mutations specific to the sporulation apparatus were rare. Of over 7,000 survivors examined, three types of mutations with prominent morphogenetic defects were found. Two mutants showed no sporulation. Forty-four mutants produced a mixture of normal conidia and aleuriospores (pigmented, thick-walled conidia). Preliminary genetic analysis suggested that the production of aleuriospores was conditioned by two complementary genes. Two mutants produced fewer conidia per conidiophore and the conidia did not

detach readily from mature conidiophores. This phenotype was stably expressed among segregating progeny. Intercrossings among these morphogenetic mutants are in progress to delineate the genetic control of sporulation.

A721

COMPARISON BETWEEN A PENNSYLVANIA POPULATION AND THE PHYSIOLOGIC RACES OF USTILAGO HORDEI BY ISOZYMES. Christ, B.J., D. M. Petrunak and R. Hellmann. Department of Plant Pathology, The Pennsylvania State University, University Park, PA 16802

Thirty-three random haploid sporidial isolates of Ustilago hordei derived from a population in PA were examined for enzyme variation by starch gel electrophoresis and compared to 12 isolates from the collection of physiologic races derived from North Dakota. Eight enzymes produced scorable results. New alleles were identified at each of the eight loci in the PA isolates. There were no alleles in common between the two populations for four enzymes, AK, MDH, PGI and ACO. For G6P and PEP there was only one allele in the ND population but two additional alleles were identified in the PA isolates. There were two alleles for IDH in the ND population but only allele 1 was found in PA isolates along with three additional alleles. There were three alleles for PGM in the ND isolates of which alleles 1 and 2 were also identified in the PA isolates along with two additional alleles. PA isolates separated into 12 electrophoretic types (ET) and the ND isolates separated into 4 ET. ND isolates separated in a distinct cluster based on diversity and cluster analysis. Two of the clusters of PA isolates were more closely related to the ND isolates than to a third cluster of PA isolates. More variation was identified in these PA isolates than previously reported or in comparison to results from other smut species.

A722

HETEROKARYON INCOMPATIBILITY IN *RHIZOCTONIA SOLANI* AG 8. H. A. Yang, K. Sivasithamparam and P.A.O'Brien*. Soil Science and Plant Nutrition, School of Agriculture, The University of Western Australia, Western Australia 6009; *Department of Biological Science, Murdoch University, Western Australia 6150

Homokaryons were recovered by regenerating protoplasts prepared from heterokaryotic field isolates of *R. solani* AG 8. Tufts of dense aerial hyphal growth were formed between compatible homokaryons. All homokaryons obtained were able to form tufts with their parent isolates and with other heterokaryotic field isolates of AG 8. Synthesized heterokaryons formed tufts with both of the contributing homokaryons. Homokaryons from isolates originating from different geographic areas in Australia produced vigorous tufts. The results indicated the existence of a heterokaryon incompatibility system in *R. solani* AG 8, but the genetic basis might be different from those in other anastomosis groups reported.

A723

NRS1, A MIDDLE-REPETITIVE SEQUENCE LINKED TO VIRULENCE GENES IN *Nectria haematococca*. Hong-Gi Kim[1] and H.Corby Kistler[2]. [1]Department of Agricultural Biology, Chungnam National University, Daejeon 305-764 Korea, and [2]Plant Pathology Department,University of Florida, Gainesville, 32611 USA.

A repetitive DNA sequence called Nrs1 has been identified from strain T-2 of *Nectria haematococca*. Nsr1 sequences were identified on 11 *Hin*dIII restriction fragments in T-2 DNA by Southern hybridization. Nrs1 did not hybridize to DNA from strain 6-36. When strain T-2 and 6-36 were crossed, 10 of the 11 *Hin*dIII fragments segregated in a 1:1 ratio in random ascospore progeny and defined three linkage groups. When chromosome-sized DNAs from T-2 were separated by pulsed field gel electrophoresis (PFGE), three bands on Southern blots hybridized to Nrs1. These results indicate a correspondence between genetic linkage groups and bands separated by PFGE. Polymorphic DNA sequences corresponding to known virulence determinants (genes for pisatin demethylase and cutinase) were tested for linkage to Nrs1 loci. Two pisatin demethylase loci were linked to each other and to several Nrs1 loci. This linkage group corresponds to a 1.6 million base pair chromosome containing the previously described pisatin demethylase loci *Pda*1 and *Pda*2.

A724

A PRELIMINARY GENETIC LINKAGE MAP OF *PHYTOPHTHORA INFESTANS*. A.T. Dyer[1], P.W. Tooley[2], F. Govers[3], M. Carras[2], J.J. Galindo[3], and W.E. Fry[1]. Dept. of Plant Pathology, Cornell Univ., Ithaca, NY 14853[1], USDA-ARS, Ft. Detrick Bldg. 1301, Frederick, MD 21701[2], Dept. of Phytopathology, Agriculture Univ., 6709 PD Wageningen, Netherlands[3].

Random DNA fragments selected from genomic libraries, a cDNA library, and DNA fragments from characterized *Phytophthora infestans* genes were used as probes in the search for restriction fragment length polymorphisms. Three isozymes, mating type, and 37 RFLP markers were analyzed for recombination in the progeny of a sexual cross between two diploid Mexican isolates. The mapping programs MAPMAKERII and JOINMAP were used for the construction of the genetic linkage map. With the data gathered so far, seven linkage groups involving 15 RFLP markers could be detected.

A725

EFFECTIVENESS OF STRIPE RUST RESISTANCE IN NORTH AMERICAN WHEAT CULTIVARS. Roland F. Line and Xianming Chen, USDA-ARS Washington State Univ, Pullman, WA 99164-6430

Resistance to stripe rust (*Puccinia strifformis*) is primarily seedling resistance (SR) and high-temperature, adult-plant resistance (HTAPR). SR is race specific and effective at all stages of plant growth and temperatures. When used in locally adapted cultivars, new races circumvent that SR, usually within three years after cultivar release, and SR is no longer effective. Use of SR in a multiline cultivar has provided protection for > 10 years. HTAPR has been effective against all races for > 30 years. Most cultivars grown where stripe rust occurs now have HTAPR, and use of HTAPR has prevented wide-spread losses. Seedlings of cultivars with HTAPR are susceptible. As plants become older, they become more resistant at the higher temperatures. They remain susceptible at low temperatures. At the high temperatures, flag leaves are most resistant. Resistance can be reversed by changing the temperature. Genes for SR and HTAPR have been identified and their inheritance has been determined.

A726

RESPONSE TO WHEAT LEAF RUST OF *Aegilops* spp. COLLECTED IN ISRAEL. Y. Anikster, D. L. Long and J. Manisterski. Institute for Cereal Crops Improvement, Tel Aviv University, Tel Aviv, Israel and USDA-ARS Cereal Rust Lab, University of Minnesota, St. Paul, Minnesota 55108, U.S.A.

Species of *Aegilops* (*Triticum*) hybridize with wheat and are used as sources of rust resistance in bread wheat breeding programs. Variability in 1450 accessions of 8 *Aegilops* species collected in Israel in response to wheat leaf rust (*Puccinia recondita* f. sp. *tritici*) was determined at Tel Aviv, Israel, and St. Paul, Minnesota. Ninety-seven percent of the *A. speltoides* (wetland habitat) accessions were resistant to leaf rust. Three of the dryland habitat species; *A. bicornis*, *A. kotschyi* and *A. searsii*; had very little or no rust resistance. Variable degrees of rust resistance were found in *A. longissima*, which grows in the Negev desert and coastal plain; *A. sharonensis*, which grows on the coastal plain but only on sandy soils; and *A. variabilis* and *A. ovata*, which inhabit diverse ecologic locations over large areas.

A727

RESISTANCE TO CERCOSPORA LEAF SPOTS IN PEANUT GENOTYPES DERIVED FROM CROSSES WITH WILD *ARACHIS* SPP. B.B. Shew, M.K. Beute*, and H.T. Stalker. Departments of Crop Science and Plant Pathology*, North Carolina State University, Raleigh 27695-7616.

Ten genotypes derived from crosses between wild *Arachis* spp. and virginia peanut were planted with four control genotypes in 4 row (3.7 m) x 3.3 m plots; each plot was bordered by 7.3 m of soybeans. Weekly

estimates of disease incidence and defoliation were used to calculate AUCs for early leaf spot (AUC-E), late leaf spot (AUC-L), and defoliation (AUC-D). All test genotypes had smaller AUC-Es than the most resistant control, and AUC-Ls in seven test genotypes were not different from a highly resistant control. Although ranks of genotypes by AUC-E and AUC-L were only weakly correlated (r=.27; p=.01), some genotypes had combined resistance to leaf spots and defoliation far superior to that in commercial cultivars.

A728

BREEDING FOR RESISTANCE TO ASCOCHYTA BLIGHT OF CHICKPEA. W. J. Kaiser, F. J. Muehlbauer, R. W. Short, J. L. Coker, R. M. Hannan, and B. C. Hellier. USDA, ARS, Washington State University, Pullman 99164-6402

Ascochyta blight of chickpea (Cicer arietinum) incited by Ascochyta rabiei was observed in the USA at Pullman, WA in 1983. By 1984, ascochyta blight was found in over 50% of the commercial fields in the Palouse region of eastern WA and northern ID. In 1986, Didymella rabiei (syn. Mycosphaerella rabiei), the teleomorph (sexual stage) of A. rabiei was discovered on overwintered infested chickpea debris from a field near Genesee, ID. The fungus is heterothallic. Ascospores from the teleomorph are important in the long distance spread of the pathogen. In 1987, over 4,500 ha of chickpeas in the region were devastated by ascochyta blight. Chickpea germplasm from different countries was screened for resistance to pathotypes of the fungus in the Palouse region and sources of resistance were identified. Blight resistance has been incorporated into large-seeded kabuli cultivars which are being increased for release to growers.

A729

RUST REACTIONS OF INTERSPECIFIC PENNISETUM HYBRIDS. J. P. Wilson, USDA-ARS Forage and Turf Research Unit, Coastal Plain Experiment Station, Tifton, GA 31793-0748.

Thirty three accessions, representing 15 different species of wild Pennisetums of the tertiary gene pool of pearl millet, were highly resistant to rust, incited by Puccinia substriata var. indica. Pollen was collected from 13 of these species and crossed onto the susceptible P. glaucum inbred Tift 23DB (2n=14). Putative hybrids were identified by morphology and confirmed by somatic chromosome numbers. Most of the crosses were unsuccessful. One hybrid (2n=16) was obtained from a P. glaucum x P. hohenackeri (2n=18) cross. The hybrid was susceptible to rust in a seedling inoculation. The P. hohenackeri parent reproduces sexually and did not segregate for susceptibility. Thus it is likely that the resistance was suppressed by the pearl millet cytoplasm or genome. Forty-eight hybrids (2n=25) were obtained from four P. glaucum x P. flaccidum (2n=36) crosses. These hybrids were resistant to rust in seedling inoculations and may be useful sources of resistance for pearl millet.

A730

EXPRESSION OF COMPONENTS OF RESISTANCE TO SEPTORIA NODORUM BLOTCH OF WHEAT AT DIFFERENT GROWTH STAGES. Catherine A. Kuwite and G.R. Hughes, Department of Crop Science and Plant Ecology, University of Saskatchewan, Saskatoon, SK, Canada. S7N 0W0.

Components of resistance to septoria nodorum blotch of wheat were studied in 12 winter wheat cultivars. These cultivars all show restricted lesion development in seedling tests. One or more of the components incubation period, infection frequency, percentage leaf necrosis and lesion type, were measured on the third leaf, the flag leaf and the heads of plants inoculated with a single isolate of S. nodorum under controlled environmental conditions. Differences existed among cultivars for each component measured on infected leaves, but not for components measured on infected heads. In general, the relative differences among cultivars for each component were similar at the third and flag leaf growth stages, although the level of expression of resistance was lower at the flag leaf stage. Cultivars differed in reaction to foliar infection with Red Chief being the most resistant; none of the cultivars were resistant to head infection.

A731

SCREENING OF LENTIL FOR RESISTANCE TO ANTHRACNOSE (*COLLETOTRICHUM TRUNCATUM*). L. Buchwaldt#, R.A.A. Morrall*, C.C. Bernier# and B. Vandenberg*. #University of Manitoba, Winnipeg, MB R3T 2N2 Canada. *University of Saskatchewan, Saskatoon, SK S7N 0W0 Canada.

Anthracnose of lentil is a relatively new but severe disease in some areas of Manitoba and Saskatchewan. Indianhead, a black seeded forage cultivar, with moderate resistance was seeded in the field with 549 accessions from the world lentil collection. Two weeks after inoculation with a virulent isolate (882) 17 accessions were rated as resistant. A screening method was developed in the greenhouse. After retesting of the 17 accessions with isolate 882 and two other isolates only two accessions, both black seeded, were resistant. Screening of breeding lines from different crosses has shown that some lines from crosses with Indianhead were moderate resistant as well as lines from crosses with speckled seeded french cultivars. Trials are in progress to evaluate the effectiveness of the resistance under field conditions.

A732

THE ROLE OF CARBON ASSIMILATION IN RESISTANCE TO *VERTICILLIUM ALBO-ATRUM* IN ALFALFA. B.W. Pennypacker, D.P. Knievel and K.T. Leath. Dept. of Agronomy, Penn State Univ., and USDA-ARS U.S. Regional Pasture Res. Lab, Univ. Park, PA 16802

Growth suppression was documented in resistant alfalfa infected with *V. albo-atrum* (Vaa). This could be due to increased hydraulic resistance, which would affect cell expansion, or to the energy demands of resistance mechanisms. The effect of net photosynthesis (Pn) on resistance was examined factorially using 2 levels of Vaa (infected and non-infected) and 3 photon flux densities (PPFD). Plants were stubble inoculated, grown for 6 weeks, and then subjected to the PPFD treatments. Significant pathogen X light X week interactions were detected in leaf dry weight, aerial biomass and *in vivo* rubisco activity. Resistance to Vaa was lost in plants grown under the lowest PPFD treatment, indicating that resistance mechanisms in alfalfa have specific photosynthate requirements.

A733

HISTOLOGICAL RESISTANCE MECHANISMS IN ALFALFA INFECTED WITH *VERTICILLIUM ALBO-ATRUM*. B.W. Pennypacker and K.T. Leath. Dept. of Agronomy, Penn State Univ. and USDA-ARS U.S. Reg. Pasture Lab, Univ. Park, PA 16802

Two resistant alfalfa clones were stubble inoculated with *V. albo-atrum* (VAA), grown for 3 mo., cut back, then sampled weekly for 6 weeks. VAA was confined by vascular occlusions to the crown of clone WL-5 for 2 wks and clone 1079 for 4 wks. Vascular differentiation was disrupted and vascular bundle dissolution occurred in stems of 1079 by week 5. Confinement of VAA to the crown until late in the growth period appeared to account for resistance in 1079. WL-5 had a second resistance response. Hypertrophied xylem parenchyma crushed infected xylem vessel elements in the stems and the hypertrophied cells were often positive for suberin. VAA in both clones was encased in a material that was positive for suberin and lignin and may be a form of lignituber. Lignitubers have not previously been reported in xylem vessels.

A734

RESIDUAL RESISTANCE TO PUCCINIA CORONATA F.SP. AVENAE IN OATS. J.M. Windes and W.L. Pedersen. University of Illinois, Department of Plant Pathology, Urbana 61801

Eight nearly-isogenic oat lines (isolines), provided by K.J. Frey, Iowa State, each with a single resistance gene to *Puccinia coronata* f. sp. *avenae* (*P.c.a.*), were compared with the recurrent parent, C237-89, for residual gene resistance using both whole plants and detached leaves. Isolines were inoculated with an isolate of *P.c.a.* virulent on all eight isolines and the recurrent parent. Latent period, urediniospore production, pustule length, pustule area, total number of pustules per leaf, and pustules per cm^2 of leaf area were measured. Coefficients of variation ranged from 24.4 to 92.0% for whole plants and 15.4 to 67.1% using the detached leaf method. Single degree of freedom contrasts of isolines compared with the recurrent parent were significant ($P > .05$) for latent period, urediniospore production, pustule length, and total number of pustules.

A735

SCREENING FOR RESISTANCE TO ELSINOE AMPELINA IN GRAPEVINE SEEDLINGS. D. L. Hopkins and J. W. Harris, Central Florida Research and Education Center, University of Florida, Leesburg, FL 34748

In Florida, grapevines must have resistance to anthracnose (Elsinoe ampelina (d By) Shear) for profitable production. Resistance in grape breeding lines is currently evaluated using natural infection in the vineyard, a procedure which is slow and expensive. Therefore, a greenhouse method for screening for resistance to anthracnose resistance in grape seedlings was developed. Abundant spores were produced on potato dextrose agar in 3-4 weeks. The seedlings, in the 2-3 true leaf stage, were inoculated by misting with a spore suspension. The most appropriate spore suspension was found to be approximately 1 x 10^6. The best symptoms were obtained when seedlings were placed in a moist chamber for 72 hours in the greenhouse at 24-30 C. Using this method for screening selfed 'Blue Lake' progeny, the expected ratio of resistant to susceptible seedlings of 3:1 was obtained.

A737

GENETIC CONTROL OF RESISTANCE TO SEPTORIA NODORUM IN DURUM WHEAT. Hong Ma and G.R. Hughes, Department of Crop Science and Plant Ecology, University of Saskatchewan, Saskatoon, SK, Canada. S7N 0W0.

Resistance to S. nodorum (derived from Triticum timopheevii, PI 290518) in durum wheat (T. durum) line S12-1, is expressed as restricted lesion development. The genetic control of this resistance was studied in controlled environment seedling tests using the parental, F1, F2, F3 and BC generations of the cross S12-1 x cv. Sceptre. Plants were inoculated at the three-leaf stage with a single-pycnidial isolate of S. nodorum. Resistance in cv. S12-1 was monogenically controlled and susceptibility was incompletely dominant to resistance. In tests with Langdon-Chinese Spring D genome disomic substitution lines, only chromosome 3A was associated with resistance. The resistant gene identified is considered to be located on chromosome 3A.

A738

GENETICS OF RUST RESISTANCE IN SEVEN NAVY BEAN GERMPLASM RELEASES. J. R. Stavely, Microbiology & Plant Pathology Lab., ARS, USDA, Beltsville, MD 20705-2350.

BelMiDak-Rust Resistant (RR)-1 through -7, type II, navy bean lines were released by ARS, USDA, and the Michigan and North Dakota Experiment Stations in 1991. Parental sources of resistance included PI 181996 that has resistance to all 58 available races of the bean rust fungus (Uromyces appendiculatus), a navy line having the Up_2 gene for resistance to 23 races transferred from bush snap beans, and navies having the Ur_3 gene effective against 22 races. Parental plants of each of the seven lines were crossed with susceptible cultivars. Progeny tests indicated that all seven lines are homozygous for a complex locus from PI 181996 (effective against all races) of which the Ur_3 gene is an allele. Only BelMiDak-RR-1 and -2 carry the Up_2 gene. They are the first United States dry beans to have Up_2. All seven are the first having PI 181996 resistance.

A739

INHERITANCE OF RESISTANCE TO BLACK ROT IN A CABBAGE BY BROCCOLI CROSS. L.E.A.Camargo, P.H.Williams, and T.C.Osborn.

The inheritance of resistance to *Xanthomonas campestris pv campestris* was studied in an F2 population derived from a cabbage by broccoli cross of two inbred cultivars (Badger Inbred-16 and OSU CR-7, respectively). Primary leaves were inoculated by a clipping method and lesion length was measured 12 days after inoculation. The distribution of lesion length was studied for parental, F1, and F2 plants. The number of genetic factors governing resistance was estimated both by a formula of WRIGHT discussed by CASTLE (Science 54:223) and by analyzing means and variances of lesion length. By both methods one recessive gene controlling resistance sufficed to explain the segregation data. Although the population was segregating for leaf area, there was no correlation between lesion length and leaf area, indicating that lesion length is a suitable parameter for assessing resistance. We are using RFLPs to map this gene in relation to genes that condition resistance at the cotyledon and adult plant stage.

A742

RAPD ANALYSIS OF COLLETOTRICHUM GLOEOSPORIOIDES. 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 gloeosporioides from diverse hosts and geographic origins were examined for molecular diversity using RAPDs. These isolates had previously been characterized for VCG and mtDNA RFLP phenotypes. Most isolates examined represented unique VCGs including multiple isolates for a given mtDNA RFLP phenotype. Several two- and four base decamer RAPD primers successfully amplified multiple DNA fragments of the various isolates. RAPD phenotypes were similar among isolates with the same mtDNA RFLP phenotype, even among isolates from different VCGs, but were distinct among isolates with different mtDNA RFLP phenotypes. Minor polymorphisms were identified among isolates with a single mtDNA RFLP phenotype (representing different VCGs) with some RAPD primers.

A746

RELATEDNESS OF RUSTS OF CEREALS AND GRASSES FROM rDNA SEQUENCE ANALYSIS P. J. Zambino and L. J. Szabo. USDA-ARS Cereal Rust Laboratory, Department of Plant Pathology, University of Minnesota, St. Paul, MN, 55108.

The internal transcribed spacer (ITS) region of the ribosomal gene of 49 isolates representing 10 rust species was PCR amplified from genomic DNA extracted from ca. 0.02 mg urediniospores or teliospores, and then sequenced. Aligned sequences of 465 bases were clustered using distance, maximum-likelihood, and parsimony protocols of the programs PHYLIP and PAUP. All analyses showed the following as distinct clusters: 1) six formae speciales of *P. graminis*; 2) four formae speciales of *P. coronata* and the coronate, microcyclic *P. mesnieriana*; 3) *P. striiformis* and the grass/barberry rust *P. montanensis*; 4) *P. hordei* and the microcyclic *Uromyces scillarum*; and 5) isolates of *P. recondita sensu* Cummins from bread and durum wheats (sequences were identical). Isolates of *P. recondita* from wheat, rye, and *Agropyron smithii* were more divergent than members of other clusters.

A749

DEVELOPMENT OF RIBOSOMAL DNA PCR PRIMERS FOR USE IN STUDYING *PRATYLENCHUS* SYSTEMATICS. R. J. MEYER and R. N. HUETTEL. Nematology Laboratory, USDA, ARS, Bldg. 011A, Rm. 153, BARC-W, 10300 Baltimore Avenue, Beltsville, MD, 20705-2350.

We have begun a molecular systematic study of the plant-parasitic nematode genus *Pratylenchus*. The first part of this study will involve examining a portion of the ribosomal DNA (rDNA) that has been found to be useful in the taxonomy of other organisms. Because there is no sequence information on the species under investigation, polymerase chain reaction (PCR) primers from other organisms are required in order to amplify the rDNA. Primers based on the rDNA sequences of the nematode *Caenorhabditis elegans* and yeast have been tested. Surprisingly, the primers from yeast produced a better yield and superior specificity as compared with the primers based on *C. elegans*. These results suggest that, unlike what has been found in other eukaryotes, it may not be possible to develop generalized primers for systematic studies in nematodes.

A751

COMPARISON OF DOUBLE-STRANDED RNA COMPONENTS AND VIRULENCE AMONG ISOLATES OF *RHIZOCTONIA SOLANI* AG-1 IA AND AG-1 IB

C. S. Kousik, J. P. Snow, and R. A. Valverde. Department of Plant Pathology & Crop Physiology, Louisiana Agricultural Experiment Station, Louisiana State University Agricultural Center, Baton Rouge, LA 70803.

Double-stranded RNA (dsRNA) was detected in 22 of 35 (63%) *Rhizoctonia solani* AG-1 IA and AG-1 IB isolates screened. Most AG-1 IA isolates had seven dsRNA components with molecular sizes ranging from 1.3-9.3 kb, whereas most AG-1 IB isolates had a large 12 kb dsRNA. Not all isolates of AG-1 IA or IB obtained from the same soybean field had dsRNA. The dsRNA were stable through six successive subculturings. Cell fractionation of *R. solani* revealed that the dsRNA were located in the fungal cell cytoplasm. DsRNA was not detected in two isolates of AG-1 IB and one isolate of AG-1 IA after 1 wk of growth at 35 C; however, the dsRNA components of one isolate of AG-1 IA were not lost at 35 C. The presence or absence of dsRNA in *R. solani* AG-1 IA or IB isolates did not correlate with virulence, mycelial growth or phenol oxidase activity of the isolates. Extensive variation in the electrophoretic band patterns of dsRNA were observed among the isolates of *R. solani* belonging to AG-1 IC, AG-2,3,4,5,7, and AG-BI. The dsRNA of these AG's appeared to be different from those of AG-1 IA or IB.

A752

Comparison of the intervening sequences flanking the 5.8S ribosomal RNA genes of *Leptosphaeria maculans* isolates from different pathogenicity groups. V.M. Morales and J.L. Taylor. NRC-PBI, 110 Gymnasium Pl.,Saskatoon, Sask. S7N OW9. Canada

The 5.8S rDNA and its flanking intervening sequences from 9 isolates of *L. maculans* were amplified by PCR and sequenced. Five of the isolates were highly virulent to *Brassica* species, two were weakly virulent and the two others were isolated from the cruciferous weed *Thlaspi arvense.* Alignment of the sequences showed that they were almost identical within a pathogenicity group regardless of their geographical origin, but very distinct from the other groups. Phylogenetic analysis of the sequences confirmed that the isolates were distributed into three groups of closely related but distinct organisms, with the *Thlaspi* isolates being positioned closer to the weakly virulent than to the highly virulent isolates.

A753

GENOTYPING OF *COLLETOTRICHUM* SPECIES USING A REPETITIVE DNA ELEMENT AND MITOCHONDRIAL DNA PATTERNS. S. Freeman, M. H. Pham, and R. J. Rodriguez. Dept. of Plant Pathology, University of California, Riverside, CA 92521.

A molecular taxonomic method is proposed for grouping isolates of the genus *Colletotrichum* into defined species using a nuclear repetitive DNA element (GcpR1) and mitochondrial (mt) DNA restriction enzyme patterns. To perform these analyses, GcpR1 was radiolabeled and hybridized to Pst I digested nuclear DNA. MtDNA was analyzed by digesting total genomic DNA with Hae III. Nuclear and mtDNA patterns from ten species of *Colletotrichum* were distinctly different from each other when analyzed by these approaches. Isolates of *C. coccodes, C. fragariae, C. acutatum, C. lindemuthianum, C. magna* and *C. obiculare* showed very similar intraspecies banding. Individual isolates of *C. graminicola* (from maize), *C. graminicola* (from sorghum), *C. gloeosporioides* (from strawberry) and *C. musae* possessed unique genotypes.

A755

MOLECULAR EVOLUTION OF MAIZE CHLOROTIC MOTTLE VIRUS ISOLATES FROM NEBRASKA, HAWAII, AND PERU. K. S. Shafer, T. O. Powers, R. C. French and S. G. Jensen. University of Nebraska-Lincoln, Lincoln, NE 68583-0722.

The geographic distribution of maize chlorotic mottle virus (MCMV) has led to questions of its origin and the genetic relatedness of MCMV isolates. Isolates from Nebraska, Hawaii, and Peru are being studied to determine nucleotide similarities of MCMV within and between the regions. Four polymerase chain reaction (PCR) primers were constructed for the coat protein gene of the virus. cDNA of two segments of the coat protein gene, each approximately 240 nucleotides, were PCR amplified and compared. Restriction enzyme analysis revealed a correlation with the geographic regions. Preliminary sequence data of the amplified segments yields four phylogenetically informative sites which show a relatedness between the Hawaiian and Peruvian isolates. All nucleotide differences occur in the third base of the codon and do not result in amino acid changes.

A756

USE OF A DIAGNOSTIC IMMUNOASSAY FOR *SEPTORIA TRITICI* TO ASSESS LEAF BLOTCH CONTROL BY FLUSILAZOLE. C. M. Smith, M. C. Joerger, L. Hirata, J. Paterson, and J. J. Willey. Du Pont Agricultural Products, E. I. du Pont de Nemours and Co., P.O. Box 30, Newark, DE 19714.

A novel diagnostic immunoassay was used to identify *Septoria tritici*, incitant of leaf blotch, in wheat plants and assess control of this disease by flusilazole. In growthroom tests, wheat plants were grown at 27°C and treated at age 14 days with flusilazole at 100, 20, and 5 g a.i./ha. Treated plants and untreated controls were inoculated 24 hours after fungicide application with *S. tritici* pycnidiospores and maintained at 20°C under high humidity. At 20–28 days after inoculation, foliage was rated visually for leaf blotch and assayed using the *S. tritici* diagnostic. Visual disease ratings were closely correlated with the level of *S. tritici* detected by the immunoassay. Based on results of both the visual assessments and diagnostic assay, flusilazole provided excellent control of leaf blotch. These results demonstrate the value of the diagnostic for detection of *S. tritici* in wheat and the utility of flusilazole for leaf blotch control.

A757

DEVELOPMENT OF RHIZOCTONIA ROOT AND CROWN ROT ON SUGAR BEET PLANTS OF DIFFERENT AGES. Cheryl A. Engelkes[1], Carol E. Windels[2], and Todd E. Cymbaluk[2], [1]Biocontrol of Plant Diseases Laboratory, USDA-ARS, Beltsville, MD 20705 and [2] University of Minnesota, Northwest Experiment Station, Crookston, MN 56716.

Sugar beet (*Beta vulgaris* L.) roots were inoculated with two cultures of *Rhizoctonia solani* (from sugar beet and pinto bean) when plants were from 6- to 12-wk-old in 1990-1991 field trials. Root rot indices (0-7 scale) were about two disease ratings higher in 1991 than in 1990. At 8 wk after inoculation in 1991, root rot indices averaged 6.8, 6.3, 5.8, and 4.3 for plants inoculated when 6-, 8-, 10-, and 12-wk-old, respectively. When data are averaged across plant age, the culture from pinto bean caused more root rot (6.4) than the culture from sugar beet (5.2). On agar, the culture from pinto bean grew 0.01-8.0 mm/24 hr faster at 25-35 C than the culture from sugar beet. Average weekly air temperatures were in this range in 5 of 8 wk following inoculation both years. Tolerance to *R. solani* increased with increasing root age at time of inoculation, even when AG-2-2 cultures differed in virulence.

A758

DEVELOPMENT OF AN ANTIBODY-BASED DIAGNOSTIC KIT TO DETECT MATURE VENTURIA INAEQUALIS ASCOSPORES. L.P. Berkett, A.R. Gotlieb, and J.A. Bergdahl. University of Vermont, Plant and Soil Science Department, Burlington, VT 05405

Current management of apple scab is based on effective control of primary infections. To efficiently schedule fungicide applications, growers need to know when ascospores are mature and when environmental conditions fulfill infection requirements. The development of a technique that would enable the apple grower to accurately and rapidly determine ascospore maturity in their own orchard would lead to more informed decisions on the necessity of fungicide applications. We have developed an 'on-farm' antibody-based kit to monitor mature ascospores of V. inaequalis. This kit combines 3 features: (1) maturation of ascospores under natural orchard conditions; (2) release of ascospores and capture on an assay medium; and (3) detection of mature ascospores with an immunoassay.

A759

IDENTIFICATION OF DNA SEQUENCES SPECIFIC TO *HELMINTHOSPORIUM SOLANI*. L. M. Delserone, K. M. Wilhelm, C. L. Merida, and R. Loria, Cornell University, Dept. of Plant Pathology, Ithaca, NY 14853

DNA sequences specific for the silver scurf fungus, *Helminthosporium solani*, would be useful for its detection in soil and plant tissue. Repetitive sequences were identified (Phytopath. 81:1239) from a genomic library of a characterized *H. solani* strain. All sequences hybridized with 12 geographically diverse *H. solani* isolates. Thirty-two of 35 repetitive sequences were species-specific; they did not cross-hybridize with *Botrytis cinerea, Colletotrichum coccodes, Fusarium solani,* or *Rhizoctonia solani.* In preliminary analyses, a sequence has been identified that did not cross-hybridize with any of the fungal or bacterial potato pathogens or saprophytes tested. Application of the polymerase chain reaction will be used to improve efficiency of soil and plant assays.

A760

ELISA DIAGNOSTIC KIT DEVELOPMENT FOR THE DETECTION OF PHIALOPHORA GREGATA IN SOYBEAN. X. Q. Jiang, B. K. Fleener, L. L. Scholbrock, and J. A. Berry, Pioneer Hi-bred Int'l, Inc., P.O. Box 1004, Johnston, IA 50131.

Pathogenic isolates of Phialophora gregata were grown in shake culture, harvested, and lyophilized. Equal protein mixtures of the fungi were used as antigen for polyclonal antibody production. The antiserum was tested by double diffusion against the antigens, unrelated pathogenic and saprophytic fungi, and healthy plant tissue. The precipitation lines coalesced among the P. gregata isolates. There were no precipitation lines to the unrelated fungi or healthy soybean leaf extract. The antiserum was further purified into IgG and conjugated with alkaline phosphatase. The completed ELISA kit was challenged by fungal cultures of three P. gregata isolates, six soybean saprophytes, 16 pathogenic fungal isolates, and healthy soybean leaf extracts. The kit showed strong specificity to P. gregata detecting the sample from 10 pg/ml of total fungal protein. Finally, the kit was used in detecting pathogen infected plant samples. The results indicate that the polyclonal ELISA kit developed is an efficient tool for the diagnosis of P. gregata.

A761

DEVELOPMENT OF *USTILAGO HORDEI*-SPECIFIC DNA PROBES. Karla J. Dugan and John E. Sherwood, Dept. Plant Pathology, Montana State University, Bozeman, 59717.

Ustilago hordei is the causative agent of barley covered smut. The primary method of control has been through fungicide seed treatment even though a number of resistance genes have been identified. This resistance appears to be encoded by monogenic genes resulting in a gene-for-gene relationship with *U. hordei* avirulence genes. Since the mechanisms causing resistance are poorly understood, *U. hordei*-specific DNA probes should be useful in analyzing this plant-pathogen interaction. A *U. hordei* genomic DNA plasmid library was constructed, and potentially useful probes were selected by dot blot and Southern hybridization techniques. Probes were tested for specificity to *U. hordei*, copy number, and lack of hybridization to barley genomic DNA. We intend to use these probes for the detection and analysis of the *U. hordei* infection process and mechanisms of host resistance.

A762

TILLETIA TELIOSPORES THAT DISPLAY CHARACTERISTICS OF BOTH TILLETIA CONTROVERSA AND T. CARIES. B. W. Russell and D. Mills, Department of Botany and Plant Pathology, Oregon State University, Corvallis 97331.

Autofluorescence of teliospore reticulations and the inability of T. controversa teliospores to germinate at 16° C have been employed to differentiate the teliospores of T. controversa from T. caries . Seven samples of reticulated teliospore from from wheat in Turkey and Oregon were tested for germination at 4° and 16° C, and for the autofluorescence of the reticulated wall layer of mature teliospores upon exposure to 485 nm light. Four of the teliospore samples displayed fluorescent reticulations characteristic of T. controversa. However, two of these fluorescing teliospore samples from Oregon consistently germinated at 16° C in 5 to 10 days. Our results show that 2 of the 7 teliospore samples had the germination characteristics of T. caries, but the fluorescence properties of T. controversa.

A763

NEW SEROGROUPS OF *XANTHOMONAS ORYZAE PV. ORYZAE* DETECTED WITH MONOCLONAL ANTIBODIES. F. U. Rehman, A. A. Benedict, and A. M. Alvarez. Departments of Plant Pathology and Microbiology, University of Hawaii, Honolulu, Hawaii 96822.

Serological diversity in *Xanthomonas oryzae* pv. *oryzae* (Xoo) was found with the monoclonal antibodies (mAbs) Xoo-7, Xoo-8, and Xoo-9 made to Asian strains of Xoo. These Xoo specific mAbs recognized two new serogroups (IIb and V) when tested with 259 Xoo strains representing various rice growing areas of the world. Monoclonal antibody Xoo-7 reacted to a group of 29 strains of Xoo that included 14 of 36 strains from Nepal, 13 of 69 from India, 1 of 84 from Philippines and 1 of 2 from Colombia. The reactivity of mAb Xoo-7 with Xoo strains was reciprocal to the reactivity of a previously reported mAb, Xco-2, except for three strains that were negative with both antibodies. A second mAb Xoo-8 reacted with two avirulent Indian Xoo strains, T1 and T2, in addition to the 29 strains that reacted with the mAb Xoo-7. A third mAb Xoo-9 reacted only with avirulent strains T1 and T2. The mAbs Xoo-7 and Xoo-8 belonged to the isotypes IgG3, recognized lipopolysaccharide antigens, and gave bright fluorescence in an immunofluorescence colony staining technique. Monoclonal antibody Xoo-9 belonged to the isotype IgG2a. These data suggest the presence of serologically distinct groups of Xoo strains in Nepal and India.

A765

A LEAF-RUST EPIDEMIC OF HYBRID POPLAR ALONG THE LOWER COLUMBIA RIVER, CAUSED BY MELAMPSORA MEDUSAE. G. Newcombe and G.A Chastagner, Wash. State Univ., Puyallup Res. and Ext. Center, Puyallup, WA, 98371.

Melampsora medusae caused an epidemic of leaf rust of hybrid (Populus trichocarpa x deltoides) poplar along the lower Columbia River in the fall of 1991. Although native to North America, M. medusae had not previously attacked hybrid poplar in the Pacific Northwest. The initial disease focus was a commercial plantation of 11 clones planted in monoclonal blocks of 5 to 50 ha, near Scappoose, OR. By early fall, 6 hybrid clones infected by M. medusae were defoliated and received a Schreiner rust severity rating of 100. Four other clones were moderately rusted (i.e., a rust rating of 25 to 50) and were not prematurely defoliated. One clone was only lightly rusted (i.e., a rust rating of 0 to 25). Ramets of the same clones as those in Scappoose were less severely rusted in other commercial plantations and nurseries within a 100 km radius of Scappoose.

A766

DEVELOPMENT OF A NUCLEIC ACID HYBRIDIZATION ASSAY FOR DETECTION OF A CLOSTEROVIRUS ASSOCIATED WITH THE SWEET POTATO VIRUS DISEASE COMPLEX. S. Winter, A. Purac and R.I. Hamilton, Agriculture Canada Research Station, 6660 NW Marine Drive, Vancouver, British Columbia, Canada V6T 1X2.

A closterovirus was associated with sweet potato feathery mottle potyvirus in sweet potato infected with the sweet potato virus disease complex (SPVD). The closterovirus, latent in single infection, renders disease indexing difficult and impedes the international exchange of germplasm. Disease-specific dsRNAs from closterovirus-infected tissues were used as templates to synthesize cDNA which was subsequently cloned in the Escherichia coli plasmid, Bluescript. A cDNA clone which hybridized to the 3' end of the genome as well as to subgenomic RNAs in closterovirus-infected tissues was selected by dot-blot hybridization. Single-stranded RNA probes, synthesized in Bluescript containing phage polymerase promoters T3 and T7, were more sensitive than cDNA in tests of infected samples from greenhouse and field plants. For comparative tests, probes were labelled by incorporating digoxigenin-labelled dUTP into transcribed RNA or DNA via random-primed synthesis. Labelled nucleic acid hybrids were detected using alkaline phosphatase-conjugated sheep anti-digoxigenin antibody (Fab-fragments) and the chemiluminescent substrate AMPPD. Application of the hybridization assay for virus detection and disease control is discussed.

A767

IDENTIFICATION AND RELATEDNESS OF PERSISTENTLY APHID-TRANSMITTED VIRUS ISOLATES FROM FORAGE LEGUMES IN THE SOUTHEAST UNITED STATES. V. D. Damsteegt[1], A. J. Russo[2], A. L. Stone[1], and O. P. Smith[1]. [1]USDA/ARS, Bldg. 1301, Ft. Detrick, Frederick, MD 21702, [2]Mount St. Mary's College, Emmitsburg, MD 21727.

Immunological techniques, vector transmission, symptomatology, and dsRNA analyses were used to identify and characterize several persistently aphid-transmitted virus isolates. Most isolates were obtained from symptomless white clover (*Trifolium repens*); all produced distinct reddened leaves in subterranean clover (*T. subterraneum*). More than half of all isolates reacted to polyclonal antisera to soybean dwarf virus; others reacted to bean leaf roll virus (BLRV) antiserum. The legume isolates differed in reactivity to monoclonals produced to SDV-Y. All known SDV strains were transmitted by *Aulacorthum solani* only; all U.S. isolates were transmitted by *Acyrthosiphum pisum* and *Myzus persicae* but not by *A. solani*. To date, dsRNA analysis suggests the legume isolates are more related to SDV-Y than to SDV-D.

A768

UTILITY OF PCR FOR DIAGNOSIS OF *ASPERGILLUS* SPECIES. N.P. Keller[1], J. Owen, D. Bhatnagar[2] and T.E. Cleveland[2]. Dept. Plant Pathology and Microbiology, Texas A&M University, TX 77843[1]; USDA/ARS, Southern Regional Research Center, P.O. Box 19687, New Orleans, LA 70179[2].

The amplification of DNA sequences by polymerase chain reaction (PCR) has great potential as a diagnostic tool for identification of fungal species and individuals within a species. We have used PCR technology to successfully distinguish species of *Aspergillus* Section *Flavi* , several distantly related aspergilli and between individual isolates within a species. We have identified certain parameters in the PCR technology including choice of primers, thermocycler, and DNA preparation that contribute to successful reproducibility. Paired primers made from *Aspergillus* DNA sequences were less successful in distinguishing isolates than single primer applications. We will discuss the relative merits of primer choice in resolving individuals within a species and the applicability of these observations to other pathogenic fungi.

A769

A 30 base pair REGION IN THE MITOCHONDRIAL SMALL SUBUNIT rRNA GENE AS A SPECIFIC DNA PROBE FOR *VERTICILLIUM DALIAE*. K.-N. Li, T. L. German and D. I. Rouse. University of Wisconsin, 1630 Linden Dr., Madison, WI 53705

Our goal is to develop a method for more reliable quantitation of *Verticillium dahliae* in soil or *in planta*. Polymerase chain reaction (PCR) was used to amplify a region of about 700 base pair (bp) in the mitochondrial small subunit rRNA gene from four species of *Verticillium* and five other fungal genera. Restriction fragment length polymorphism (RFLP)analysis showed that this region differed among the genera, among species of *Verticillium* and also among isolates of *V. dahliae*. PCR products from isolates of *V. dahliae, V. albo-atrum* and *V. tricorpus* were cloned and sequenced. DNA sequence comparison revealed a region of 30 bp that are identical among *V. dahliae* isolates but have at least 6 bp difference between species. This region has been evaluated as a specific DNA probe for detecting *V. dahliae*.

A770

SEROLOGICAL STUDIES OF GEMINIVIRUSES INFECTING BEANS IN CENTRAL AND SOUTH AMERICA. M. Cancino, A. M. Abouzid, E. Hiebert, D. E. Purcifull, and F. J. Morales*. Dept. Plant Pathology, University of Florida, Gainesville, FL 32611 and *CIAT, Cali, Colombia.

Two monoclonal antibodies (broad range=BR and narrow range=NR) prepared to bean golden mosaic virus (BGMV) isolates from Guatemala (Guat) and Dominican Republic (DR) were tested against the following geminiviruses: abutilon mosaic, BGMV isolates from Brazil and Florida, BGMV-Guat and -DR isolates, Euphorbia mosaic, Rhynchosia mosaic, squash leaf curl, soybean yellow mosaic, and tomato mottle (TMoV). Monoclonal BR reacted efficiently in Western blot and ELISA tests with all tested geminiviruses, indicating this monoclonal has a broad spectrum of reactivity. Monoclonal NR reacted only with BGMV-DR and -Guat isolates. Polyclonal antibodies prepared to TMoV coat protein expressed in *E. coli* reacted with all the above geminiviruses and a geminivirus in Florida infecting the Brassicaceae. The serological specificity of the polyclonal antiserum prepared to *E. coli* expressed TMoV replicase (AL1) protein is being studied.

A771

USING MASTER GARDENER VOLUNTEERS IN AN EXTENSION PLANT CLINIC. Pottorff, Laura Pickett. Colorado State University Cooperative Extension, Jefferson County, Golden, CO 80401.

Colorado has two Cooperative Extension plant clinics that provide an essential service to citizens. The Colorado State University campus-based clinic, located in Ft. Collins, specializes in plant pest diagnosis of agricultural crops. A second clinic, located in Jefferson County (Denver metro area) receives horticultural plant samples. Clinics of this nature currently are facing budget cutbacks throughout the U.S. The demand for the type of service offered by these clinics however, is increasing. The Jefferson County branch of the Colorado State University Plant Diagnostic Clinic was able to approach this problem by utilizing horticulturally trained volunteers. Master Gardeners with one or more years of extension volunteer experience are eligible to receive indepth training in plant disease, insect and abiotic problem diagnosis. Twenty trained Master Gardeners volunteered more than 400 hours and diagnosed 26% of the samples received by the Jefferson County Clinic, saving taxpayers approximately $3,500 in equivalent hourly wages.

A773

DEVELOPMENT OF A DNA PROBE FOR THE DETECTION OF *CORYNEBACTERIUM SEPEDONICUM*. J. Williams and D. Mills. Department of Botany and Plant Pathology, Oregon State University, Corvallis, Oregon 97331-2902

Unique DNA clones of the *Corynebacterium sepedonicum* genome (causal agent of potato ring rot) were made by generating a DNA genomic library of *C. sepedonicum*, subtracting out DNA by homologous binding of DNA from related species that are known to have common sequences, and subsequently cloning this unique DNA into a vector. *C. sepedonicum* DNA (tester DNA) was sheared, fractionated, and *EcoR*I adaptors were ligated to the blunt ends of the fragments. These fragments were allowed to hybridize (ratio 50:1) to a pool of biotinylated, *Hae*III-cut DNA from *C. michiganense*, *C. insidiosum*, and *C. fasciens* (driver DNA). Tester:driver hybrids and excess driver DNA were precipitated out using streptavidin, leaving DNA enhanced for unique tester DNA. Two more rounds of subtraction, with excess driver DNA enriched the target sequences 100-1000 fold. This unique *C. sepedonicum* DNA was amplified twice by PCR using a 16-mer synthetic primer, homologous to one strand of the *EcoR*I adaptor. The resulting DNA was then cloned into the TA cloning vector pCR1000 (Invitrogen). A *Not*I fragment of these clones, containing the insert, was used to probe a slot blot containing *C. sepedonicum* and five related species, *C. fasciens*, *C. insidiosum*, *C. michiganense*, *C. rathayi*, and *C. oortii*. Clones with specific homology to *C. sepedonicum* have been isolated and are presently being screened for specificity to strains of both *C. sepedonicum* and related species.

A774

DETECTION OF *STREPTOMYCES IPOMOEA* IN MIXED CULTURES, SOIL, AND SWEETPOTATO FIBROUS ROOTS WITH A SEROLOGICAL ASSAY. T. R. Weicht, J. W. Moyer and J. B. Ristaino. Dept. of Plant Pathology, North Carolina State University, Raleigh, NC 27695-7616.

Polyclonal antisera produced to *S. ipomoea* was cross-absorbed with a saprophytic *Streptomyces* species. Pure cultures, mixed cultures of *S. ipomoea* plus saprophytic *Streptomyces* spp., *S. ipomoea*-amended autoclaved soil, or macerated lesions were placed on nitrocellulose membranes that were overlaid on antibiotic-amended water agar. After incubation at 32 C for 7 days, colonies were probed with antisera conjugated with alkaline phosphatase. Antiserum was specific for many pathogenic isolates of *S. ipomoea* when tested against 14 saprophytic *Streptomyces* spp., but cross reacted with 3 isolates of *S. scabies*. *S. ipomoea* was also detected in mixed cultures grown on agar media. The number of colonies of *S. ipomoea* detected (y) in autoclaved soils were positively correlated with the expected (x) number of colonies ($\log(y) = -1.73 + 1.17\log(x)$, $R^2 = 0.95$). *S. ipomoea* was detected from 2-3 wk old lesions, but bacteria interfered with detection from older lesions. The technique will provide a useful tool for future ecological studies in soil.

A775

RFLP ANALYSIS OF ISOLATES OF *HELMINTHOSPORIUM SOLANI*. L. M. Delserone, K. M. Wilhelm, C. L. Merida, and R. Loria, Cornell University, Dept. of Plant Pathology, Ithaca, NY 14853

To evaluate the genetic diversity of *Helminthosporium solani* and to investigate the epidemiology of silver scurf of potato, restriction fragment length polymorphisms (RFLPs) have been identified for use in detection of specific isolates in soil and in plant tissue. An *Eco*RI genomic library of a characterized *H. solani* strain was constructed in pUC18. Repetitive sequences were identified in high stringency hybridizations with total *H. solani* DNA (Phytopath. 81:1239), and were screened against geographically diverse *H. solani* isolates. Some sequences indicated that isolates from the eastern US and Canadian provinces were genetically similar, as were a group of isolates from the western US and Canada. This geographical isolation of genotypes is consistent with the pattern of seed tuber production and distribution.

A776

FUSARIUM SPECIES ASSOCIATED WITH BANANA FRUITS ROT AND THEIR POTENTIAL TOXIGENICITY, R. F. Vesonder, A. Logrieco, A. Bottalico, C. Altomare, and S. W. Peterson, USDA, ARS, Mycotoxin Research Unit, Microbial Properties Research Unit, National Center for Agricultural Utilization Research, 1815 North University Street, Peoria, Illinois 61604

Banana fruits exhibiting signs of decay were collected from markets in the United States and Italy, and fungi were isolated from the lesions Fusarium moniliforme, F. subglutinans, and F. semitectum var. majus were the most commonly isolated species. When the isolated fungal strains were re-inoculated into bananas or inoculated on maize kernels, the cultures were negative for zearalenone, zearalenols and trichothecenes. No fumonisins, fusarin C or fusaric acids were detected in cultures made from bananas purchased in the U. S. Moniliformin (up to 267 mg/kg) was detected in maize kernel culture of F. subglutinans from bananas. No mycotoxins were detected in naturally infected banana fruits. Although no mycotoxins were detected in the extracts from corn cultures of F. subglutinans and F. semitectum var. majus, the extracts were toxic to brine shrimp and mice.

A777

MICROBIAL CONTAMINATION OF ALFALFA SPROUTS CAUSED BY SEED INFESTATION. Harold E. Moline, USDA, ARS, Horticultural Crops Quality Laboratory, Bldg. 002, Room 113, BARC-W, Beltsville, Maryland 20705-2350.

Poor shelf life of alfalfa sprouts was traced to a high level of seedlot contamination with Erwinia herbicola. Roots of sprouting alfalfa seeds turned brown and sprout containers developed foul odors. Bacterial exudate was evident on roots, although the bacteria did not kill the sprouts. Germination of seeds on water agar plates revealed more than 90 percent contamination with yellow pigmented bacteria subsequently identified as E. herbicola. Surface sterilization of seeds with sodium hypochlorite or ethanol reduced the level of contamination to 30 percent, but did not reduce the sprout problem. This high level of contamination was associated with bacteria carried inside the seed coat. Since the grower relied on surface sterilization of seeds to free this lot of contaminating bacteria, we had to recommend that the seed lot be discarded. There were no effective means to decontaminate it.

A778

SENSITIVITY OF GILBERTELLA PERSICARIA TO FUNGICIDES. C. Ginting, E. I. Zehr, and R. W. Miller, Department of Plant Pathology and Physiology, Clemson University, Clemson, SC 29634

Efficacy of six fungicides against Gibertella persicaria (Eddy) Hesseltine was evaluated in vitro on Synthetic Mucor Agar (SMA) at 26 C. Without fungicide amendment or with either fosetyl-Al (100 $\mu g\ ml^{-1}$) or triforine (400 $\mu g\ ml^{-1}$), the radius of fungal colonies was ca. 30 mm at 24 hr. At 4 $\mu g\ ml^{-1}$, dicloran or iprodione almost completely inhibited colony growth (<16 mm radius at 96 hr), and vinclozolin limited colony radius to 13 mm at 24 hr. Captan at 40 $\mu g\ ml^{-1}$ limited fungal growth to 13 mm radius at 24 hr. In all treatments sporangiospores germinated, but germ tubes swelled and lysed on medium containing effective concentrations of dichloran, iprodione, and vinclozolin. Stable, resistant variants were isolated from SMA plates containing iprodione (40 & 100 $\mu g\ ml^{-1}$).

A779

FUNGICIDAL ACTIVITY OF CHLORINE AGAINST GILBERTELLA PERSICARIA. C. Ginting, and E. I. Zehr, Department of Plant Pathology and Physiology, Clemson University, Clmeson, SC 29634

Efficacy of sodium hypochlorite (NaOCl) against Gilbertella persicaria (Eddy) Hesseltine was determined in vitro. NaOCl was diluted in phosphate buffer (pH 6.7-6.8) to prepare specific available chlorine concentrations determined by ferrous ammonium sulfate titration. One-ml suspension of 5×10^4 sporangiospores ml^{-1} was mixed in 49 ml chlorine solution for 5 min, neutralized with sodium thiosulfate, and 0.1 ml was plated on potato-dextrose agar supplemented with rosebengal (40 $\mu g\ ml^{-1}$), and streptomycin and chloramphenicol (60 $\mu g\ ml^{-1}$ each). After 2 days at 26 C, colony numbers were 22, 9, 7, and 0 at 0, 1, 2, and $\geq$ 4 $\mu g\ ml^{-1}$ available chlorine, respectively. A 20-min exposure at 1 and 2 $\mu g\ ml^{-1}$ resulted in 7 and 3 colonies, respectively. At 24 $\mu g\ ml^{-1}$ chlorine for 15, 60, or 120 sec, colony numbers were 10, 0.6, and 0, respectively. Temperatures between 1 and 21 C during a 20-min exposure at $\geq$ 2 μg ml-1 had no effect on chlorine activity.

A780

EFFICACY OF CURING CITRUS FRUITS FOR CONTROL OF GREEN MOLD IS ENHANCED BY SURFACTANTS. R. R. Stange, Jr., and J. W. Eckert. Department of Plant Pathology, University of California, Riverside, CA 92521.

Yellow lemons were injury-inoculated with *Penicillium digitatum* and incubated at 20 C for 22 h. All fruit were dipped in 100 ppm chlorine, rinsed with water, and then dipped for 30 s in either water, or a 0.01% (wt/vol) aqueous solution of Tergitol NP-10, Tween-20 , or sodium lauryl sulfate (SDS). Fruit were cured 18 h at 32 C. After 4 weeks in storage at 15 C percent decay was: water control, 20.9; Tergitol NP-10, 13.4; Tween-20, 8.9; and SDS 7.9. Decay reductions for Tween-20 and SDS were significant ($P \leq 0.05$). In other studies, deposition of resistance-associated compounds in injuries was not enhanced by Triton X-100 nor by chitosan, cellobiose, phosphonate, autoclaved citrus pectin, H_2O_2,or calcium chloride; hovever, *P. digitatum* (10^6 spores/mL) and crab-shell chitin (10mg/mL) did stimulate their accumulation.

A781

POSTHARVEST DECAY OF MUSKMELON IN RELATION TO PECTOLYTIC ENZYMES OF THREE FUNGAL PATHOGENS. *B. D. Bruton, USDA-ARS, Lane, OK 74555, W.S. Conway and K.C. Gross, USDA-ARS, Beltsville, MD 20705; and C.E. Sams, Univ. of Tenn., Knoxville, TN 37901.*

Muskmelon fruit tissue at 20, 30, 40 and 50 da postanthesis was subjected to partially purified pectolytic enzyme preparations (PE) from Diaporthe melonis (DM), Fusarium semitectum (FS), and Rhizopus stolonifer (RS) and a pectinase standard. The 50 da fruit were mature fruit (40 da) that had been stored for 10 da. The inner mesocarp was more susceptible to enzymatic maceration than the hypodermal/epidermal tissue (H/E) regardless of fruit age. Percentage maceration of H/E from PE of DM, FS, and RS, respectively, was 2, 13, and 36% on 40 da fruit and 21, 13, and 18% on 50 da fruit. Using the DM enzyme, very little tissue maceration was observed on 20, 30, and 40 day old fruit as compared to the other fungal enzymes. After 10 da storage, the DM enzyme macerated a greater percentage of fruit tissue than that of FS, and was similar to RS. DM, a quiescent infecting fungus, causes serious losses after harvest.

A782

VARIATION IN FUMONISIN LEVELS IN MAIZE HYBRIDS. R. A. Shelby[1], S. E. Holt[1], and D. G. White[2]. Department of Plant Pathology, [1]Auburn University, AL 36849, and [2]University of Illinois, Urbana, IL 61801.

Fumonisins are secondary metabolites of *Fusarium moniliforme* growing on maize which have been shown to be toxic to several species of livestock. A monoclonal antibody which indicates total fumonisins makes possible the rapid screening of large numbers of samples by competitive indirect immunoassay (CI-ELISA). This assay typically measures total fumonisins at approximately an 8-fold higher value than fumonisin B_1 measured alone by other methods. To test susceptibility to fumonisin contamination, 15 commercial corn hybrids were planted in a randomized complete block design with two replications at 18 locations in 11 states. Hybrids had mean total fumonisin concentrations ranging from 10 to 26 PPM. Mean total fumonisin for the locations ranged from .5 to 48 PPM. Significant differences (P=0.05) were detected among both hybrids and locations. Fumonisin concentrations tended to be higher at the more southerly locations.

A783

COMPARISON OF COMPETITIVE IMMUNOASSAY AND THIN-LAYER CHROMATOGRAPHY METHODS FOR ANALYSIS OF FUMONISIN IN MAIZE. R. A. Shelby[1], and G. E. Rottinghaus[2]. [1]Department of Plant Pathology, Auburn University, AL 36849, and [2]Veterinary Medical Diagnostic Laboratory, University of Missouri, Columbia 65201.

Grain samples from 214 individual plots of maize hybrids from 1991 Missouri yield trials were milled, divided into two subsamples, and analyzed for levels of fumonisin by different methods in two separate laboratories. The competitive immunoassay (CI-ELISA) which measured total fumonisins indicated an 8-fold higher fumonisin level on average than did reverse-phase thin-layer chromatography (RP-TLC) which measured only fumonisin B_1. Qualitatively, the methods agreed on presence or absence of fumonisin in 167/214 samples and disagreed in 47/210 samples. ELISA was positive for 31 negative TLC tests, where ELISA was negative for 16 positive TLC tests. Mean fumonisin levels were 0.5PPM measured by RP-TLC and 4.2 PPM measured by CI-ELISA. Generally, ELISA appears to be more sensitive, but detects other fumonisin-related molecular structures, which are not known at this time.

A784

CHARACTERIZATION OF PISATCHIO NUTS CONTAMINATED WITH AFLATOXIN. M. A. Doster and T. J. Michailides, Department of Plant Pathology, University of California, Berkeley/Kearney Ag. Center, Parlier 93648.

It has been shown previously that abnormal pistachio nuts with split hulls (early splits) frequently are contaminated before harvest by aflatoxins produced by Aspergillus flavus and A. parasiticus. We determined that between 16 and 69% of the early splits from various orchards had shrivelled hulls. A. flavus or A. parasiticus were isolated from 1.3% of the kernels from shrivelled early split nuts but from only 0.5% of the nonshrivelled early splits that had normal-appearing hulls. Shrivelled early splits had approximately 100 times more aflatoxin than nonshrivelled early splits, while no aflatoxins were detected in normal pistachios with intact hulls. We believe that shrivelled early splits split earlier in the summer than the others and so are exposed a longer time for the fungi to infect and produce aflatoxin. Also, a characteristic staining of the pistachio shell along the

suture was associated with 66% of the shrivelled early splits. The separation of pistachio nuts having shrivelled hulls or stained shells from normal nuts should be easy and would allow a reduction of aflatoxin in processed pistachio nuts.

A785

PRODUCTION OF GEOSMIN BY FUNGI IN STORED GRAIN AND IN CULTURE. D. B. Sauer and L. M. Seitz, USDA-ARS, U.S. Grain Marketing Research Laboratory, Manhattan, KS 66502.

Geosmin is a compound that imparts musty-earthy odors to drinking water, fish, and occasionally to other food products. It is produced mainly by certain aquatic or soil-inhabiting blue-green algae and actinomycetes, but has also been found in moldy or musty grain. Geosmin was produced in grain sorghum stored at 17% moisture and 25 C for 9 weeks and at 19% moisture for 4 weeks, with *Aspergillus candidus* being the dominant fungus in both cases. When pure cultures of *A. candidus* and other common grain storage fungi were grown on moist autoclaved rice or corn, only *Penicillium cyclopium* produced geosmin and strong earthy odors. Liquid cultures of the actinomycete *Streptomyces tendae* produced much higher levels of geosmin than did any of the grain storage fungi. When minimal media such as Czapek-Dox broth were supplemented with methionine, geosmin production was reduced and principal odor compounds were disulfides and trisulfides.

A786

INFLUENCE OF ITURIN A ON MYCELIAL WEIGHT AND AFLATOXIN PRODUCTION BY ASPERGILLUS FLAVUS AND ASPERGILLUS PARASITICUS IN SHAKE CULTURE. M. A. Klich, A. R. Lax, and J. M. Bland, USDA, ARS, Southern Regional Research Center, P.O. Box 19687, New Orleans, LA 70179.

Iturin A, a peptidolipid produced by Bacillus subtilis, inhibits growth of a large number of fungi. In this study, the effects of iturin A were evaluated on nine isolates of Aspergillus flavus and seven isolates of A. parasiticus in liquid shake culture. The mycelial dry weight of the A. flavus isolates was not significantly influenced by iturin A, however, there was a significant reduction in mycelial dry weight for two of the A. parasiticus isolates. Aflatoxin production was significantly reduced in five of the A. flavus isolates and three of the six aflatoxigenic A. parasiticus isolates. For the other seven isolates, aflatoxin levels were either unchanged or significantly increased in the presence of iturin A. These results indicate that iturin A does not consistently reduce growth or aflatoxin production of these fungi in pure culture.

A789

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

The nucleocapsid gene from a dahlia isolate of tomato spotted wilt virus (TSWV-L) was cloned from purified viral RNA using reverse transcriptase, and PCR amplified using two specific primers. *In vitro* translation revealed a 29 Kd protein similar in size to that found in extracts of infected plants. Immunoprecipitation with antiserum against the N protein confirmed this as coat protein. The gene was ligated into the vector pBI121 under the control of the CaMV 35S promoter. Shoot regeneration protocols were developed for three cultivars of chrysanthemum, Iridon, Hekla, and Polaris. *Agrobacterium tumefaciens* strain EHA105, a disarmed version of the broad host range strain A281, was used to transform the gene into cultivar Iridon. Kanamycin-resistant shoots were recovered and are being analyzed for gene expression and virus resistance.

A790

SUBLIMINAL INFECTION OF *SAINTPAULIA IONANTHA* BY TOBACCO MOSAIC VIRUS. M. A. Sulzinski, D.D. Jurkonie and C. S. Adonizio. Department of Biology, University of Scranton, Scranton, PA 18510.

Saintpaulia ionantha was previously reported to be immune to tobacco mosaic virus (TMV) infection. In this study, we mechanically inoculated fifteen cultivars of *S. ionantha* with 0.2 mg TMV/ml phosphate buffer. Two weeks post-inoculation, tissue was harvested and assayed for TMV infection by (a) bioassay on the local lesion host *Nicotiana tabacum* "Samsun NN"; (b) ELISA to detect TMV coat protein antigens; and (c) indirect immunofluorescence microscopy of *S. ionantha* separated mesophyll cells. There was evidence of limited TMV infection in each of the fifteen cultivars. Our results suggest that *S. ionantha* is not immune to TMV infection, and that this host undergoes subliminal infection by TMV.

A791

DETECTION OF COAT PROTEIN OF WHEAT SPINDLE STREAK MOSAIC VIRUS IS POSITIVELY CORRELATED WITH CHARACTERISTIC SYMPTOM EXPRESSION. J. E. Carroll[1], G. C. Bergstrom[1], and S. M. Gray[2]. [1]Dept. of Plant Pathology, and [2]USDA/ARS, Cornell University, Ithaca, NY 14853.

Serological detection of coat protein of wheat spindle streak mosaic virus (WSSMV) was used to assess the infection of wheat after inoculation with infectious soil containing the vector *Polymyxa graminis*. 'Augusta' wheat seeds were sown in pots of autoclaved, composted soil mix overlaying a thin layer of dried, WSSMV-infested, field soil. After seedling emergence, plants were maintained at 15 C (day)/7 C (night) for 1 wk. They were then removed, washed, transplanted into autoclaved, composted soil mix, and maintained for 10 wk under the same regime. Half of the pots were vernalized for 8 wk in an outdoor cold frame during winter and were then returned to the growth chamber for 4 wk. Virus-like symptoms occurred on vernalized and non-vernalized plants, although characteristic, chlorotic spindle streaks occurred only on vernalized plants. Coat protein detection by ELISA or western blotting was positively correlated with the presence and intensity of characteristic symptoms.

A792

CULTURED CELLS INFECTED WITH TOMATO SPOTTED WILT VIRUS USED FOR VIRUS AND VECTOR STUDIES. G. L. Schuster, R. S. Halliwell, F. L. Mitchell, and P. R. Porterfield. Texas Agricultural Experiment Station, Texas A&M University, College Station, TX 77843.

Peanut leaves testing positive for tomato spotted wilt virus (TSWV) were collected, surface sterilized and placed on a support medium to promote callus growth. Initial observations detected significant differences in the callus growth rate between healthy and diseased cultures, but after multiple subcultures, the growth differences were not as obvious. Subculturing was preformed at 4-week intervals and followed by enzyme-linked immunosorbent assays. The virus was detected in 80% of the cultures, with an average positive O.D. reading of 1.55 at 490 nm. Virus particles were observed in ultra thin sections (60-90 nm) in the outer layer of callus cells. The virus was rarely observed in the middle or bottom callus cells. The TSWV infected callus provides a convenient source of TSWV for serological studies. In addition, thrips (*Frankliniella* sp.) which are vectors of TSWV can be maintained temporarily on the infected callus cells (transferred to water agar) for virus acquisition studies.

A793

USE OF A POLYMERASE CHAIN REACTION (PCR)-BASED ASSAY FOR WHEAT SOILBORNE MOSAIC VIRUS (WSBMV) TO EVALUATE RESISTANCE IN HARD RED WINTER WHEAT (*TRITICUM AESTIVUM* L.). R. E. Pennington, J. L. Sherwood, and R. M. Hunger. Department of Plant Pathology, Oklahoma State University, Stillwater, OK 74078-9947.

A reverse transcription (RT)-PCR-based assay was developed for detection of WSBMV RNA in *T. aestivum*. Primers for cDNA synthesis and PCR amplification were designed based on the sequences of WSBMV RNAs 1 and 2 (Shirako and Wilson, personal communication). Sensitivity of the assay was highest using primers specific for the coat protein coding region of RNA 2.

The RT-PCR assay and ELISA were used to monitor WSBMV in resistant (Hawk and Newton) and susceptible (Vona and Sage) cultivars (cvs) in the field. By three months after planting, WSBMV was detected in all root samples, although at lower titers in resistant cvs, and in most foliar samples from susceptible cvs. WSBMV was not detected in foliage of resistant cvs. These results are consistent with previous evidence (Phytopathology 81:1216) that resistance is due to inhibition of systemic movement of the virus.

A794

COMPARISON OF BIOASSAY INDEXING AND ELISA FOR THE DETECTION OF GRAPEVINE LEAFROLL VIRUS IN GRAPEVINE SELECTIONS. A. Rowhani, D. A. Golino and M. Cunningham, Department of Plant Pathology, USDA-ARS and Foundation Plant Materials Service, respectively, University of California, Davis, CA 95616.

Results of the field index for grapevine leafroll disease on Vitis vinifera cv. Cabernet Franc was compared with the ELISA test developed for two different serologically distinct clostroviruses associated with the disease (GLRaV-types II and III). All the tested vines had different undetermined disease status. In 1989-1990,100 selections were tested: 46 had leafroll disease of which 19 were only on Cabernet Franc; 5 were only ELISA positive; and 22were positive by both tests. In 1990-1991, 91 candidates were tested: 36 were infected with leafroll disease of which 3 were positive only on Cabernet Franc; 4 were only ELISA positive; and the remaining 29 were positive by both tests. These results suggests that further work is needed on the efficiency of both tests.

A795

ENTRY OF INGESTED PLANT VIRUSES INTO THE HEMOCOEL OF THE PLANT VIRUS VECTOR, Diabrotica undecimpunctata howardii. **R. Y. Wang, R. C. Gergerich, and K. S. Kim. Department of Plant Pathology, University of Arkansas, Fayetteville, 72701.**

Some plant viruses are found in the hemocoel of beetle vectors after feeding on infected plants. Immunofluorescent and electron microscopy were used to detect the presence of ingested viruses in the hemocoel of the spotted cucumber beetle, Diabrotica undecimpunctata howardii. Southern bean mosaic virus and the cowpea strain of tobacco mosaic virus, both known to be present in the hemocoel, and bean pod mottle virus and tobacco ringspot virus that are not found in the hemocoel were studied. After a 24-h acquisition period on virus-infected leaves or on drops of purified virus, hemolymph was assayed on local lesion hosts for the presence of virus, and the fore-, mid- and hindgut of the beetles were prepared for microscopy. Virus was found only in the lumen of the gut for viruses which did not occur in the hemocoel. For beetles in which virus was recovered from the hemocoel, virus was found both in the lumen of the gut and in the epithelial cells of the midgut. This suggests that plant viruses enter the hemocoel through the cells of the midgut and not through the cuticle-lined fore- and hindgut.

A796

RETENTION OF TOMATO SPOTTED WILT VIRUS (TSWV) ANTIGEN BY CULTURED TOXORHYNCHITES AMBOINENSIS MOSQUITO CELLS AND INHIBITION OF VIRAL INFECTIVITIES IN PLANTS BY THE CELL EXTRACTS. H. T. Hsu, and T. R. Chen*, USDA-ARS, Beltsville, MD and *ATCC, Rockville, MD.

Fluorescent antibody staining revealed the presence of viral antigens in cultured mosquito cells inoculated with the NC4 strain (L-serotype) of TSWV. Inoculated cells fluoresced intensely during the first 4 days, but diminished notably thereafter. The fluorescence in these cells declined substantially when passaged once, and disappeared upon further subcultivations. Viral antigens were not detected in mosquito cells inoculated with the Igg strain (I-serotype) of TSWV. TSWV inocula (in 0.1 M histidine-0.01 M $MgCl_2$, pH 6.4) recovered from a 60 min inoculation of mosquito cells were highly infectious when tested in Nicotiana benthamiana. Extracts of mosquito cells inhibited TSWV infection in plants. About $10^{1.6}$ to 10^2 fold reduction in infectivity and a 5 to 7 day delay in local lesion development were observed when TSWV inocula (in 0.1 M phosphate-0.01 M Na_2SO_3, pH 7.2) contained a 10^{-3} dilution of cell homogenates.

A799

BEAN POD MOTTLE COMOVIRUS (BPMV): NEW HOSTS AND BEHAVIOR IN JACKBEAN (*Canavalia ensiformis* L.). J.M. Theuri and O.P. Sehgal, Department of Plant Pathology, University of Missouri, Columbia, MO 65211

New systemic hosts of BPMV include: Leguminosae - *Calapogonium cearalearum, Canavalia ensiformis, Macroptilium lathyroides, Phaseolus acutifolius, Trigonella foenum-graecum, Vigna aconitifolia, V. caracalla, V. vexillata, V. wilmsii;* Chenopodiaceae - *Chenopodium foetidum.* BPMV causes a mild systemic mottle on Jackbean leaves. The specific infectivity (SI) of BPMV isolated from inoculated primary Jackbean leaves at 48 days postinoculation (p.i.) was ca. 15% that of the virus isolated at 5 days p.i. This SI decline was correlated with a progressive breakdown of encapsidated RNA-1. These results are largely similar to BPMV behavior in *P. vulgaris* cv Pinto (a local lesion host) but the SI decline of BPMV in Jackbean leaves was considerably slower. The cause for this is unclear but an earlier onset of senescence in the infected Pinto leaves may have some bearing on this phenomenon.

A800

PRODUCTION OF TRANSGENIC CITRUS PLANTS EXPRESSING THE CITRUS TRISTEZA VIRUS COAT PROTEIN GENE. A. Gutiérrez-E., G.A. Moore, C. Jacono, M. McCaffery, and K. Cline. Horticultural Sciences Department, Institute of Food and Agricultural Research, University of Florida, Gainesville, FL 32611.

Citrus tristeza virus (CTV) is the most devastating citrus virus worldwide. Recent studies in a number of laboratories have shown that protection against a virus may be achieved by the introduction of the cloned viral coat protein (CP) gene into transgenic plants. Experiments are underway in our laboratories to enhance the frequency of *Agrobacterium*-mediated *Citrus* transformation and to produce transgenic plants expressing the CTV CP. *Agrobacterium* strain/vector plasmid combinations that allow efficient transformation of citrus have been identified and protocols for shoot regeneration have been improved. The CTV CP gene has been isolated, sequenced, and cloned into a custom engineered plant expression vector (JL Slightom, Gene 100:251-255). Transgenic plants expressing the CTV CP have been identified based on ß-glucuronidase expression, PCR analysis, and immunoblot analysis with antibody to the CP.

A801

MOLECULAR CHARACTERIZATION OF BEAN CALICO MOSAIC GEMINIVIRUS. A. O. Loniello[1], R. T. Martinez[1], M. R. Rojas[1], R. L. Gilbertson[2], J. K. Brown[3], and D. P. Maxwell[1]. [1]Univ. of Wisconsin, Madison, WI 53706, [2]Univ. of California, Davis, CA 95616, and [3]Univ. of Arizona, Tucson, AZ 85721.

Whitefly-transmitted geminiviruses from the Western Hemisphere have a ssDNA genome composed of two circular components, DNA-A and DNA-B. Bean calico mosaic geminivirus (BCMoV) was described by Brown et al. (Plant Dis. 74:81) in 1990 as a new whitefly-transmitted geminivirus from Sonora, Mexico. Symptoms on BCMoV-infected beans are similar to those caused by bean golden mosaic geminivirus (BGMV). Thus, it is important to determine if BCMoV is a new virus or a strain of BGMV. A polymerase chain reaction method was used to amplify a 1.1-kb fragment of DNA-A. This fragment was cloned and partial sequence of AL1 ORF was obtained. Nucleotide comparisons with other bean-infecting geminiviruses gave identities less than 80%. Three putative full-length clones (2.6 kb) of DNA-B were obtained. The Common Region sequence was determined for one of these DNA-B clones, and was 45%, 60%, and 61% identical to the Common Region sequences of a BGMV from Guatemala, BGMV from Brazil, and bean dwarf mosaic geminivirus from Colombia, respectively. The Common Region of BCMoV had the greatest nucleotide identity (86%) with squash leaf curl geminivirus isolate R from California. These results indicate that BCMoV is a distinct bean-infecting geminivirus.

A802

COMPARISON OF GENOME AFFINITIES AND EPITOPE PROFILES OF WHITEFLY-TRANSMITTED GEMINIVIRUSES. J.K. Brown, Dept. of Plant Sciences, Univ. of Arizona, Tucson, AZ 85721; M. M. Swanson, and B. D. Harrison, Scottish Crop Research Inst., Invergowrie, Dundee, Scotland, DD2 5DA UK.

The relationships between whitefly-transmitted geminiviruses from five different continents was assessed by DNA hybridization assay and/or in triple antibody ELISA with a panel of monoclonal antibodies. Differential hybidization profiles of virus isolates were generated from dot blot hybridization experiments using sulfonated viral DNA probes and stringent post-hybridization conditions (56C,0.1X SSC). Results were assessed visually and scored on a scale of 0 to 6 relative to intensity and limiting dilution of reactions. Epitope profiles for virus isolates were developed following ELISA with Mab's raised against capsid antigen of ACMV or ICMV. Panels of Mab's were selected based upon the ability to detect distinguishable and/or unique epitopes. In general, Mab epitope profiles, and hybridization profiles using DNA-1 component (or equivalent) probes both reveal greater similarities among American viruses than between the American viruses and those from other continents; hybridization tests with DNA-2 component probes are predominantly virus-specific. Reported percent similarities based on computerized alignment and comparison of available DNA sequence data generally corroborates these findings. These data suggest that for American geminiviruses, evolution may have either proceeded convergently from different progenitor viruses, or divergently from one ancestral form, with DNA-2 diverging to a greater extent than DNA-1 for bipartite geminiviruses.

A803

ISOLATION OF VIRUSES ASSOCIATED WITH CHERRY TWISTED LEAF, APRICOT RING POX, AND APRICOT PIT POX DISEASES AND THEIR RELATIONSHIP TO APPLE STEM PITTING VIRUS. Y. P. Zhang[1], G. I. Mink, M. G. Tiffany, and W. E. Howell, Departments of Plant Pathology, [1]University of California, Davis, CA 95616, and Washington State University, Prosser, WA 99350.

Twisted leaf disease in sweet cherry and ring pox and pit pox diseases in apricot are found almost exclusively in stone fruit orchards located primarily in the state of Washington and British Columbia. None of the causal agents had been isolated, but each appears to spread by unidentified vectors. Recently, we sap-transmitted viruses from each of these diseased trees to two selections of Nicotiana occidentalis. The diseased source trees were previously tested to have no other known viruses except sour cherry green ring mottle virus which was not isolated in parallel experiments. Based on symptoms on the herbaceous hosts and the dsRNA species purified from woody hosts (cherry and apricot) and the inoculated herbaceous hosts, the three virus isolates appear to be similar. All three stone fruit virus isolates reacted positively with antiserum prepared to apple stem pitting virus (ASPV) in ELISA tests, and dsRNA profiles of the stone fruit virus isolates and ASPV are similar. These results suggest that ASPV and viruses from the stone fruit trees are related.

A804

ELECTROPORATION MEDIATED TRANSFORMATION OF *PHYTOPHTHORA*. R. S. Redman and R. J. Rodriguez. Department of Plant Pathology, University of California, Riverside, CA 92521.

Growing hyphal tips of the filamentous fungi *Phytophthora palmivora* and *Phytophthora megasperma* were transformed by electroporation with the plasmid pHA1.3 which confers hygromycin resistance. Growing hyphal tips were mechanically damaged with glass beads and electroporated at a constant capacitance of 25 μF (micro-Farad) with varying voltage and resistance ranges. In addition, the effects of temperature, plasmid DNA concentration on transformation efficiency and stability of transformants were also investigated. Transformation efficiencies as high as 300 transformants/μg DNA were observed in *P. palmivora*. A general protocol for the stable, electroporation mediated transformation of *Phytophthora* will be given.

A806

FUNCTIONAL ANALYSIS OF THE *COCHLIOBOLUS HETEROSTROPHUS* MATING TYPE LOCUS BY GENE DISRUPTION. S. K. Christiansen, O. C. Yoder and B. G. Turgeon. Dept. of Plant Pathology, Cornell University, Ithaca, NY 14853

Mating type in *C. heterostrophus* is determined by one locus with two idiomorphs (*MAT-1* and *MAT-2*), which have been cloned and sequenced. Gene disruptions were done to identify functional ORFs and to determine the phenotype conferred by each gene. For *MAT-2*, an internal fragment of the idiomorph was cloned and transformed into a*MAT-2* strain. Southern analysis of 6 transformants showed 2 ectopic and 4 homologous integration events. Transformants with ectopic integrations were fully fertile whereas those with homologous integrations were sterile, indicating that the product of the predicted ORF is necessary for sexual development. Mating ability of sterile transformants was restored by transformation with a wildtype copy of *MAT-2*. Race T (T-toxin producing) strains with *MAT-2* disruptions, although sterile, were still fully virulent on corn, indicating that disruption of the *MAT-2* gene in this fungus has no effect on either pathogenicity or ability to produce T-toxin. Similar experiments with *MAT-1* are in progress.

A807

INTERSPECIFIC CROSSING BY MANIPULATION OF MATING TYPE GENES IN *COCHLIOBOLUS*. S. K. Christiansen, O. C. Yoder and B. G. Turgeon. Dept. of Plant Pathology, Cornell University, Ithaca, NY 14853

We have cloned mating type genes from *C. heterostrophus, C. carbonum,* and *C. victoriae*. In all three, mating type is determined by a single locus (*MAT*) with two alternate forms (idiomorphs) *MAT-1* and *MAT-2*. Cross hybridization and sequence analyses have revealed that the genes are highly similar among the three, yet these species cannot normally be crossed with each other and are pathogens of different hosts. One of our goals is to cross fungal species which parasitize different plants and obtain progeny in which genes for different pathogenic specificities are segregating. Toward this end, we have shown that when an idiomorph from any of the 3 species is transformed into a *C. heterostrophus* strain of opposite mating type, the recipient is homothallic and a dual mater, indicating that *MAT* genes are interchangeable among species. Furthermore, when the *MAT-1* gene of *C. carbonum* is transformed into *C. heterostrophus*, the transformant is able to cross with a *C. carbonum MAT-2* strain. This suggests that it may be possible to produce interspecific progeny by manipulation of *MAT* genes.

A808

AN ELECTROPHORETIC KARYOTYPE OF FUSARIUM MONILIFORME. Keying Yan and M. B. Dickman, Department of Plant Pathology, University of Nebraska, Lincoln, NE 68583-0722.

An electrophoretic karyotype of Fusarium moniliforme has been obtained using contour clamped homogeneous electric field (CHEF) gel electrophoresis. Eleven chromosome bands were clearly resolved, one of which may be a doublet. The estimated sizes of these molecules based on migration relative to Saccharyomyces cerevisiae and Schizosaccharomyces pombe ranged from 0.7 to 6.9 megabases, with a total genome size of approximately 41 megabases. DNA blot analysis was used to develop a preliminary physical map with both heterologous and homologous probes. In addition, karyotypes of six F. moniliforme strains representing diverse geographical locations and differing in host preference were compared. No obvious chromosome polymorphisms were detected. An established karyotype will assist our continuing studies of stability and movement of foreign DNA in fungi at the chromosome level.

A809

A TRANSFORMATION SYSTEM FOR *SCLEROTINIA SCLEROTIORUM*. K.M. Pilskalns and D.C. Sands, Department of Plant Pathology, Montana State University, Bozeman, Montana, 59717.

A transformation system was developed for *Sclerotinia sclerotiorum*, a plant pathogenic fungus currently being developed as a bioherbicide. To our knowledge, this is the first report of transformation of this fungus. Low frequency transformation was achieved by polyethylene glycol/$CaCl_2$ precipitation with vector DNA which was complexed with cationic liposomes. The vector used was pDH25 which carries the hygromycin B phosphotransferase gene flanked by *Aspergillus nidulans* trpC terminator and promotor sequences. The mitotic and meiotic stability of the fungal transformants is being investigated along with ways of increasing transformation frequency.

A810

GENETIC TRANSFORMATION AND DETECTION OF CONIFER ROOT ROT PATHOGEN *FUSARIUM OXYSPORUM* F. SP. *PINI*. D.A. Varley[1], J.N. Bruhn[2], and G.K. Podila[1], [1]Dept. of Biological Sciences and [2]School of Forestry and Wood Products, Michigan Technological University, Houghton, MI, 49931.

Fusarium oxysporum f. sp. *pini* Schlecht. emend Snyd. & Hans. is an important root rot pathogen of conifer seedlings. While chemical methods are used to control this pathogen, their efficacy is questionable. Alternative preventive methods of control are needed. We are testing the possibility of using altered strains of *F. oxysporum* f. sp. *pini*, as a means of biocontrol of root rot of conifer seedlings. In order to use altered strains of *F. oxysporum* f. sp. *pini*, we need methods to monitor and distinguish between altered and wild type strains of *F. oxysporum* f. sp. *pini*. We have developed a protoplast transformation system for *F. oxysporum* f. sp. *pini*. Hygromycin B resistance gene was used as a selection marker, while ß-glucuronidase (GUS) and firefly luciferase (LUX) genes were used as reporter genes to transform *F. oxysporum* f. sp. *pini*. Using the marker and reporter genes, we have developed a sensitive and rapid screening process to monitor the altered strains of *F. oxysporum* f. sp. *pini*.

A812

COTRANSFORMATION OF THE SOYBEAN FUNGAL PATHOGEN *CERCOSPORA KIKUCHII*. R. G. Upchurch, USDA/ARS, Plant Pathology, North Carolina State Univ., Raleigh, NC 27695-7616.

Wild-type, cercosporin-plus and cercosporin-minus mutant strains of the soybean fungal pathogen *Cercospora kikuchii* have been successfully cotransformed with the benomyl resistance-conferring plasmid pCKB4 and a plasmid containing the *E. coli* β-glucuronidase gene (*gusA*). Approximately one-quarter of the transformants initially selected on benomyl had β-glucuronidase (GUS) activity and could be selected directly on medium containing the substrate 4-methyl-umbelliferyl-β-D-glucuronide (MUG). Both markers were stably maintained after eight consecutive transfers on medium without benomyl. The number of copies and sites of *gusA* insertion into the genome varied among the transformants. The analysis of *gusA* expression during infection of soybean by *C. kikuchii* will be presented.

A814

SEXUAL REPRODUCTION IN Pythium sylvaticum; CHROMOSOMAL REARRANGEMENTS AND INHERITANCE OF MOLECULAR MARKERS. F.N. Martin, Plant Pathology Department, University of Florida, Gainesville, FL 32611.

Sexual reproduction in P. sylvaticum has been investigated by crossing opposite mating types that contain differences in chromosomal sized DNA (separated by CHEF electrophoresis) and a number of molecular markers. While both isolates had the same apparent number of chromosome sized DNAs (eleven), differences in their relative sizes were observed. F_1 and back crossed progeny contained primarily mixtures of parental karyotypes, but some isolates also contained unique bands not present in the parents. Molecular markers for RFLP analysis were constructed from portions of the ribosomal DNA, random clones of the nuclear DNA, and random amplified polymorphic DNAs. The relationship between the inheritance of these markers and chromosomal rearrangements will be discussed.

A815

CHARACTERIZATION OF THE *NAR* GENE ASSOCIATED WITH AFLATOXIN B1 BIOSYNTHESIS IN *ASPERGILLUS PARASITICUS*. P.K. Chang, F. Trail, R. Rasooly and J. Linz, Michigan State University, East Lansing.

Aspergillus parasiticus produces the toxic secondary metabolite, aflatoxin. Colonization of several economically important crops by the fungus results in aflatoxin contaminated food supplies. An understanding of the regulation of aflatoxin production will be used in developing novel control methods. An early step in the biosynthetic pathway of the toxin is the conversion of norsolorinic acid to averantin. Previously, the *nar* gene was isolated from *A. parasiticus* by complementation of an aflatoxin-deficient, norsolorinic acid accumulating strain to aflatoxin production, using nitrate reductase as a selectable marker. The *nar* gene has now been sequenced and the introns and upstream regulatory elements localized. The role of the *nar* gene in aflatoxin biosynthesis is currently being studied by gene disruption. The functional organization of the *nar* promoter region will be examined by fusion to a reporter gene.

A817

ELECTROPHORETIC KARYOTYPE FOR COLLETOTRICHUM GLOEOSPORIOIDES F.SP. AESCHYNOMENE. C.R. Cisar and D.O. TeBeest. Department of Plant Pathology, University of Arkansas, Fayetteville, AR 72701.

Chromosomal-sized DNA molecules from a laboratory strain of Colletotrichum gloeosporioides f.sp. aeschynomene were prepared using a modification of the method of Orbach et al. (Mol. Cell. Biol. 1988, 8:1469-73) and separated by clamped homogeneous electric field electrophoresis (CHEF). The DNA was transferred to nitrocellulose and hybridized with a variety of molecular probes. The probes include a cutinase gene from Colletotrichum gloeosporioides, a ß-tubulin gene from Neurospora crassa, a ribosomal protein gene, S14, from chinese hamster, a rRNA gene repeat from N. crassa, a glutamate dehydrogenase gene from N. crassa, a glyceraldehyde-3-phosphate-dehydrogenase gene from Glomerella cingulata and randomly selected clones from a Glomerella cingulata/Colletotrichum gloeosporioides cDNA library. The mitochondrial chromosome is also identified using a mitochondrial DNA probe from Colletotrichum orbiculare.

A818

CLONING AND SEQUENCE OF A BAMHI FRAGMENT OF TILLETIA CARIES IDENTIFIED BY HOMOLOGY WITH THE B "WEST" MATING TYPE GENE OF USTILAGO HORDEI. B. W. Russell and D. Mills, Department of Botany and Plant Pathology, Oregon State University, Corvallis 97331-2902.

Fungal mating type genes, or idiomorphs, are highly conserved within a species but may also have sequence homology with idiomorphs from closely related species. Hence, comparison of the nucleotide sequences of the idiomorphs of Tilletia caries and T. controversa may be useful in determining their taxonomic relationship. BamHI-digested total genomic DNA of T. caries was probed with the 1.4 Kb insert from pBH101 contained part of the b "west" mating type gene of Ustilago hordei. A single 1.4 Kb fragment which hybridized with the probe was identified and cloned from T. caries. The T. caries fragment had homology with a 1.4 Kb BamHI fragment of T. controversa as well. Unexpectedly, this clone hybridized with 2 to 3 chromosome 2bands when probed onto blots of CHEF separated chromosomes of both T. caries and T. controversa. The 1.4 Kb fragment is currently being sequenced.

A819

ELECTROPHORETIC KARYOTYPES OF *GIBBERELLA FUJIKUROI* MATING POPULATIONS A-F. J.-R. Xu & J. F. Leslie, Department of Plant Pathology, Kansas State University, Manhattan, Kansas 66506-5502.

Gibberella fujikuroi can be divided into six different mating populations with anamorphs in *Fusarium moniliforme*, *Fusarium subglutinans*, and *Fusarium proliferatum*. We have resolved chromosomes of 24 representative strains using contour-clamped homogeneous electric field (CHEF) gel electrophoresis. These strains all have 12 chromosomes ranging in size from 0.8-12 Mb. Putative doublet bands have a higher density and can hybridize to more numerous random RFLP probes than do bands that represent single chromosomes. Each mating population has a distinct karyotype that is different from the karyotype of the other mating populations. Among these strains, strains within a mating population have a uniform karyotype. The estimated genome size for mating populations A, B, D & F is 45-50 Mb, and 50-55 Mb for mating populations C & E. When Southern blots are probed with random RFLP probes derived from strain A-00102, high levels of deletion and genome reorganization can be detected between strains belonging to different mating populations.

A820

SURVEY OF DOUBLE STRANDED RNA FOUND IN *OPHIOSTOMA* SPECIES. S. Iverson, T. Harrington, W.C. Zimmerman, P. Wendler, and R.L. Farrell, Repligen Sandoz Research Corporation, Lexington, MA and Department of Plant Pathology, Iowa State University, Ames, IA.

Isolates of the Ascomycete, *Ophiostoma piliferum*, from Virginia, U.S.A. and S. Carolina, U.S.A., *O. piceae* from Portugal, Finland, Tasmania, Australia, and Virginia, U.S.A., *O. pluriannulatum* from Virginia, U.S.A. and South Carolina, U.S.A. and *O. ips* from S. Carolina, U.S.A. were grown in stationary flasks containing malt yeast extract media. Double stranded RNA (dsRNA) was extracted directly from mycelia with a glycine-sodium phosphate buffer and phenol:chloroform. It was further purified by cellulose chromatography. dsRNA was confirmed by RNase and DNase digestion and gel electrophoresis. Four of ten *O. pilifera* isolates and four of twenty *O. piceae* isolates examined contained dsRNA. No dsRNA was detected in the seven *O. ips* isolates examined or the two *O. pluriannulatum* isolates examined. All isolates positive for dsRNA exhibited different banding patterns with the number of bands ranging from two to nine per isolate. Results will be presented contrasting strains containing dsRNA versus those without as to growth in culture and other characteristics.

A821

ESTIMATION OF GENETIC VARIABILITY AMONG RACES OF *PHYTOPHTHORA MEGASPERMA* F. SP. *GLYCINEA* (PMG) USING RANDOMLY AMPLIFIED POLYMORPHIC DNA. T. E. Chase and Z. Liu. Plant Science Department, South Dakota State University, Brookings, SD 57007.

Phytophthora megasperma f. sp. *glycinea* (Pmg), the causal agent of root rot in soybean, consists of at least 25 physiological races. To estimate genetic variability of Pmg races, the Randomly Amplified Polymorphic DNA (RAPD) assay is being used to generate genome specific patterns of DNA fragments. A set of 7 primers has been found to detect DNA polymorphisms among 14 Pmg races and some South Dakota isolates. PCR amplification with primer A13 (Operon Technologies) divides 14 Pmg races into 4 groups. Most combinations of two different primers in a single amplfication generated DNA fragments that were not produced when each primer was used separately. Preliminary results indicate that RAPD will be useful for molecular genetic studies of Pmg races.

A822

IDENTIFICATION OF WHEAT SEPTORIA FUNGAL PATHOGENS BY SIMPLE DNA HYBRIDIZATION. P.P. Ueng, E.A. Geiger and G.C. Bergstrom* USDA-ARS, BARC-West, Plant Molecular Biology Lab., Beltsville, MD 20705 and *Department of Plant Pathology, Cornell University, Ithaca, NY 14853.

Mycosphaerella graminicola (Anamorph Septoria tritici), Phaeosphaeria nodorum (Anamorph Stagonospora nodorum, Septoria nodorum) and Septoria avenae are important leaf fungal pathogens in wheat. Species can be readily distinguished by DNA hybridization. With genomic DNA isolated from fungal mycelia as probes, these species could be specifically identified by dot blot DNA hybridization. Restriction fragment length polymorphism (RFLP) profiles of these fungi conferred distinct multiple band patterns when a hypervariable tandemly repeated sequence from bacteriophage M13 was used as a probe. The unique distribution of major bands in RFLP profiles were characteristic among these Septoria species. With some endonuclease enzyme restrictions, additions or deletions of certain minor band(s) in RFLP profiles could be used to identify different isolates of the same fungal species.

A823

MOLECULAR VARIATION IN AUSTRALIAN ISOLATES OF RHIZOCTONIA SOLANI. P.A. O'Brien, H. Yang#, J.E. Barton, and K. Sivasithamparam#. School of Biological and Environmental Sciences, Murdoch University, Murdoch WA 6150 Australia. #Department of Soil Science and Plant Nutrition, University of Western Australia, Nedlands WA 6009, Australia.

Isolates of Rhizoctonia solani Kuhn were analyzed by RFLP analysis using random cloned fragments as probes, by RAPD-PCR, and by plasmid content. All of the anastomosis groups (AG), and pectic zymogram groups could be identified by RFLP, and RAPD-PCR analysis. For some groups (e.g., AG2), there was a high level of variability between the isolates, whereas in other groups (e.g., AG8) there was very little variation between the isolates. Plasmids were detected in 30-50% of isolates analyzed. Isolates from the same group, and the same geographical region had the same plasmids, whilst isolates from the same group and different regions had different plasmids. The plasmids are not homologous to sequences in the nuclear or mitochondrial genomes.

A824

AMPLIFICATION OF SPECIES-SPECIFIC PHYTOPHTHORA DNA SEQUENCES BY PCR. J. Schoelz, T. Ersek, and J. English, Dept. of Plant Pathology, University of Missouri, Columbia 65211.

Interactions among Phytophthora spp. colonizing common host tissues are difficult to evaluate because of morphological similarities and possible parasexual events among species. To overcome these constraints, previously developed species-specific DNA fragments from P. parasitica and P. citrophthora were cloned and sequenced. Oligonucleotide primer sequences thereby determined were used to amplify species-specific DNA sequences by the polymerase chain reaction (PCR) techniques. Electrophoretic patterns of PCR products enabled distinction between these two species to be made. These primers are being evaluated for their abilities to amplify, and distinguish between, DNA of these Phytophthora spp. in co-colonized plant tissues.

A825

USE OF PCR FOR DETECTION OF MYCOSPHAERELLA FIJIENSIS AND M. MUSICOLA, THE FUNGI CAUSING SIGATOKA LEAF SPOTS, IN BANANA LEAF TISSUE. A. Johanson & M. J. Jeger, Natural Resources Institute, Chatham Maritime, Chatham ME4 4TB, United Kingdom.

Two forms of Sigatoka leaf spots affect bananas and plantains. Yellow Sigatoka is caused by the fungus *Mycosphaerella musicola* and black Sigatoka by *M. fijiensis*. The fungi can be difficult to isolate from plant material, and it is often not possible to distinguish between the diseases by symptoms or fungal morphology alone. DNA sequence information of the transcribed spacer regions between the mature 18S and the 28S rRNA subunits from several *Cercospora* species was used to design oligonucleotide primers which would produce a specific product when fungal DNA was amplified by the polymerase chain reaction (PCR). A species-specific DNA product was also produced when infected plant material was amplified using these primers. This method does not require culturing of the fungus and has potential use for diagnosis and monitoring, particularly in areas where the black Sigatoka pathogen is not yet present.

A827

THE EFFECT OF FERTILIZER ON ARMILLARIA INFECTED LODGEPOLE PINE GROWN IN THE GREENHOUSE. K.I. Mallett and D.G. Maynard, Forestry Canada, Northern Forestry Centre 5320, 122 St. Edmonton, Alberta, T6H 3S5.

A greenhouse experiment was conducted to determine the effect of differing levels of nutrient concentration on infection of lodgepole pine by Armillaria ostoyae. Six-month-old lodgepole pine trees were inoculated with two different isolates of A. ostoyae and were fertilized with either full- or quarter-strength Hoagland's solution. The nutrient concentration (mg/kg dry weight basis) in the roots, shoots, and leaves were determined in the infected and healthy trees ten months after inoculation. There were more Armillaria-infected trees in the full-strength treatment than in the quarter-strength treatment; however, there was the same amount of mortality in the two treatments. There was a higher concentration of nitrogen, phosphorus, and sulfur in the needles of the trees treated with full-strength Hoagland's solution compared to the quarter-strength treated trees. Higher concentrations of potassium, sodium, and phosphorus were found in the stems and needles of infected trees compared to the healthy trees regardless of the fertilizer treatment. There was no difference in the concentrations of nitrogen, sulfur, calcium, magnesium, iron and aluminium in the infected compareded to the healthy trees.

A828

THE BIOCHEMICAL TAXONOMY OF AFRICAN *ARMILLARIA*. C. Mohammed, Oxford Forestry Institute, South Parks Road, Oxford, OX1 3RB, United Kingdom

Studies at Oxford have concentrated on the taxonomic investigation of African *Armillaria* species using various biochemical and molecular techniques: protein, isoenzyme and DNA analysis (Southern blotting and hybridisation, Random Amplified Polymorphic DNA (RAPD)). Analysis of the 60 West, Central, East and South African *Armillaria* isolates available at the beginning of 1991 has resulted in the definition of 3 main biological groups and several sub-groups. This biochemical taxonomic classification corresponds closely with results from parallel taxonomic investigation and species characterisation by researchers using other methods (observation of vegetative mat morphology in culture, fruiting in culture, ability to fruit, morphology of fruitbodies, study of monospore isolates, pairing tests).

A829

ARMILLARIA ROOT DISEASE IN TEA PLANTATIONS IN KENYA. Philip M. Wargo, USDA Forest Service, Hamden, CT 06514 and James M. Onsando, Tea Research Foundation of Kenya, Kericho, Kenya.

Armillaria root disease causes significant damage in tea plantations in Kenya and is especially severe in recently established small farm plantations of 0.5 to 1.5 ha in size. Severity is related to land status prior to conversion to tea and is greatest on former forest land. Spread occurs primarily by direct root contact with infected roots of former trees (primary inoculum) or other tea roots (secondary inoculum). Infection from rhizomorphs occurs infrequently. Increased damage in these young plantations may be related to infection from secondary inoculum. Younger plantations are clonal and the genetic similarity of susceptible adjacent plants enhances spread. Because older plantations are derived from seed, adjacent plants may not be genetically similar and therefore may differ in susceptibility. Both A. heimii and A. mellea have been isolated from tea, but the taxonomy of the major disease causing species has not been determined.

A830

CLONAL DEVELOPMENT OF *ARMILLARIA* SPECIES IN SEVERAL FOREST TYPES. J.J. Worrall, SUNY College of Environmental Science and Forestry, Syracuse NY 13210.

In several forest types of the Northeast, the size and distribution of *Armillaria* clones was assessed by somatic incompatibility tests among isolates from roots or rhizomorphs. *Armillaria calvescens*, *A. ostoyae*, *A. gemina* and *A. bulbosa* were represented in the study. The greatest distances between isolates of any one clone were 44 m and 39 m for *A. ostoyae* and *A. gemina*, respectively, but most clones were apparently much smaller. Clone territories abutted and interdigitated in some cases but overlapped only at their edges. When pairs of isolates were obtained from the wood vs. superficial rhizomorphs of a tree, they were usually the same clone. However, in a few cases they were different clones or even different species of *Armillaria*. In spruce-fir forests, canopy gaps partly attributable to Armillaria root rot often contained more than one clone. The numerous small clones and a comparison of clone status with stand history are consistent with the concept that sexual reproduction is, in some cases, an epidemiologically important part of the life cycle.

A831

VEGETATIVE INCOMPATIBILITY AND SEXUAL SYSTEMS OF *ARMILLARIA* FROM TROPICAL AFRICA. Guillaumin J.J.,[1] Abomo-Ndongo S.,[1] Mohammed C.[2]
[1] Centre INRA de Clermont-Ferrand, Unité de Mycologie, 12 Avenue du Brézet, 63039, Clermont-Ferrand, France
[2] Oxford Forestry Institute, South Parks Road, Oxford, OX1 3RB, United Kingdom

The results of culture pairing tests with 25 isolates (originating from 15 different African countries) divided them into 4 groups: i) *Armillaria heimii* (syn. *A.fuscipes*) *sensu lato* - a large group (17 isolates) from Central, West and East Africa, ii) a small but distinct group of 4 isolates - the African form of *A.mellea*, iii) 3 isolates from high altitude Kenya, iv) 1 isolate (also from high altitude Kenya). Isolates of groups 1 and 2 fruited in culture and single spore isolates were obtained from the fruit bodies. Single spore isolates *A.heimii* of West and Central African origin all had a cottony haploid-like morphology (well known in European *Armillaria*). In pairing tests single spore isolates of different geographical origin were compatible giving rise to a crustose isolate typical of a diploid. Pairings between haploid isolates from a same fruit body suggest a heterothallic bipolar sexual system. Single spore isolates from both *A.mellea* and *A.heimii* of East African origin were crustose. This and other research (including cytological studies) has led the authors to propose the existence of homothallism in African *Armillaria*.

A832

FRUITING IN CULTURE OF *ARMILLARIA* ISOLATES FROM TROPICAL AFRICA. Intini M.,[1] Guillaumin J.J.,[2] Abomo-Ndongo S.,[2] Mohammed C.[3]
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[2] Centre INRA de Clermont-Ferrand, Unité de Mycologie, 12 Avenue du Brézet, 63039, Clermont-Ferrand, France
[3] Oxford Forestry Institute, South Parks Road, Oxford, OX1 3RB, United Kingdom

Twenty nine *Armillaria* isolates from 15 different African countries were included in artificial fruiting trials in both Clermont-Ferrand, France and Florence, Italy. These isolates belonged *a priori* to 3 main groups: i) the African form of *A.mellea*, ii) *A.heimii sensu lato* (a large group with considerable variability), iii) isolates from the highlands of Eastern Africa different to those of the two previous groups. Different substrates and environments were tested. 23 out of the 29 isolates have fruited in culture. These isolates all belong to the *A.mellea* or *A.heimii* groups. No isolates of the Eastern highlands group have fruited. African *A.mellea* fruit bodies have a morphology closely resembling that of the prominently ringed, honey coloured European *A.mellea sensu stricto*. *A.heimii* fruit bodies obtained in culture can be very variable although distinguishable from that of *A.mellea* isolates by the poor development or absence of a ring and a more delicate cap speckled with scales or warts. The most typical morphology seen when fruiting isolates of this *A.heimii* group does conform with the description of *A.heimii* given by Pegler.

A833

RAISING MONOCLONAL ANTIBODIES TO EUROPEAN SPECIES OF *ARMILLARIA*. C. Mohammed, R. Priestley, F.M. Dewey, Department of Plant Sciences, South Parks Road, Oxford, OX1 3RB, United Kingdom

The most currently used method of species identification for the genus *Armillaria* is based on the somatic incompatibility shown in pairing tests between isolates of different species. The method is fairly long and tedious and can be a victim of subjective interpretation. The development of an immunologically based user friendly identification kit, both objective and rapid, would greatly facilitate *Armillaria* research. Specific proteins for *A.mellea* and *A.ostoyae*, revealed on SDS PAGE gels, have been excised, treated for the removal of SDS and injected into mice. The monoclonal antibodies raised have been screened against antigens from all European species of *Armillaria* and from other fungal genera, including the most common wood rotting fungi. MAbs have been found which are i) genus specific and ii) *A.ostoyae* specific.

A834

EPIDEMIOLOGY OF ARMILLARIA ROOT DISEASE IN RED PINE PLANTATIONS. J.N. Bruhn, J.B. Pickens, and [1]J.D. Mihail, School of Forestry and Wood Products, Michigan Technol. Univ., Houghton, MI 49931 and [1]Dept. of Plant Pathol., Univ. of Missouri, Columbia, 65211.

A complete census of Armillaria root disease in three red pine (*Pinus resinosa*) plantations has been conducted since symptoms were first observed in 1986. Each study site was divided into 12 sub-plots (500 m^2). Disease progress was characterized by the monomolecular function (29 of 36 subplots) or the Gompertz function (7 of 36 subplots). Cumulative air temperature degree days was used as a surrogate for elapsed time because of the temperature dependency of biological activity and the long winters in the study area. Clones of *A. ostoyae* have been identified by vegetative confrontation of isolates derived from symptomatic trees and from stump basidiomata. Maps of *A. ostoyae* clones suggest little overlap. Thus it is possible to compare rates of disease progress within individual clones. Plantation-wide disease progress rates can differ greatly from rates determined for individual clones.

A835

DELINEATION OF CLONES OF *ARMILLARIA OSTOYAE, A. CALVESCENS* AND *A. GEMINA*. D. M. Rizzo and T. C. Harrington. Depts. of Plant Pathology, Univ. of Minnesota, St. Paul, 55108 and Iowa State Univ., Ames, 50011.

Diploid isolates of *Armillaria* were collected from a 50 X 25 m plot at two sites in the White Mountains, New Hampshire to identify clones. All living and dead trees were examined for rhizomorphs, mycelial fans and decay. Isolates were identified to species using diploid-haploid pairings and to clones using both somatic incompatibility and four isozyme markers. At a predominantly spruce-fir site, only *A. ostoyae* was identified; at least 18 of the 40 sampled trees were pathogenically colonized. Isolates from the 40 trees were divided into six clones; the largest clone had colonized 17 trees. At a mixed conifer-hardwood site, another six clones of *A. ostoyae* were identified among isolates from 32 trees; at least 13 of the trees were pathogenically colonized. The largest clone occupied an area at least 25 m diam. and colonized 14 trees. *Armillaria calvescens* (29 trees) and *A. gemina* (31 trees) were also isolated from the conifer-hardwood site, mostly from epiphytic rhizomorphs on hardwoods; isolates of these species were each divided into two clones. At both sites, there was little intermingling of clones of the same species; however, clones of different *Armillaria* species overlapped considerably.

A836

CULTURAL CHARACTERISTICS AND BASIDIOME MORPHOLOGY OF *ARMILLARIA GALLICA AND A. OSTOYAE* FROM KOREA. Jae Mo Sung, Jo Young Cha, and T. C. Harrington. College of Agriculture, Kangweon National University, Chuncheon 200-701, Korea, and Department of Plant Pathology, Iowa State University, Ames 50011.

We have identified three species of *Armillaria* (*A. ostoyae, A. gallica* and *A. mellea*) in Kangweon Province, Korea through haploid-haploid and haploid-diploid pairings with North American and European tester strains. Basidiome tissue and single-basidiospore isolates were obtained from 18 basidiomes collected from nine forest sites on 11 species of hardwoods and two species of conifers, including *Pinus koraiensis*. Both *A. ostoyae* and *A. gallica* were identified among isolates from the 18 basidiomes. Basidiomes and cultures of the two species were morphologically distinguished. Fluorescence microscopy showed that hyphae from single basidiospores were haploid and hyphae from basidiome tissues were diploid. Field observations suggest that colonized stumps of *Quercus* spp. and rhizomorphs play important roles in the epidemiology of Armillaria root rot in pines planted on cutover hardwood sites.

A837

EVALUATION OF THE ROOT DISEASE INDICATOR USED IN THE FOREST HEALTH MONITORING PROGRAM. F.A. Baker, Department of Forest Resources, Utah State University, Logan, UT 84322-5215; C.G. Shaw, III, D.W. Omdal, and P.M. Wargo, USDA Forest Service.

The Forest Service's Forest Health Monitoring program seeks to quantify the incidence and severity of root diseases as an indicator of forest health. The program's proposed technique examines for evidence of root disease the main, primary root on a tree's north and south sides out 1 m from the base. We simulated this technique on data collected from excavating 11 ponderosa pines, 20 lodgepole pines, 16 true and Douglas-firs on one site and 48 on another, and 32 red spruce. Detection probability was 0.84 on the 16 true and Douglas-firs involved in a root disease complex, 0.75, 0.58 and 0.68, respectively, for lodgepole pine, true and Douglas-firs, and red spruce infected with Armillaria sp.; and 0.52 for ponderosa pines infected with Heterobasidion annosum. The probability of detection increased as more roots were sampled. We do not consider the 2 root technique to be an accurate or precise method to detect infection by root disease and the required excavations significantly damages roots.

A838

PATCH DYNAMICS IN PINE STANDS INFECTED BY ARMILLARIA ROOT DISEASE. J.E. Lundquist, Rocky Mountain Forest and Range Experiment Station, U.S. Forest Service, Fort Collins, CO. 80526.

The frequency and size of Armillaria root disease (caused by Armillaria sp.) patches in 1763 lowveld Pinus spp. and Eucalyptus spp. stands in Lebowa, South Africa were characterized using roadside and aerial surveys. Root disease occurred in 42% of the stands examined. Host species and disease incidence were: Pinus elliottii (72%), P. khesiya (50%), P. roxburghii (50%), P. carabaea (45%), P. taeda (27%), P. patula (8%) and Eucalyptus spp. (0%). Disease progress curves for P. elliottii indicated that disease severity was related to stand age and that disease developed in four distinct phases. Proportion infected stand area increased rapidly to 1.63% by age 6, remained constant between ages 6-20, increased to 5.23% between ages 20-29, and decreased abruptly to 2.19% between ages 30-40, when stands are usually harvested. Proportion of infected P. elliottii stands increased rapidly to a plateau of 72% by age 10 and remained constant ($y = 0.83 - 0.61/x$, $r^2 = .30$, $p \leq 0.01$) to at least age 40. The number of previous rotations harvested on a site and their host species also influenced disease progression.

A841

PRODUCTION OF THE COMMON SCAB INDUCING PHYTOTOXIN, THAXTOMIN A, BY *Streptomyces scabies*. Eric C. Eckwall[1], Martin J. Babcock[2], and Janet L. Schottel[2]. Depts. of [1]Plant Pathology, and [2]Biochemistry, Univ. of Minnesota, St. Paul, MN, 55108, USA.

Streptomyces scabies is a gram-positive, filamentous soil bacterium which causes scab lesions on potatoes (*Solanum tuberosum*). Lesions resembling those resulting from natural infection can be induced by the application of thaxtomin A, a 4-nitroindol-3-yl containing 2,5-dioxopiperazine. This phytotoxin was produced by *S. scabies* strain RB2, a pathogenic isolate from Becker, Minnesota, in both oatmeal broth and oatmeal agar media. In liquid shake culture, synthesis of thaxtomin A occurred during late log/early stationary phase, as detected by thin layer chromatography. Production was repressed by 0.5% glucose and excess levels of tryptophan and tyrosine. This production method provides an easy means of obtaining thaxtomin A, which was previously available only through culturing *S. scabies* on surface sterilized potato slices.

A842

INTERACTIONS BETWEEN THE BIOLOGICAL CONTROL AGENT *PSEUDOMONAS FLUORESCENS* STRAIN A506 AND *ERWINIA AMYLOVORA* IN PEAR BLOSSOMS. M. Wilson and S.E. Lindow. Department of Plant Pathology, University of California, Berkeley, CA 94720.

Pseudomonas fluorescens strain A506 is an effective biological control agent of fire blight of pear in northern California. When pear blossoms (cv. Comice) were spray inoculated with the biological control agent, A506 reached populations of approximately 10^6 cfu/blossom on both the stigmatic and nectarial surfaces. When the biological control agent was coinoculated with the pathogen, *Erwinia amylovora*, A506 colonized the stigmatic and nectarial surfaces, but did not significantly reduce the population of *E. amylovora*. When blossoms were inoculated with the biological control agent 72h in advance of the pathogen, populations of *E. amylovora* on the stigma were reduced by 1000-fold and populations of *E. amylovora* on the nectary were reduced by approximately 100-fold. Disease development was suppressed in these blossoms pretreated with the biological control agent. Although *P. fluorescens* strain A506 was unable to reduce *E. amylovora* populations by competitive exclusion, it did significantly reduce *E. amylovora* populations when applied to blossoms in a pre-emptive manner, particularly on the stigmatic surface. *P. fluorescens* strain A506 apparently excludes *E. amylovora* by pre-emptive utilization of resources, either nutritional or physical, required for pathogen multiplication.

A843

CONTROL OF CROWN GALL IN APPLE (*MALUS*) ROOTSTOCKS USING COPAC E AND TERRAMYCIN. M. L. Canfield[1], C. Pereira[2], and L. W. Moore[1], Departments of Botany and Plant Pathology[1] and Statistics[2], Oregon State University, Corvallis, OR 97331-2902.

Copper and antibiotic compounds were evaluated for control of crown gall disease in apple rootstocks. Rootpruned EMLA 7, Mark, and seedling rootstocks were dipped in 2.5, 5 or 10% Copac E or 9, 18, or 36 g^{L-1} Terramycin. Trees were then dipped in a 10^9 cfu ml^{-1} mixture of *Agrobacterium tumefaciens* strains B49C/83 and D10B/87. Noninculated controls were dipped in water. The experiment was conducted at four nursery sites, two in Washington and two in Oregon. Both Copac E and Terramycin reduced disease incidence and similar results were observed at all four sites. Terramycin was more effective than Copac E in preventing infection. For example in Mark rootstock, the most susceptible of the three rootstocks, mean disease incidence in the pathogen inoculated trees at the four sites ranged from 45 to 58%; whereas disease incidence was from 26 to 36% and 10 to 15% for trees treated with 10% Copac E and 36g L^{-1} Terramycin, respectively. Mortality of the three rootstocks was higher with increasing concentrations of both Copac E and Terramycin. EMLA 7 rootstocks were the most severely affected and at one site the 10% Copac E treated trees had 80% mortality as compared to 20% in water treated controls.

A844

REACTION OF RICE CULTIVARS TO DIFFERENT INOCULUM DENSITIES OF *XANTHOMONAS ORYZAE PV. ORYZAE* IN NEPAL. T. B. Adhikari[1] and T. W. Mew[2]. [1]Institute of Agriculture and Animal Science, Nepal, [2]International Rice Research Institute, The Philippines.

Rice cultivars exhibited a range of susceptibility to *Xanthomonas oryzae* pv. *oryzae* (hereafter, Xoo) in surveyed sites and bacterial blight nurseries. Susceptibility of three selected rice cultivars (IR24, Sabitri, and Laxmi) was evaluated after inoculation with five densities of different Xoo strains by the leaf clipping method. Disease severity, assessed by measuring the lesion length at seven and 14 days after inoculation, differed significantly among rice cultivars. Regardless of inoculum density, Laxmi had significantly less disease than IR24 and Sabitri. Inoculum density x cultivar interactions were significant. When exposed to high inoculum (10^9 cfu/ml), disease development over time and lesion expansion were lower in Laxmi than in either Sabitri or IR24. Thus, Laxmi may be a source of resistance to Nepalese strains of Xoo.

A845

KERNEL BLIGHT OF BARLEY IN NORTHCENTRAL MONTANA. C. Martinez-Cano, S.L. Siemsen and D.C. Sands. Montana State University, Bozeman, MT 59717.

Kernel blight, a disease of unknown etiology, commonly occurs on barley in northcentral Montana. *Pseudomonas syringae, Xanthomonas campestris pv. translucens, Erwinia herbicola, Bacillus polymyxa, Pseudomonas sp., Pyrenophora teres,* and *Alternaria sp.* were frequently found in symptomatic seed. Since no single microorganism was consistently isolated from blighted kernels and symptoms could not consistently be reproduced in the field or greenhouse with the pathogens, other factors were explored. Kernel blight symptoms have been obtained by inoculation of microorganism(s) under high humidity only during the kernel development stages from early milk to hard dough. Of the saprophytes, *Erwinia herbicola* isolates have been shown to be potentially effective inhibitors in vitro of *Xanthomonas campestris pv. translucens* and *Pseudomonas syringae*, two cereal pathogens. The mechanism of antagonism may involve acid production as well as undetermined inhibitory compounds.

A846

DIVERSITY OF FOUR SPECIES OF *XANTHOMONAS* AS DETERMINED BY CELLULAR FATTY ACID ANALYSES.
N.C. Hodge, A.R. Chase, and R.E. Stall. Department of Plant Pathology, University of Florida, Gainesville, FL 32611.

Approximately 1,000 strains of *Xanthomonas albilineans, X. campestris, X. fragariae,* and *X. maltophilia* were subjected to fatty acid (FA) analyses. Strains of 10 pathovars of *X. campestris* from ornamentals were included. Quantitative variance among FA profiles enabled identification of the four species with 100% accuracy. Dendrogram cluster analysis placed strains of *X. albilineans* remotely from those of the other three species. Whereas the strain profiles of each species or pathovar were distinct, strains within *X. albilineans, X. fragariae,* and *X. maltophilia* were homogeneous by their consistent FA ratios. Pathovars of *X. campestris* that had conserved profiles were *fittonia, hederae, maculifoliigardeniae, malvacearum, pelargoniae,* and *zinniae*. FA profiles of *X. campestris* pathovars *begoniae, dieffenbachiae, fici,* and *poinsettiicola* had quantitatively diverse FA profiles. These heterogeneous pathovars did not form discrete subgroups, but intercalated randomly with one another on the dendrogram.

A847

ENTRY OF *CLAVIBACTER MICHIGANENSIS* SUBSP. *MICHIGANENSIS* INTO TOMATO PLANTS THROUGH PRUNING WOUNDS. W. M. Carlton, M. L. Gleason, and E. J. Braun. Dept. of Plant Pathology, Iowa State University, Ames, Iowa, 50011.

In a factorial field experiment (pruning X inoculation) conducted in 1990 and 1991, 8-wk-old, caged tomato transplants (cv. Jet Star) were spray-inoculated with a 10^4 cfu/ml suspension of a mixture of three strains of rifampicin-resistant *Clavibacter michiganensis* subsp. *michiganensis*. Uninoculated treatments remained unsprayed. Two weeks after inoculation, all axillary branches below the first flower cluster were removed manually from plants in the pruned treatments. Mature fruits were harvested and weighed weekly. Disease severity was estimated periodically as percent diseased foliage and percent stem tissue colonized by the pathogen. Inoculation significantly ($P=0.05$) increased disease severity and reduced yields in both 1990 and 1991. Pruning had no significant effect on disease severity or yield in 1990. In 1991, however, pruning significantly increased disease severity and significantly reduced yield in inoculated treatments.

A848

SOIL MOISTURE EFFECTS ON SYMPTOM DEVELOPMENT OF BACTERIAL RING ROT OF POTATOES. Bailey, L., Powelson, M.L., and Christensen, N.W., Department of Botany and Plant Pathology, Oregon State University, Corvallis, OR 97331-2902.

Field microplot studies were conducted in Corvallis, Oregon in 1990 and 1991 to determine effects of water application rates on severity of foliar symptoms and incidence of tuber symptoms of bacterial ring rot of potatoes. Treatments consisted of two cultivars, Russet Burbank and Norland, four inoculum levels, 0, 10^2, 10^6, and 10^9 cells/ml of *Corynebacterium sepidonicum*, and three rates of water, deficit , moderate, and excessive. Treatments were arranged factorially in a split-plot design with water as whole plots and cultivars x inoculum concentrations as subplots. Total water measured (irrigation and rainfall) was 27, 47, and 50 cm in 1990 and 16, 27, and 49 cm in 1991. There were no main-effect interactions for either disease or yield . The differences between cultivars was significant. Foliar symptoms were more severe in Norland than in Russet Burbank, whereas the incidence of tuber symptoms was higher in Russet Burbank than in Norland. Rates of applied water had no effect on development of bacterial ring rot symptoms.

A849

IMMUNODETECTION OF *XANTHOMONAS CAMPESTRIS* PV. *CAMPESTRIS* IN THE GUTTATION FLUID OF LATENTLY INFECTED CABBAGE SEEDLINGS. G.T. Mochizuki and A.M. Alvarez, Dept. of Plant Pathology, University of Hawaii, Honolulu, HI 96822.

The hypothesis that *Xanthomonas campestris* pv. *campestris* (*Xcc*) could be recovered from the guttation fluid of latently infected cabbage seedlings, was tested using three inoculation methods and assayed using a monoclonal antibody (mAb) based immunofluorescent colony staining technique (IFC) to recover viable cells of the pathogen present in low populations. Ten day old cabbage seedlings were inoculated with *Xcc* by applying 1 to 10 cfu directly into guttation droplets on hydathodes, by infiltrating 10^4 cfu into the root zone, or by vacuum infiltrating the seed and growing out the seedlings. Plants were kept in growth chambers at 22C to retard symptom expression. Guttation was induced and the droplets were collected at 48 hr intervals and assayed using IFC and standard dilution plating. Recovery of *Xcc* was intermittent and varied from 2 to over 2000 cfu per seedling. The IFC assay using *Xcc*-specific mAb X21-FITC was more sensitive and reproducible than standard plating techniques. The minimum times for the appearance of *Xcc* in guttation fluid for hydathode, root, and seed inoculation methods were 2, 9 and 20 days, respectively. These data suggest the possible role of undetected infection and transmission of *Xcc* in the seedbed.

A850

RESPONSE TO ERWINIA INOCULATION IN PLANTS DERIVED FROM TISSUE CULTURE AND TUBERS OF THREE POTATO CULTIVARS. S. A. Rindels[1], P. L. Spinski[2] and D. J. Gallenberg[1]. Departments of Plant Science[1] and Horticulture[2], South Dakota State University, Brookings, SD 57007.

Plants derived from both tissue culture and tubers of three potato cultivars were tested in greenhouse and field studies for their response to stem inoculation with two strains of Erwinia carotovora ssp. atroseptica (Eca). In the field, there were significant responses to cultivar and plant source. Disease severity was greater in Red Pontiac than in Russet Burbank or Norchip. Tuber plants were affected more than tissue culture plants. In the greenhouse, bacterial strain was significant, as was the cultivar x plant source interaction. Eca strain G424 induced greater severity than SR8. Although disease severities were similar for Russet Burbank plants, whether from tissue culture or tubers, Norchip plants from tissue culture had greater disease severity, while Red Pontiac tuber plants were affected more.

A851

BLACK ROT STRAINS WITH ATYPICAL PIGMENTATION ISOLATED FROM COMMERCIAL CRUCIFER SEED. *A.R. Poplawsky* and W. Chun, Plant Pathology Division, PSES, University of Idaho, Moscow ID 83843

In laboratory tests of commercial crucifer seed, washes are plated on the semi-selective agar medium FS and examined for the presence of the black rot pathogen *Xanthomonas campestris* pv. *campestris* (*Xcc*). We recently isolated suspect *Xcc* strains from each of four western seed lots which were blue on FS instead of the expected blue-green color. All four strains were positive for starch hydrolysis and EPS production, but two were reduced and two were negative for xanthomonadin pigment. All four strains produced typical symptoms on cauliflower, and had membrane protein profiles identical to a known *Xcc* strain. Each of the atypical strains was restored for pigment production by one of three different, previously cloned xanthomonadin genes. We conclude that these are strains of *Xcc* which are reduced or negative for pigmentation, and that pigment loss occurred via at least three different, independent events. The inherent variability in pigmentation of *Xcc* requires that non-pigmented, but otherwise typical appearing isolates be considered as suspect *Xcc* in diagnostic assays.

A852

SEED TRANSMISSION OF ERWINIA STEWARTII IN CORN UNDER FIELD CONDITIONS. C. C. Block, D. C. McGee and J. H. Hill. Department of Plant Pathology, and Seed Science Center, Iowa State University, Ames, IA 50011.

A rifampicin and nalidixic acid resistant isolate of Erwinia stewartii (9A) was recovered from 9 of 4,058 corn seedlings. Infected seed of two varieties, the dent corn inbred, A632, and the sweet corn, 'Hybrid Pride of Canada' (HPC), was obtained by systemically infecting plants with isolate 9A in the greenhouse. Percent seed infection was 79% for A632 and 41% for HPC. Seed was harvested in late April and planted in the field 4 and 6 weeks later: 2800 seeds of A632 and 3200 seeds of HPC. Insect-proof screens precluded transmission to the plants by the corn flea beetle vector. E. stewartii (9A) was recovered from 7 of 1307 emerged plants of A632 (0.54%) and from 2 of 2751 emerged plants of HPC (0.07%). Transmission occurred at a much higher frequency in the greenhouse, 4.3% for A632 and 1 % for HPC. In a second test, no seed transmission was detected from 12,000 seeds harvested in September, 1990 from Iowa corn fields. Half of the 12,000 seeds came from severely diseased, naturally infected plants and half from inoculated plants. Seed infection levels were much lower, though, and averaged about 5 percent.

A853

STREPTOMYCIN RESISTANCE AND COPPER TOLERANCE AMONG STRAINS OF PSEUDOMONAS CICHORII IN COMMERCIAL CELERY SEEDBEDS. Ken Pohronezny, Mark Sommerfeld, and Richard N. Raid, University of Florida, EREC, and A. Duda & Sons, Belle Glade, FL 33430.

Strains of Pseudomonas cichorii were isolated from diseased celery plants at four seedbed sites, representing >95% of Florida's celery industry. Seventy per cent of 87 strains tested in vitro were resistant to 200 μg/ml streptomycin. The prevalence of resistance ranged from 0 to 100% among the four sites. Resistance to streptomycin has been persistent in this area, despite little streptomycin use by the industry over the last 15 years. Strains were also qualitatively assayed for tolerance to 0.64 mM $CuSO_4$ on a low-copper-complexing medium. Thirty-two per cent of the strains were classified as sensitive (<30% growth), 44% as moderately tolerant (30 to 60% growth), and 25% as tolerant (>60% growth). When exposed to 1.2 g/L copper hydroxide bactericide for 4 hr, populations of representative tolerant strains were reduced from 10^8 to only 10^6 cfu/ml. In contrast, populations of sensitive strains typically were less than 10 cfu/ml. Widespread copper tolerance is probably related to heavy selection pressure from long-term copper use by the celery industry.

A854

CHONDROSTEREUM PURPUREUM, A POTENTIAL MYCOHERBICIDE FOR RED ALDER IN BRITISH COLUMBIA FORESTS. S. F. Shamoun and R. E. Wall. Forestry Canada, Pacific Forestry Centre, 506 W. Burnside Road, Victoria, British Columbia V8Z 1M5, Canada.

Chondrostereum purpureum, the causal agent of silver leaf disease of orchard crops, is also being evaluated for its beneficial potential as a mycoherbicide for red alder (Alnus rubra Bong.), a major competitor in conifer plantations throughout British Columbia (B.C.). Three plots consisting of at least 60 saplings were selected near French Beach, Vancouver Island, B.C. for field testing. Twenty trees in each plot were girdled with a knife at breast height (130 cm) and ten of the girdling wounds were subsequently inoculated with mycelia of C. purpureum and sealed with Parafilm (R). The ten control wounds received only agar before being sealed. Eight months after inoculation, downward necrosis (DN) and percent dieback (PD) were recorded and subjected to analysis of variance. Mean DN and PD were 4.0 cm and 27%, respectively. This was significantly different than the lack response in the controls both measurements; control trees completely recovered from the girdling effects. Girdling stress also triggered attack from endophytic fungi, particularly Melanconium sp., on the main stems and branches of trees inoculated with C. purpureum. The results indicate that C. purpureum has potential as a mycoherbicide for red alder, either alone or in combination with predisposing mechanical, environmental, or chemical stresses or endophytes.

A855

A SENSITIVE HYDROPONIC SEEDLING BIOASSAY FOR THE BIOHERBICIDES, Colletotrichum truncatum and Alternaria cassiae. R.E. Hoagland; USDA-ARS, Southern Weed Science Lab.; Stoneville, MS 39887

A bioassay to measure bioherbicidal efficacy of spore preparations of the pathogens C. truncatum and A. cassiae on two weed species, hemp sesbania (Sesbania exaltata) and sicklepod (Cassia obtusifolia), respectively, was developed. The system utilized 4-day-old hydroponically dark-grown seedlings sprayed with spore suspensions. Root and shoot lengths were monitored non-destructively over time under conditions of dark growth, 90-100% RH, and 25°C. Shoot-growth inhibition and stem collapse (death) were directly related to spore concentration applied. Generally, these pathogens at 10^3 to 10^4 spores/ml caused significant shoot growth inhibition within 25-30 h and seedling death within 40-50 h. This bioassay has also been useful in studying herbicide-pathogen interactions, and may be extended to determine bioherbicidal efficacy of different pathogen isolates, pathovars, or spore formulations.

A857

AMMONIA PRODUCTION BY BACTERIAL ANTAGONISTS AND SENSITIVITY OF FUNGAL PATHOGENS. R. G. Linderman and J. L. Marlow. USDA-ARS Hort. Crops Res. Lab., Corvallis, OR 97330.

Rhizosphere bacterial antagonists of fungal pathogens varied in the production of excess ammonia (NH_3), from none to over 600 ug/ml in 72 h. NH_3 production was substrate specific and generally increased with substrate concentration, was detectable within 24 h of colony establishment, and continued at varied rates during a 6-day assay. Some bacteria produced NH_3 equally well at 15 or 21°C, but others only at 21°C. Presumably, high NH_3 producers had more constitutive deaminating enzymes than low producers. Isolates of pathogenic fungal genera varied in sensitivity to NH_3 (from high to low): Aphanomyces, Rhizoctonia, Phytophthora, Pythium, Pyrenophora, Thielaviopsis, Colletotrichum, Cylindrocladium, and Fusarium; species within genera and isolates within species also varied in sensitivity. Implications of NH_3 production by rhizosphere bacteria are discussed in relation to biocontrol.

A859

PATHOGENICITY OF SCLEROTIALESS MUTANTS OF *SCLEROTINIA SCLEROTIORUM*. C. A. Hertoghe, R. V. Miller, and D. C. Sands. Montana State University, Bozeman, MT. 59717

Six putative mutants of *Sclerotinia sclerotiorum* were generated by chemically mutating protoplasts for the purpose of creating a biological control agent which could not overwinter. One mutant, SL7, failed to produce sclerotia under any circumstances and others demonstrated temperature dependent responses or reversion. Virulence of SL7 rivals that of various wild type isolates on bean, sunflower, dandelion and spotted knapweed. This is the first report of a pathogenic sclerotialess mutant of *Sclerotinia sclerotiorum*. This strain has the potential as a biological control agent because of this non-survival trait.

A860

FORMULATION OF A POTENTIAL BIOCONTROL AGENT FOR CALAMAGROSTIS CANADENSIS. Richard S. Winder, Simon F. Shamoun, and Charles E. Dorworth. Forestry Canada, Pacific Forestry Centre, 506 W. Burnside Road, Victoria, British Columbia, V8Z 1M5, Canada.

A new Colletotrichum sp. was formulated to enhance virulence on Calamagrostis canadensis (Michx.) Beauv., a reforestation weed in Canada. Conidia were dissolved in a 0.01 M tannic acid solution and rinsed to remove the matrix. In vitro matric inhibition was eliminated; in vivo germination was lacking and coverage was poor. Tween 20 (0.02% v/v) completely inhibited germination, as did skimmed milk. Soy milk and 25% (v/v) Aloe extract did not affect in vitro germination; germination in Aloe extract in vitro was normal but there was no significant damage at rates of up to 1 x 10^8 conidia m^{-2}. Mycelia from four 10% dextrose liquid cultures or conidia from four potato dextrose agar cultures were incoroporated into a 2% (w/v) sodium alginate, 20% (w/v) kaolin clay, and 150 mg streptomycin sulfate/ml solution, solidified in 0.25 M $CaCl_2$, dried, and ground. Plants watered until runoff and dusted with 0.01 g m^{-2} conidial powder exhibited no significant damage. Mycelial dust caused 36 ± 1% leaf area damage (LAD) after 48h dew. This increased to 53 ± 3% when 2% vegetable oil surfactant (VS) was the wetting agent. With 16 h dew, spray until runoff of 132g/L hyphae in a solution of 0.5% sodium alginate resulted in ca. 30% biomass reduction and LAD after one week. It is possible to combine the liquid formulation with VS and the powdered formulation to attempt further improvement.

A861

A COMPUTERIZED IMAGE ANALYSIS PROGRAM FOR AGRICULTURAL APPLICATIONS. M. Mulesky[1], B. Chism[1], M. Piermarini[2], and C. Hagedorn[1]. [1]Department of Plant Pathology, Physiology and Weed Science, and [2]Department of Electrical Engineering, Virginia Tech, Blacksburg, VA 24061.

A computer program was developed which utilizes Tag Image File Format (TIFF) to accumulate information on root and foliar samples photographed or placed directly on a cased image scanner. Data accumulated is stored in a Paradox® compatible database for further manipulation using various standard spreadsheet and statistical software. Practical applications of the program include the study of seasonal leaf area development, yield analyses, pesticide spray drift patterns, insect and disease damage, and projected plant root surface area.

A862

FIELD EVALUATION OF POTENTIAL CONTROL AGENTS FOR SOYBEAN CYST NEMATODE ON SOYBEAN. Susan L. F. Meyer and Robin N. Huettel, Nematology Laboratory, USDA, ARS, Bldg. 011A, Rm. 153, BARC-W, 10300 Baltimore Avenue, Beltsville, MD, 20705-2350. Cooperator: Crop Genetics International, Hanover, Maryland.

Novel control agents for soybean cyst nematode (SCN) were evaluated during the 1991 growing season. Microplot treatments were: SCN sex pheromone (bioregulator 1), a related compound (bioregulator 2), a mutant fungus, autoclaved fungus, fungus plus '1', fungus plus '2', aldicarb, and a control with no nematicides. Bioregulators 1 and 2, three related compounds, aldicarb, a resistant soybean cultivar, and an untreated susceptible cultivar were tested in field rows. In the microplots, the greatest reduction in midseason cyst numbers resulted from treatment with fungus plus '2'. Cyst numbers at harvest were higher compared to controls than at midseason. Highest microplot yields were from aldicarb-treated plants and lowest from untreated controls. In the row tests, all treatments resulted in reduced midseason cyst counts compared to the susceptible cultivar. Highest yields were from the resistant cultivar and from several bioregulator treatments.

A863

BIOLOGICAL CONTROL OF POSTHARVEST CHILE PEPPER FRUIT ROT PATHOGENS WITH *PICHIA GUILLIERMONDII*. C.L. Biles and M.M. Wall, Department of Entomology, Plant Pathology and Weed Science and Department of Agronomy and Horticulture, New Mexico State University. Las Cruces, NM 88003.

Chile peppers are susceptible to several pre- and postharvest fruit rots. Experiments were conducted to determine the effectiveness of the yeast, *Pichia guilliermondii*, in controlling fruit rot caused by *Alternaria alternata*, *Rhizopus stolonifer*, *and Phytophthora capsici* on field harvested green chile peppers and peppers at different maturity stages. When comparing *P. guilliermondii* treated and non-treated green chile peppers, Alternaria fruit rot was decreased by 82% and Rhizopus rot was decreased by 96%. In contrast, inconsistent biocontrol was exhibited against *P. capsici*. Alternaria rot was decreased at all fruit maturity levels with a 68%, 70%, 90%, and 78% decrease at the respective maturity stages, 100% green, 10% red, 50% red and 100% red. Rhizopus rot was completely controlled at all maturity levels except the 100% red stage in which 85% control was observed with one isolate and only 6% control by another. Phytophthora fruit rot was controlled at the 100% red level only in which two *P. guilliermondii* isolates exhibited 74% and 33% control.

A864

APPLICATION OF CANDIDA GUILLIERMONDII IN COMMERCIAL CITRUS WAXES FOR BIOCONTROL OF PENICILLIUM ON GRAPEFRUIT. R. G. McGuire. USDA, ARS, Subtrop. Hort. Res. Sta., Miami, FL, 33158.

Commercial processing of fruit may alter surface microflora at the same time that it increases susceptibility to disease through injury. This is especially true with quarantine treatments that seek to eradicate insect pests with heat or chemicals. A yeast, Candida guilliermondii, that is antagonistic to Penicillium was applied to the surface of grapefruits in citrus waxes to test biocontrol. Although the solvents of many commercial waxes were biocidal, FMC 705 and FMC 223 (FMC Corp., Lakeland, FL) were satisfactory carriers for the yeast. C. guilliermondii survived for 2 mo at 12 C within a film of dried wax on filter disks. When applied to grapefruits at concentrations designed to produce surface populations of 10^4 and 10^6 cfu/cm^2, populations stabilized over 2 mo at 12 C around 10^5 cfu/cm^2. Populations of undescribed yeasts on fruit were originally 10^3 cfu/cm^2 before quarantine treatment of 3 h at 48 C reduced them to 4 x 10^1. Fruit subsequently coated with wax alone decayed within 60 days, but fruit on which C. guilliermondii had been applied in wax remained sound.

A865

INFLUENCE OF PHENAZINE-PRODUCING AND PHENAZINE-MINUS STRAINS OF *PSEUDOMONAS FLUORESCENS* ON BIOCONTROL ACTIVITY AND GROWTH OF *TRICHODERMA HARZIANUM*.
L. M. Dandurand, G. R. Knudsen, D. J. Eschen, and B. J. Thiel. Plant Pathology Division, University of Idaho, Moscow, Idaho 83843.

Trichoderma harzianum ThzID1, formulated with alginate and polyethylene glycol as a granular seed coating, reduced *Aphanomyces* root rot of pea in growth chamber experiments. *Pseudomonas fluorescens* 2-79RN10, which produces a phenazine antibiotic, also reduced root rot, but control was less than with ThzID1 alone. Disease control was significantly less when seeds were treated with ThzID1+2-79RN10, compared to ThzID1 alone. Root rot was not reduced by the phenazine-minus mutant *P. fluorescens* B46, and treatment with ThzID1+B46 resulted in the same level of disease control as ThzID1 alone. The results suggest that phenazine production by 2-79RN10 inhibited biocontrol activity of ThzID1. However, in separate studies of ThzID1 hyphal growth from coated pea seeds in

soil, addition of 2-79RN10 did not affect hyphal density or colony radius compared toThzID1 alone, over 5 days. Hyphal growth from peas treated with ThzID1+B46 was greater than growth from seeds treated with ThzID1 alone.

A866

EVALUATION OF BIOLOGICAL AND CHEMICAL TREATMENTS FOR CONTROL OF SCLEROTIUM ROLFSII ON APPLE SEEDLINGS. K. E. Conway and P. P. Abbasi, Department of Plant Pathology, Oklahoma State University, Stillwater, OK 74078-9947.

Fungal organisms tested were: Trichoderma harzianum isolates TH86 and TH110, T. aureoviride TA224 and TA357, Gliocladium virens (GL21), and Laetisaria arvalis (LA). Bacteria were Pseudomonas cepacia (PC) and Bacillus subtilis (BS) used either alone or as PC combined with TH110, TA224, TA357 or GL21 and BS with LA. Chemicals were Lorsban 15G and Flutolanil 50 WP. Organisms were suspended in a gel matrix and applied to the entire length (30 cm) of 1-yr-old apple seedlings prior to planting on 15 April 1991. Trees in control and chemical treatments were also coated with gel prior to planting. Chemicals were applied to soil next to trees on 17 May, 17 June and 15 July. Tree mortality on 17 October was greatest in GL21, TH86, TA224 and BS treatments (76, 72, 70, and 70% respectively). Mortality in controls was 65%. Most effective were Flutolanil, Lorsban, LA, TH110, and PC combined with TA224 and TA357 (20, 30, 48, 56, 56 and 54%, respectively).

A867

THE RHIZOSPHERE BACTERIAL COMMUNITY OF FOUR PEA CULTIVARS: EFFECTS OF *PSEUDOMONAS CEPACIA* AMMD. E. B. King and J. L. Parke, Department of Plant Pathology, University of Wisconsin-Madison, 1630 Linden Dr., Madison, WI 53706.

To determine if the introduction of a biocontrol agent changes community composition of pea rhizosphere bacteria and to determine if pea host cultivar influences community composition, bacterial isolates from the rhizosphere of each cultivar with and without seed treatment with *Pseudomonas cepacia* AMMD were sampled at 2 wks and again at 8 wks after planting. Isolates were characterized according to their responses to 35 physiological attribute tests. The community associated with *P. cepacia* AMMD treated samples was significantly different from the community from nontreated samples for two of four cultivars at the first sampling time, but not at the second sampling time. Bacterial communities from the four cultivars were indistinguishable with multivariate analyses. Communities sampled at 2 wks were readily distinguishable from communities sampled at 8 wks after planting.

A868

BIOCONTROL ACTIVITY AND PATHOGENICITY OF BINUCLEATE RHIZOCTONIA ON SOYBEAN. F. U. Khan, B. Nelson, and T. Helms*. Depts. of Plant Pathology and Crop and Weed Science*, North Dakota State University, Fargo, ND 58105.

Three cultures of binucleate *Rhizoctonia* (BNR) (AGK) were evaluated for biocontrol of *R. solani* and for pathogenicity on seven soybean cultivars in the greenhouse. Soil was infested with a virulent culture of *R. solani* AG2-2 and seed was planted in direct contact with mycelia of BNR. Germination was recorded in ten days and survival, height and disease severity were recorded after three wk. All BNR significantly increased germination and survival, and decreased disease severity on all cultivars. There was no cultivar x BNR interaction indicating cultivar nonspecificity of biocontrol activity. There were no significant differences in disease control among the three BNR. BN8-2, BN8-3 and BN4 reduced disease severity (1 to 5 scale)to 1.94, 1.96 and 2.0, respectively, compared to 3.77 in the control. BNR did not reduce plant height as compared to treatments with no BNR and no *R. solani*, and did not cause lesions. These results suggest a significant biocontrol potential of BNR for control of *R. solani* on soybean.

A869

CRUCIFEROUS AMENDMENTS, CHITIN, AND PAECILOMYCES LILACINUS REDUCE POPULATIONS OF VERTICILLIUM DAHLIAE IN SOIL. David P. Morgan and T. J. Michailides, Department of Plant Pathology, University of California, Berkeley/Kearney Agricultural Center, Parlier 93648.

Amendments of dried cabbage, broccoli, or chitin at a concentration of 1% w/w reduced the number of viable microsclerotia of Verticillium dahliae up to 100% after incubation of naturally infested soils in sealed bottles for 15 days. In addition, a 68% reduction of V. dahliae microsclerotia occurred after amending 3 x 10^6 conidia of Paecilomyces lilacinus per g of soil. All of these experiments included soils at field capacity (-35 kPa) and soils were incubated at temperatures ranging from 10-30 C. Increasing soil incubation temperatures decreased the time needed to achieve LD_{50} for the cabbage-, broccoli-, and chitin-amended soils. However, when P. lilacinus was added to the soils, levels of viable microsclerotia of V. dahliae were reduced more at 10 C (68% reduction) than at 30 C (23% reduction). Cruciferous amendments, chitin, and P. lilacinus could perhaps be used in controlling Verticillium wilt in commercial crops.

A870

BIOLOGICAL, CHEMICAL AND PHYSICAL PROPERTIES OF COMPOSTED YARD WASTES AS INDICATORS OF MATURITY AND PLANT DISEASE SUPPRESSION. M. E. Grebus, C. Marugg and H. A. J. Hoitink. Dept of Plant Pathology, Ohio State Univ., Wooster, OH 44691.

Maturity guidelines for compost prepared from yard wastes high in grass clipping content (33%, v/v) and produced by a temperature feedback-controlled windrow composting system (forced aeration) were determined by monitoring chemical, physical and biological properties during the process. Maturity was characterized by a decrease in the rate of respiration of the compost based on oxygen consumption, increases in cation exchange capacity, ash content and concentrations of available plant nutrients (P, K, Ca, Mg), a decrease in total carbon/nitrogen ratio, and finally, the accumulation of nitrate nitrogen. Radish and ryegrass were reliable indicators of maturity in plant growth bioassays, but watercress and cucumber were not. Composts of all maturity levels consistently induced suppression to Pythium damping-off. Suppression of Rhizoctonia damping-off increased with maturity in some batches but this effect was not consistent.

A871

USING MILD STRAIN CROSS PROTECTION TO MANAGE PAPAYA RINGSPOT VIRUS IN HAWAII. S. A. Ferreira, K. Y. Pitz, R. F. L. Mau, and L. Sugiyama, University of Hawaii, HI and D. Gonsalves, Cornell University, Geneva, N.Y.

Papaya ringspot virus (PRSV) is a non-persistent aphid transmitted virus that causes serious economic losses in papaya and a few cucurbit hosts in Hawaii. Initial studies with the Line 8 cultivar, which is no longer grown commercially, indicated a potential for using mild strain cross protection in papaya. Consequently, a series of demonstration trials were installed at 4 locations on Oahu to further evaluate the potential for cross protection in Line 8 and the commonly planted cultivars, Waimanalo, Kamiya, and Sunrise. The severity of mild strain symptoms on foliage and fruit was seasonal and differences were observed between cultivars. At times, symptoms were virtually indistinguishable from those of the severe strain. For part of the year, mild strain symptoms on the cultivar Sunrise were too severe for acceptable commercial production. Incidence of severe strain in cross protected orchards, or superinfection, varied by location and cultivar. After 16 months, superinfection rates at all test locations ranged from 0 to 10.6%, enabling growers to produce papayas in these locations economically.

A872

A GENOMIC REGION REQUIRED FOR PYOLUTEORIN PRODUCTION BY *PSEUDOMONAS FLUORESCENS* PF-5. J. Kraus and J. E. Loper, Dept. Botany and Plant Pathology, Oregon State University, and USDA-ARS, Horticultural Crops Research Laboratory, 3420 N.W. Orchard Ave., Corvallis, OR 97330.

P. fluorescens Pf-5 inhibits the growth of *Pythium ultimum* due to the production of pyoluteorin, an antifungal compound. Six Plt$^-$ mutants, deficient in pyoluteorin production, were obtained by Tn*5* mutagenesis of Pf-5. The Plt$^-$ mutants defined three groups, based on the size of the *Eco*RI fragment of genomic DNA containing a Tn*5* insertion. *Eco*RI fragments, containing Tn*5* and flanking sequences, were cloned from mutants representing each of the three groups. Five cosmid clones, which hybridized to one to three of the cloned *Eco*R1 fragments, were identified from a genomic library of Pf-5. The five cosmids contained overlapping regions of cloned genomic DNA. The original Tn*5* insertions responsible for the Plt$^-$ phenotype were localized to 17 kb within the cloned region required for pyoluteorin production.

A873

ANTIBODY, TRANSFORMANT, AND CHEMICALLY-INDUCED MUTANT FOR TRACKING *GLIOCLADIUM VIRENS* GL-21 AND *TALAROMYCES FLAVUS*. D. R. Fravel, S. Mischke, and R. D. Lumsden. Biocontrol of Plant Diseases Laboratory, USDA, ARS, Beltsville, MD 20705.

Antibodies were raised in turkeys to a purified 33-kDa protein associated with gliotoxin production in *G. virens* (GL-21). Antisera from turkey eggs allowed detection of the protein on mycelia of GL-21 grown on a nitrocellulose membrane buried in soilless mix. Antisera did not react with mycelia of other resident fungi. GL-21, and/or a benomyl-resistant transformed

strain of GL-21 were added to sterile soil. Survival was assessed by recovery on media with or without benomyl. Although there were 100-fold fewer propagules of the transformant than wild-type GL-21 in the mixture, 37% of the colonies recovered after 1 wk incubation were benomyl resistant. A benomyl-resistant biotype (Tfl-1) of *T. flavus* was obtained by chemical mutagenesis of the wild-type Tfl. Although Tfl-1 survived at half the rate of Tfl in a field test, benomyl resistance was a useful marker for tracking *T. flavus*.

A874

COMPARISON OF THE EFFICACIES OF SEVERAL BIOCONTROL AGENTS OF PYTHIUM DAMPING-OFF OF *AMARANTHUS CRUENTUS* X *CAUDATUS*. Ramsey L. Sealy, C.M. Kenerley, and E.L. McWilliams, Texas A&M University, College Station, TX 77843.

Damping-off of amaranth seedlings by species of *Pythium* Pringsheim is a problem that plagues amaranth producers and breeders. In this study, several reported biocontrol antagonists of Pythium damping-off, both singly and in combinations, were evaluated for their efficacies in controlling damping-off in *Amaranthus cruentus* L. X *caudatus* L. by *P. myriotylum* Drechsler. The only biocontrol agent or combination of agents that gave significant control in this study were *Pythium nunn* Lifshitz and a combination of *P. nunn* and *Trichoderma koningii* Oudem. Differences in our seedling production system from the systems of other researchers may explain the lack of efficacy exhibited by other antagonists.

A875

EFFECTIVE MANAGEMENT OF POWDERY MILDEW IN PUMPKIN WITH TRIADIMEFON DESPITE DEVELOPMENT OF FUNGICIDE RESISTANCE FOLLOWING TREATMENT. M. T. McGrath and M. S. Ghemawat, Department of Plant Pathology, Long Island Horticultural Research Laboratory, Cornell University, Riverhead, NY 11901-1098.

Management of *Sphaerotheca fuliginea* with triadimefon and its impact on fungicide resistance were examined in commercial and experimental plantings of pumpkin in 1991. Triadimefon was effective. Average powdery mildew severities (% symptomatic leaf area) on 12 September on adaxial and abaxial leaf surfaces were 49% and 59% for nontreated plots and 0% and 0.6% for plots treated with triadimefon 3 times (14-day) and chlorothalonil 5 times (7-day) beginning after disease detection. Fungicide resistance increased in treated pumpkin. No triadimefon-resistant isolates were detected during late July to August (epidemic start) in any field or during September in nontreated research plots or a nontreated commercial field . Resistance was found in 89% and 86% of the isolates collected in September from fungicide-treated research plots and a commercial field. Most were resistant to 200 ppm a.i. of triadimefon. Benomyl resistance shifted from 17% to 89% in the fungicide-treated commercial field and from 30% to 75% in the fungicide-treated plots.

A876

CHLOROTHALONIL SMOKE RESIDUE AS MEASURED BY ELECTRON BEAM ANALYSIS. C. Tappan[1], C. R. Krause[1], and C. C. Powell[2]. [1]U.S. Department of Agriculture (USDA), Agricultural Research Service (ARS), Application Technology Research Unit, OARDC/OSU, Wooster, OH 44691 and [2]Dept of Plant Pathology, Ohio State Univ., Columbus, OH 43210.

Electron Beam Analysis (EBA) was used to characterize and track the fate of Exotherm Termil, a chlorothalonil smoke formulation, on artificial target surfaces in a greenhouse. Fungicide particles, averaging less than 3.0 µm in diameter and as small as 0.4 µm, were found to vary significantly in number with location within a poinsettia canopy and with distance from the smoke source. Fungicide particles did not vary significantly in size either within the canopy or with distance. No measurable residue was found when the greenhouse was not tightly sealed. EBA can provide more precise information on environmental fate and behavior as it pertains to the method of fungicide application.

A877

EFFECT OF REPEATED APPLICATION OF SELECTED HERBICIDES AND INSECTICIDES/NEMATICIDES ON SOYBEAN CYST NEMATODE DENSITY. P.A. Donald, A.J. Keaster, R.J. Kremer, and B.D. Sims, 108 Waters Hall, University of Missouri, Columbia, MO 65211

In order to determine the non-target effects of long-term application of the same pesticides on soybean cyst nematode (SCN), Heterodera glycines, four herbicides and four insecticides/nematicides were tested in replicated field plots (soybean cv. Essex) with annual applications of the same combination of herbicide and/or insecticide/nematicide for three years. Soil samples were collected three times during the growing season and analyzed for SCN egg densities in 1990 and 1991. Densities of SCN at harvest were highest in herbicide-treated plots in 1990. In 1991, SCN densities at planting and harvest, SCN eggs/g root and soybean seed yields were more strongly affected by the application of insecticides/nematicides than by herbicides.

A878

EFFECTS OF SODIUM TETRATHIOCARBONATE, FOSETYL-AL, AND METALAXYL ON DEVELOPMENT OF ROOT AND CROWN ROTS OF APPLE CAUSED BY TWO *PHYTOPHTHORA* SPP. G.T. Browne and S.M. Mircetich, USDA-ARS, Department of Plant Pathology, University of California Davis 95616

Sodium tetrathiocarbonate (STC) at 200-1800 ppm a.i., fosetyl-Al (AL) at 333-4000 ppm, and metalaxyl (MET) at 20-60 ppm in commercial formulations were evaluated for control of apple root and crown rots caused by *Phytophthora cactorum* (*Pcc*) and *P. cambivora* (*Pcm*) in the greenhouse. Without fungicide, *Pcc* and *Pcm* caused moderate and severe disease (mean root rot (MRR) 51-81% and 97-98%, respectively). Disease caused by *Pcc* was reduced to MRR 0-37% by monthly MET drenches, AL sprays, AL drenches, or STC drenches at 200 ppm; the other applications of STC did not reduce disease. With *Pcm*, STC failed to reduce disease (MRR 80-100%), but efficacy of MET and AL was inconsistent. In a winter experiment, control of *Pcm* was excellent with MET (MRR 0%) and moderate with AL drenches and sprays (3000-4000 ppm, MRR 28-50%). MET and AL were less effective in a summer experiment when plants required more frequent watering. At the time of treatments, STC at 600-1800 ppm reduced incidence of *Pcc* and *Pcm* infection in leaf disk baits more than MET and AL did, but STC appears to lack persistence needed for control of the disease.

A879

AMPROPYLFOS: A SEED TREATMENT FUNGICIDE FOR SMALL GRAINS. C.J.R.Klittich, Rhône-Poulenc Agro AB, Box 11555, S-100 61 Stockholm, Sweden.

Ampropylfos is an aminophosphonic acid, the first of this chemical class to be used as a fungicide. It is safe both toxicologically and environmentally, and has a unique mode of action. At 20-50 g ai/100 kg seed, it gives excellent control of seed-borne *Drechslera* species, particularly *Drechslera graminea* and *Drechslera teres* on barley and *Drechslera avenae* on oats, fungi that are difficult to control with other fungicides. It is completely selective at three times the dose rate on small grains. Ampropylfos also gives significant control of seed-borne *Ustilago avenae*, *Ustilago hordei*, *Fusarium nivale*, *Fusarium roseum*, and *Septoria nodorum*.

A880

EFFECT OF FUNGICIDES ON PHYTOPHTHORA ROOT ROT AND PRODUCTIVITY OF CRANBERRY IN WISCONSIN. M. J. Drilias and S. N. Jeffers, Department of Plant Pathology, University of Wisconsin, Madison, WI 53706.

Metalaxyl, fosetyl-Al, and oxadixyl were evaluated during 1990 and 1991 for management of root rot of cranberry caused by *Phytophthora* spp. Microplots were established where plants had died from the disease in beds at two commercial cranberry marshes. Each plot consisted of 20 rooted cranberry cuttings (cv. Stevens) planted inside a 30-cm-diameter PVC ring embedded in the soil. In a separate trial conducted at one of the marshes, metalaxyl and fosetyl-Al were applied to plots (2 X 2 m) of established plants (cv. Ben Lear) located at the periphery of an area where plants had died. Fungicides were applied monthly from June through October in 1990 and June through September in 1991. In microplots, fungicides did not increase plant survival or growth. However, there was a significant difference among treatments in the number of plants infected with *Phytophthora* spp.; metalaxyl and oxadixyl, but not fosetyl-Al, reduced infection. On established plants, berry yield in untreated plots was greater than that in fungicide-treated plots. The number of flowers in untreated plots was significantly greater than those in fungicide-treated plots. There were significant reductions in the percentage of shoots flowering in plots treated with fosetyl-Al and in the number of flowers per flowering shoot in plots treated with metalaxyl. Fungicides had no apparent effect on disease development in any of the trials.

A881

Evaluation of Alternative Spray Materials for Disease Control in Home Rose Gardens. D. L. Clement, and J. C. Locke, Maryland Institute for Natural Resources, Cooperative Extension Service, 12005 Homewood Road, Ellicott City, MD 21042 and USDA, ARS, Florist Crops Laboratory, Beltsville, MD 20705-2350.

Sprays of materials not currently registered for disease control were compared to the conventional fungicide Funginex for blackspot and powdery mildew control on the floribunda rose cv 'Iceberg' in the 1991-92 growing season. Roses were container grown in outdoor plots under drip irrigation. Safer Insecticidal Soap, Wilt Pruf, Volck Oil, Volck Oil combined with baking soda, neem oil and neem wax were applied at weekly intervals from May through October. Funginex was the most effective control for blackspot, however, neem oil, neem wax, Safer Insecticidal Soap and the Volck Oil baking soda combination showed a substantial decrease in disease incidence. No powdery mildew infection occurred in 1991 for evaluation. None of these materials effectively controlled Japanese beetles, however, the neem oil, neem wax, insecticidal soap and oil treatments had noticeably fewer spider mites compared with the Funginex treatment.

A882

IN VITRO SENSITIVITY OF *COLLETOTRICHUM COCCODES* TO FOUR FUNGICIDES. E. Uribe and R. Loria, Cornell University, Dept. of Plant Pathology, Ithaca, NY 14853

Colletotrichum coccodes, cause of black dot of potato, was assayed *in vitro* for sensitivity to thiabendazole (TBZ), imazalil (IMA), maneb and CGA-173506 at 0, 1, 10, and 100 ppm. Radial growth, spore germination and sclerotial germination were significantly (α=0.01) affected by both fungicide type and fungicide concentration. All fungicides significantly (α=0.05) reduced radial growth after 14 days at 10 ppm and 100 ppm; IMA reduced growth at 1 ppm. All fungicides significantly (α=0.05) reduced spore and sclerotial germination at 10 and 100 ppm, although the effects on sclerotia at 10 ppm TBZ were inconsistent. CGA consistently (α=0.05) reduced spore and sclerotial germination at 1 ppm. Some fungicide treatments caused abnormal germ tube and colony morphologies and affected sclerotial production.

A883

COMPARISONS OF CALCIUM-BASED AND FILM-FORMING MATERIALS FOR CONTROL OF BROWN ROT OF PEACH CAUSED BY *MONILINIA FRUCTICOLA*. J.E. Adaskaveg, J.M. Ogawa, and A.J. Feliciano.Plant Pathology,UC Davis, CA 95616

Nutritional materials [calcium formate (CF) and calcium silicate (CS)] and film-forming, anti-transpirants [di-1-p-menthene (Vapor Gard) and an acrylic resin (Rhoplex AC-33NP)] were compared to the fungicide iprodione for control of brown rot of peach caused by *M. fructicola*. Rhoplex (20 ml/L), CF (2 g/L), and CS (2 g/L) showed no *in vitro* fungitoxicity; while Vapor Gard (20 ml/L) inhibited conidial germination. Test materials were applied to fruit with a handsprayer for the following genotypes and application times (before harvest): Elegant Lady/3, 2, and 1 wk; Fairtime/2 and 1 wk; and Bolinha and Corona/1 wk and 2 da. For the first two genotypes, the materials were compared to iprodione (applied with an air-blast sprayer 3 and 1 wk before harvest). Fruit were harvested, inoculated (non-wounded) with a 20 μl conidial suspension (25,000/ml), exposed to a wetness period (0, 4, 8, or 12 hr), and incubated for 5 days at 20C, 90% RH. Orthogonal contrasts indicated that test materials significantly reduced severity and incidence of brown rot compared to non-treated fruit for wetness periods studied. Furthermore, CF provided control similar to that of commercial applications of iprodione. For Bolinha and Corona, similar trends among treatments were observed but differences were not significant. Materials that may strengthen epidermal tissue or enhance the cuticular layer may supplement or provide alternatives to fungicides.

A884

MORTALITY OF BUNT TELIOSPORES FOLLOWING H_2O_2 FUMIGATION. J. L. Smilanick, R. D. Arrue, D. J. Henson, B. J. Goates and G. L. Peterson. USDA-ARS, 2021 S. Peach Avenue, Fresno, CA 93727

Low-moisture content vapor-phase H_2O_2 fumigation, a new cold sterilization process developed by the American Sterilizer Company (AMSCO), was tested as a quarantine treatment for *T. controversa*. Surface-borne teliospores of *T. tritici* or *T. controversa* on wheat seed did not germinate after a five-min fumigation at 46-48°C. Germination was 1 to 5% of the controls after a one-min treatment. Results with deep vacuum (38 mm Hg) or shallow vacuum (ca. 400 mm Hg) were not different. The treatment did not wet wheat seed nor influence its germinability, even if applied for more than 30 min. Preliminary tests with teliospores of *Tilletia indica* and *T. fusca* were similar. However, when intact sori were fumigated, teliospores within the sori survived, and variable mortality occurred if sori were ruptured before fumigation. Since sori are found in wheat, this treatment has insufficient activity for quarantine purposes. However, it may be a practical seed surface disinfestation process for other application where the microbes are borne superficially on seed surfaces. The process was much more potent than methyl bromide, propylene oxide, or chloropicrin fumigation.

A886

INFLUENCE OF TILLAGE METHOD ON INOCULUM DENSITY OF *PHIALOPHORA GREGATA* IN OVERWINTERING SOYBEAN RESIDUE. E.A. Adee and C.R. Grau.

Dept. of Plant Pathology. Univ. of Wisconsin, Madison, WI 53706.

The saprophytic activity of *Phialophora gregata* (Pg), causal agent of brown stem rot of soybean, is believed to be essential to the survival of this vascular pathogen. Greater disease severity and lower yields have been observed in no-till (NT) vs. conventional tillage (CT) systems. The two tillage systems were simulated in November of two consecutive years by placing soybean residue on (NT) or buried 15 cm below (CT) the soil surface. Soybean residue, 40 g of the lower 26 cm of main stems, was placed in nylon mesh litter bags (30 x 30 cm, 1.25 mm mesh). Residue of two colonization levels (heavy/light), identified on the basis of symptom severity, were used. The following May, residue was recovered from litter bags and assessed for percent of initial residue weight (WT) and colony forming units of Pg per gram of residue (CFU/g). Inoculum was significantly lower in the simulated CT vs. NT system as a result of both lower WT (57% vs. 81%) and fewer CFU/g (1,245 vs. 31,800). Weight loss was greater in residue with a heavy initial colonization level than with the light level (66% and 72% WT, respectively). Additional studies have shown CFU/g can increase over winter in lightly colonized residue that is buried (9.0×10^4 to 4.5×10^6), whereas inoculum decreased in heavily colonized residue (1.1×10^6 to 5.0×10^5).

A887

SOYBEAN CULTIVAR REACTION TO FUSARIUM SOLANI FORM A IN GROWTH CHAMBER SCREENING TRIALS. Jose Melgar and K. W. Roy. Department of Plant Pathology and Weed Science, Mississippi State University, Mississippi State, MS, 39762.

The relation of plant age at the time of inoculation and of the temperature of incubation to symptom expression was determined prior to cultivar screening. Optimum seedling age was found to be 7-10 days, and optimum temperature was found to be 25-27 C.

Twenty-eight soybean cultivars representing maturity groups V-VII were grown in a walk-in chamber and maintained at 27 C. Seedling hypocotyls were inoculated 1 week after germination by using a toothpick inoculation technique. Fourteen days after inoculation, cultivar reaction was determined using a 0-9 rating scale, where 0 = no lesion and 9 = plant killed. Significant differences in cultivar reaction occurred, and the cultivar rankings in two separate trials were similar and consistent with field reactions.

A888

INTERACTIVE EFFECTS OF SALINITY AND VERTICILLIUM ALBO-ATRUM ON YIELD AND VERTICILLIUM WILT DISEASE SEVERITY FOR TWO ALFALFA CULTIVARS. A.B. Howell, L. Francois and D.C. Erwin, Dept. of Plant Pathology, University of California, Riverside CA 92521.

The interaction between soil salinity and Verticillium albo-atrum on two alfalfa cultivars (Moapa-69 and NK-89786) was evaluated in a greenhouse. Salinity treatments were administered by drip irrigation of plants with water having average electrical conductivities of 0.8, 3.0, 5.0, or 7.5 deciSiemens/m. Seven-week-old plants were inoculated with 0, 10^4, 10^5, or 10^6 conidia/ml. No overall interaction among salinity, V. albo-atrum and the cultivars was observed for alfalfa yield. However, the differences in yield reduction of the two cultivars due to the effects of soil salinity and the combination of salinity and V. albo-atrum were significant (P=0.05). Cultivar NK-89786 was more sensitive to salinity than was Moapa-69. Salinity increased the severity of Verticillium wilt on NK-89786 when both factors were imposed together.

A889

PHAEOSPHAERIA LEAF SPOT, A NEW DISEASE OF MAIZE IN NORTH AMERICA. M. L. Carson, USDA-ARS, Box 7616, Department of Plant Pathology, and M. M. Goodman, Box 7620, Department of Crop Science, North Carolina State University, Raleigh 27695.

Phaeosphaeria leaf spot of maize, caused by the fungus *Phaeosphaeria maydis* (Henn.) Rane, Payak & Renfro(syn.=*Leptosphaeria zea-maydis* Saccas; *Metasphaeria maydis* (Henn.) Höhnel) has been found in maize breeding nurseries in the Homestead, Florida area during the last two winter seasons. Lesions are typically round to oval in shape, light tan or "bleached" in color, with distinct reddish-brown margins, measuring from 0.5 to 2.0 cm, and usually appear after

anthesis. Pseudothecia and less commonly, pycnidia of the *Phoma* anamorph were found immersed in lesions. The fungus grows slowly in culture, mainly producing psuedothecia on corn leaf agar and pycnidia on PDA. Attempts to inoculate maize seedlings in the greenhouse with the fungus have been largely unsuccessful. The disease does not appear to be widespread or particularly damaging at this time, but because of the apparent susceptibility of certain popular maize inbred lines and because it is a new disease in North America, further study is warranted.

A890

THE EFFECT OF SOIL FERTILITY ON THE DEVELOPENT OF STEM CANKER IN GREENHOUSE-GROWN SOYBEANS. J. C. Rupe, University of Arkansas, Fayetteville, 72701.

Two closely related soybean cultivars, Lee 74 and Lee non-nod (a non-nodulating strain of Lee 74), were inoculated with Diaporthe phaseolorum var. caulivora, the causal agent of stem canker. Twenty plants of each cultivar were either fertilized twice a week with a fertilizer solution (1,000 ppm of 20-20-20), or left unfertilized. The plants were inoculated two weeks after planting by inserting an infested toothpick into the lower stem and sealing the wound with petroleum jelly. Disease incidence was monitored twice a week in the greenhouse for up to 4 weeks. The experiment was conducted twice. Stem canker progressed faster and killed more plants in the non-fertilized treatments than in the fertilized treatments. This difference was greater for the Lee non-nod than for the Lee 74.

A891

INCIDENCE OF TOXIN-PRODUCING PATHOTYPES OF PYRENOPHORA TRITICI-REPENTIS IN SOUTH DAKOTA. Shaukat Ali and G.W. Buchenau. Plant Science Department, South Dakota State University, Brookings, SD 57007.

Fifty-nine isolates of Pyrenophora tritici-repentis, collected primarily from the spring wheat area of South Dakota, were evaluated for their ability to induce necrosis and/or chlorosis when inoculated onto spring wheat seedlings. Those that induced necrosis symptoms were further tested for their ability to produce the necrosis toxin. On the cultivar Celtic (susceptible to both necrosis and chlorosis), 64% of the isolates caused what appeared to be a combination of symptoms (nec+ chl+) and 12% caused symptoms of chlorosis only (nec- chl+). Only 2% caused necrosis only (nec+ cl-), and the rest produced small lesions without necrosis or chlorosis (nec- chl-). All isolates produced only small brown lesions on the resistant cultivar Erik. Culture filtrates from isolates that induced necrosis during pathogenesis caused severe necrosis when injected into leaves of Celtic but not Erik.

A892

ACREMONIUM WILT OF SORGHUM IN EGYPT. H. A. El-Shafey, M. F. Abd-El-Rahim, M. M. Refaat, E. M. El-Assiuty, and T. H. Abd-El-Moity. Plant Pathology Institute, Agricultural Research Center, Giza, EGYPT.

Acremonium (Cephalosporium) wilt, an important disease of sorghum (*Sorghum bicolor*), was first reported in Egypt in 1979. This disease is caused by *Acremonium strictum* Gams (*Cephalosporium acremonium* Corda). Wilt symptoms start as narrow reddish streaks in the vascular bundle that extend lengthwise on the lower pale green internodes and may involve the entire stalk and the leaf veins. The stalk then dries up becoming yellowish-brown and shrunken. Stalk symptoms may be modified due to invasion by secondary organisms. Wilted plants do not usually form heads, and if any are formed they usually show a 50% reduction in grain weight. Typical wilt infection was obtained by soil or stalk inoculation. The pathogen may grow on root surfaces, spread through the xylem vessels of the roots and stalks, and may even progress into the leaf veins. *A. strictum* infected grain sorghum, but not Sudan grass or broom corn, and may produce black-bundle symptoms in maize. *A. strictum* could produce sorghum wilt even when combined into the soil with *Fusarium moniliforme* and/or *Macrophomina phaseolina*. *Trichoderma harzianum* parasitized *A. strictum* and significantly decreased the incidence of wilt when added to soil infested with *A. strictum*.

A893

EVIDENCE FOR SEXUAL RECOMBINATION IN *ALBUGO CANDIDA*. Q. Liu and S.R. Rimmer, Department of Plant Science, University of Manitoba, Winnipeg, Manitoba, Canada R3T 2N2.

The occurrence of cross-fertilization between homothallic isolates of *Albugo candida* was investigated. Metalaxyl-insensitive (met^-) mutants were obtained from field plots. Segregation in S_1 and S_2 indicated that met^- was controlled by a single dominant gene. Metalaxyl-insensitivity was used as a genetic marker to detect hybrid progeny in co-inoculation between an isolate from *Brassica juncea* and an isolate from *B. rapa*. In the F_1, hybrid isolates were metalaxyl-insensitive and pathogenic only on a common suscept, and thus different from both parental isolates. In the F_2, isolates with recombinant phenotype were identified. Our study demonstrates that cross-fertilization between isolates attacking different *Brassica* species can occur and that sexual recombination can be a mechanism for genetic variation in *A. candida*.

A894

MATING BEHAVIOUR OF *ALBUGO CANDIDA*. Q. Liu and S.R. Rimmer, Department of Plant Science, University of Manitoba, Winnipeg, Manitoba, Canada R3T 2N2.

Techniques for producing oospores of *Albugo candida* in senescent cotyledons as well as in stem and floral hypertrophies of infected *Brassica* plants were developed and used to determine mating behaviour of *A. candida*. Single pustule lines (SPLs), each presumably derived from a uninucleate zoospore, were established from isolates originating from four different *Brassica* species. SPLs were inoculated either alone or in paired combinations on cotyledons and meristems of their homologous hosts. Isolates from *Brassica juncea* and *B. rapa* were self-fertile as SPLs were capable of sexual reproduction when inoculated alone. In contrast, isolates from *B. oleracea* and *B. carinata* were self-sterile as they produce few or no oospores in infected host tissues. Further study with isolates from *B. oleracea* and *B. carinata* is under way to identify isolates of opposite mating types.

A895

HISTOLOGICAL COMPARISON OF LATENT AND ACTIVE INFECTIONS OF SOYBEAN FOLIAGE BY *CERCOSPORA KIKUCHII*. C. E. Orth and W. Schuh. Department of Plant Pathology, Penn State Univ., PA 16802-4507.

Soybean foliage colonization by *C. kikuchii* can result in symptomless latent infections, or symptoms can be expressed as brown / purple angular lesions. Visible symptoms are rarely observed in the field during periods of rapid vegetative growth, GS V2 - R4. The presence of latent infections may be demonstrated by incubating detached leaves in a moist chamber at 25 C after the leaves have been either desiccated for 48 hours or treated with 11.64 % paraquat. Stroma and conidiophores formed from latent infections in 4 days. Leaves of 3 cultivars inoculated and incubated in the growth chamber at 25 C/92% RH developed both active and latent infections. Leaf disks were removed after 36 hours, 3 days, 7 days, and 14 days, embedded in paraffin, sectioned and the sections stained with Calcofluor fluorescent brightener. Leaf sections were examined using fluorescent light microscopy and SEM. Active lesions result from infection through stomates and colonization of intercellular spaces. Latent infections are subcuticular fans which are limited to areas generally less than 1 mm^2. Rapid dying of leaf tissue allowed expansion of the subcuticular mycelia laterally and vertically in the tissues.

A896

CORRELATION OF SYMPTOM TYPE WITH DIFFERENCES IN KERNEL WEIGHT, VOLUME AND DENSITY IN SORGHUM INFECTED WITH MAIZE DWARF MOSAIC VIRUS STRAIN A. J. D. Alexander, R. W. Toler, S. A. Hardy. Tex. Agr. Exp. Sta., College Station, Texas 77843.

Twenty accessions of the 1991 International Sorghum Virus Nursery were mechanically inoculated with maize dwarf mosaic virus strain A (MDMV-A). Infection levels and disease symptoms in the inoculated and control plots were recorded 5 weeks after inoculation and the grain was collected at maturity. The weights and volumes of five 100-kernel lots from each treatment of each accession were measured and kernel densities were calculated. Comparisons of kernel weights, volumes and densities between treatments were made within each accession and standardized. These results were correlated with symptoms expressed by the accessions when infected with MDMV-A. In accessions with mild to moderate mosaic symptoms, kernel volumes were slightly less and kernel densities were slightly greater relative to controls. Kernel volumes were greater and kernel densities were reduced in the accessions which displayed strong mosaic symptoms and general chlorosis. Kernel densities and especially kernel weights and volumes were reduced in the accessions which had severe chlorotic and necrotic symptoms.

A897

ENHANCED SURVIVAL OF WHITE CLOVER STOLONS OVER SUMMER BY APPLICATIONS OF BENOMYL. R. G. Pratt and G. A. Pederson, USDA, ARS, P.O. Box 5367, Mississippi State, MS 39762

In a first-year study, stolons of three white clover (Trifolium repens L.) cultivars were treated biweekly with benomyl during

summer dormancy, from June through August, to determine whether fungicide would influence stolon survival and stand regeneration in the fall. Four replicated plots of each cultivar were sprayed with benomyl and four were not sprayed. Survival of stolons and stand regeneration were evaluated in early September. The three cultivars differed significantly in numbers of stolons and live stolon tips, lengths of live versus dead stolons, and percentages of initial stands regenerated. Applications of benomyl significantly increased values for these parameters across all cultivars without significant fungicide x cultivar interactions. These results suggest that fungal pathogenesis may be an important factor for survival of stolons of white clover during summer dormancy and regeneration of new stands from stolons in the fall.

A898

YIELD LOSSES ASSOCIATED WITH MELOIDOGYNE INCOGNITA AND HOPLOLAIMUS COLUMBUS ON KENAF. J. D. Mueller and S. A. Lewis. Clemson University, Edisto Research and Education Center, P. O. Box 247, Blackville, SC 29817.

Yield losses associated with Hoplolaimus columbus and Meloidogyne incognita on 'Everglades 41' kenaf, Hibiscus cannabinus, were evaluated in field plots treated with eight nematicide regimes. Treatments included: 1) a nontreated check, 2) 0.59 or 3) 1.18 kg/ha aldicarb, 4) 1.18 kg/ha fenamiphos, 5) 0.20 kg/ha acephate, 6) 28 or 7) 56 L/ha 1,3-dichloropropene, and 8) 56 L/ha 1,3-dichloropropene + 0.59 kg/ha aldicarb. Everglades 41 was susceptible to infection by both nematodes, however, yield loss could not be attributed to H. columbus when it occurred alone. Yields were significantly suppressed in the field infested with both nematodes. 1,3-dichloropropene + aldicarb (4.86 t(m)/ha) and the high rate of 1,3-dichloropropene (4.17 t(m)/ha) had greater yields than the other treatments or the check (1.94 t(m)/ha). The high rate of aldicarb was phytotoxic fields resulting in yield reductions.

A899

SIMULATED ACIDIC RAIN EFFECTS ON SOYBEANS AND FROGEYE LEAF SPOT IN GEORGIA. Jerry T. Walker, Daniel V. Phillips, John Melin, and David Spradlin, Department of Plant Pathology, University of Georgia, Griffin, GA 30223.

Simulated acidic rains at pH 2, prepared from mixtures of sulfuric and nitric acid (2:1) and applied 7 times at biweekly intervals to cv. Kirby, did not cause significant changes in plant weights or 100 seed weights during 1989 and 1990. Sulfur levels in leaf tissue were not significantly higher than in plants irrigated with water at pH 6.8. A significant reduction in number of frogeye leaf spot lesions occurred on acidic-irrigated soybeans compared with those receiving only ambient rains. Germination of *Cercospora sojina* conidia was completely inhibited in water at pH 2; germination in water at pH 2.5 and pH 2.9 was 96% after 24 hrs.

A900

A STUDY OF DISEASE DEVELOPMENT IN RHIZOCTONIA SOLANI-INFECTED SUGARBEETS USING MAGNETIC RESONANCE IMAGING. J. M. Halloin,[1] T. G. Cooper,[2] and E. J. Potchen[2]. [1]Agricultural Research Service, USDA, SBCRU, Dept. of Botany and Plant Pathology, and [2]Dept. of Radiology, Michigan State University, East Lansing, MI 48824.

Roots of sugarbeets (Beta vulgaris) were inoculated with the root rotting pathogen Rhizoctonia solani (AG 2-2) and incubated at 30 C for 3 wks. Development of root rot was studied with spin-echo magnetic resonance imaging (MRI) at 1, 2, and 3 wks following inoculation. Transverse plane images of healthy roots were typified by the occurrence of alternating bright and dark rings, with the dark areas corresponding to areas of highest concentrations of sucrose and cellulose. Images of diseased tissues were typified by loss of contrast between the bright and dark rings, and by some loss of overall image intensity. Varying patterns of putative disease development were observed; some roots exhibited progressive broadening of single lesions, whereas others exhibited more disperse occurrences of diseased tissues. Sectioning and visual observations of the roots at the conclusion of the experiments confirmed the differing patterns of disease development. MRI is a useful method for the nondestructive study of disease development in sugarbeet roots and other plant tissues.

A901

EFFECTS OF PYRICULARIA LEAF BLIGHT ON YIELD OF PEARL MILLET FORAGE. J. P. Wilson and R. N. Gates. USDA-ARS Forage and Turf Research Unit, Coastal Plain Experiment Station, Tifton, GA 31793-0748.

The effects of leaf blight, incited primarily by Pyricularia grisea, on forage yield and quality of pearl millet were evaluated in 1990 and 1991. A range of disease severities on susceptible Tifleaf 1 was established by inoculation with P. grisea or application of chlorothalonil. Although supplemental irrigation was provided, no appreciable leaf blight developed in the dry 1990 season. In 1991, plot severities (% foliage with chlorosis and necrosis) ranged from 5 to 32.5% when harvested at 10% anthesis. Leaf blight severity was correlated ($P < 0.01$) negatively with green plot yield, dry matter yield, and digestible dry matter yield and positively with dry matter concentration. In-vitro dry matter digestibility was unaffected by disease. Linear regression indicated that within the range of severities obtained, dry matter yield decreased 15.3% with each 10% increase in leaf blight severity.

A902

DOLLAR SPOT CONTROL ON BENTGRASS USING A BROWN PATCH FORECASTING MODEL TO TIME FUNGICIDE APPLICATIONS. W.H. Shaffer, Dept. of Plant Pathology, University of Missouri, Columbia, MO 65211

This experiment was conducted on a two-year-old 'Penncross' creeping bentgrass putting green located at the University of Missouri Horticulture Research Center, New Franklin, Missouri. The green was mowed daily at 0.5 cm. Disease was from natural infection by Lanzani sp. Ten fungicide treatments and an unsprayed check were evaluated. Treatments were applied as standard protective sprays seven times on a 14 day schedule or three times as predicted by an Envirocaster™ brown patch forecasting model. Forecasted sprays were not repeated until the next infection period after the standard 14 day interval had elapsed. Treatments included per 1000 ft²: Banner 1.10 EC, 4 oz; Banner 1.10 EC 2 oz + Fore 80W 8 oz; Chipco 26019 2F. 4 oz; Daconil 2787 4.17F, 6 oz; and Prostar 50W, 2 oz. All treatments provided significant control of dollar spot except Prostar when compared with the unsprayed check. Daconil 2787 as a forecasted spray provided the least control of dollar spot while Banner plus Fore. as a calendar spray, provided the best control of dollar spot.

A903

THE EFFECT OF MOWING HEIGHT ON THE DISTRIBUTION OF *MAGNAPORTHE POAE* IN THE SOIL PROFILE AND THE DEVELOPMENT OF A DNA PROBE FOR THE DETECTION OF THIS PATHOGEN. K.A. Plumley, B.B. Clarke, B.I. Hillman, and T.E. Bunting. Dept. of Plant Pathology, Rutgers University, New Brunswick, NJ 08903.

Summer patch, caused by *Magnaporthe poae*, is a serious disease of fine turf in the United States. A correlation between reduced height of cut and increased foliar symptom development has been demonstrated. Baron Kentucky bluegrass plots (7.5 m^2) were artificially inoculated with *M. poae* and maintained at either 4 or 8 cm in a randomized, complete block design. Soil cores were removed weekly from April through August 1991 and assayed for the presence of *M. poae* using a wheat seedling bioassay. Underground movement of the pathogen was not correlated with height of cut. Since current diagnostic procedures require 6-8 weeks to identify *M. poae*, we are developing a DNA probe for rapid detection of the pathogen. DNA of *M. poae* has been used to construct a library of clones in the plasmid vector pGEM3Zf+. Clones containing inserts ranging in size up to 5 kbp have been identified and are being screened for specificity.

A904

DISTRIBUTION OF OAK LEAF SCORCH IN THE DELAWARE VALLEY, NEW JERSEY. A. B. Gould, J. M. Wells, and B. B. Clarke. Dept. of Plant Pathology, Rutgers University, PO Box 231, New Brunswick, NJ 08903.

Oak leaf scorch, caused by the bacterium Xylella fastidiosa, is becoming a problem in the New Jersey urban landscape. Relatively little is known about disease development, spread, or means of control. The disease is currently restricted in New Jersey to three counties bordering the Delaware river in the southwestern region of the state. Over a period of two years, healthy and diseased red and pin oak trees in Gloucester, Burlington, and Camden counties were sampled on a seasonal basis for populations of X. fastidiosa in xylem fluid. Bacterial populations were associated with symptom expression in oak and varied seasonally. The relatedness of the X. fastidiosa strain isolated from oak to the causal agents of Pierce's disease of grape and phony peach disease was assessed.

A905

EFFECT OF LENGTH OF IRRIGATION PERIOD ON ROOT ROT OF ITALIAN STONE PINE CAUSED BY *PHYTOPHTHORA PARASITICA*. C. M. Sandlin and D. M. Ferrin, Department of Plant Pathology, University of California, Riverside 92521

The severity of root rot was inversely related to the length of irrigation period (15 to 60 min, 3 times per wk) for container-grown Italian stone pine (*Pinus pinea*) inoculated with *Phytophthora parasitica*. Mean fresh root weights of 4-mo-old inoculated seedlings irrigated for 15 or 45 min were 13.6 and 18.4 g, respectively, and 24.4 and 25.5 g for controls; effects due to inoculation and to the interaction between inoculation and irrigation period, but not to irrigation period alone, were significant ($P < 0.05$). Similar results were obtained for 6-mo-old plants irrigated for 30 and 60 min. In a separate experiment, the percentages of total root length infected were measured for inoculated 10-wk-old seedlings irrigated for 15, 30 or 45 min; the treatment means were 25.3, 21.5 and 13.3%, respectively.

A906

COMBINED BAITING AND ELISA TO DETECT AND QUANTIFY *PHYTOPHTHORA* SPP. IN CONTAINER MEDIA. Z. Banihashemi, J.D. MacDonald and J. Stites. Department of Plant Pathology, University of California, Davis, CA, 95616

Phytophthora-infested potting medium was spread to a thickness of 2-3 mm in shallow containers and saturated with water to a depth of 5 mm above the medium surface. Fresh citrus leaf disks (5 mm dia) were floated on the water for 12-24 hr at room temperature (22-24 C). Some baits were removed, rinsed, blotted and cultured on selective antibiotic medium. Remaining baits were removed, rinsed and incubated an additional 48 hr at room temperature on moist filter paper in humidity chambers. These baits then were macerated in extraction buffer using a glass tissue grinder and the extract was assayed for *Phytophthora* using commercial ELISA kits (Phytophthora kit E, Agri-Diagnostics Associates, Cinnaminson, NJ). There was a positive relationship between the propagule density (determined by dilution-plating of soil suspensions) and the numbers of infected baits (as detected by culture-plating) or the ELISA reaction intensity of bait extracts. *Phytophthora parasitica* could be detected at population densities as low as 0.5 CFU/g dry potting medium by both methods. In experiments with artificially-infested potting media, baits also could detect <0.5 CFU of *P. capsici*, *P. cinnamomi*, *P. citricola*, *P. citrophthora*, *P. cryptogea*, and *P. drechsleri* per g dry medium.

A907

PERITHECIAL FORMATION AND SINGLE ASCOSPORE ANALYSIS OF CALONECTRIA SPATHIPHYLLI. J. Y. Uchida and M. Aragaki. Department of Plant Pathology, University of Hawaii, Honolulu, 96822.

Cross inoculation studies with Cylindrocladium spathiphylli Schoulties et al. revealed that isolates from Heliconia were avirulent to Spathiphyllum, and conversely, isolates from Spathiphyllum were avirulent to Heliconia. Recently, El Gholl and associates successfully mated C. spathiphylli isolates and proposed Calonectria spathiphylli as a new species. Its heterothallic nature was also established, and a Florida tester isolate was designated S+. Of Hawaii field isolates, fifteen from Spathiphyllum were S-, one was S+, six from Heliconia were S-, and two were S+. Crosses between single conidial Hawaii cultures from Spathiphyllum and Heliconia yielded progeny with parental and recombinant characteristics (mating reaction and host specialization), as evidence for hybridization.

A908

COLLETOTRICHUM BLIGHT OF ORCHIDS. J. Y. Uchida and C. Y. Kadooka. Department of Plant Pathology, University of Hawaii, Honolulu, 96822.

Elliptical to circular blossom and foliar spots and rots are the primary symptoms of a serious disease caused by Colletotrichum coccodes on Dendrobium. Depending on flower color, these spots vary from off-white to different shades of brown, darkening to brownish-black. Compact, pale orange to salmon-colored spore masses, in concentric ring patterns are characteristic on diseased flowers in the field. The orange spore masses and absence of soft rot distinguish this disease from Botrytis blight. Colletotrichum coccodes also causes rots on buds, spikes, and leaves, leading to heavy defoliation. Simultaneous leaf infections by Phyllosticta capitalensis and C. coccodes compound the rate of disease development in commercial Dendrobium fields. Pathogenicity of numerous isolates of C. coccodes from several orchid genera has been demonstrated.

A909

MODELING LOGNORMAL AND OTHER SPATIAL DISTRIBUTIONS OF EPIPHYTIC BACTERIA . Vern J. Elliott. USDA-ARS Crops Research Laboratory, POB 1168, Oxford, NC 27565 and Department of Plant Pathology, North Carolina State University, Raleigh, NC 27695

Simulation modeling was used to study the leaf to leaf distribution of epiphytic bacterial populations. The model evaluated bacterial multiplication under differing assumptions about site specific multiplication and survival rates. When exponential growth with a normal distribution of site specific exponential rates was simulated, a lognormal distribution resulted. If a carrying capacity was imposed, the distributions tended towards normal. A lognormal distribution was seen only if time, growth rates, and environmental conditions did not allow a significant number of individual leaf populations to reach the carrying capacity. When a population consisting of multiple species of bacteria with varying multiplication rates was simulated, leaf to leaf populations of total bacteria tended to be normally distributed. This simulation can be used to explain certain population distributions observed in actual sampling experiments.

A910

FACTORS AFFECTING SYMPTOM EXPRESSION OF BACTERIAL RING ROT IN THE FIELD IN COLORADO. A.M. Van Buren and M.D. Harrison, Department of Plant Pathology and Weed Science, Colorado State University, Fort Collins, CO 80523.

Potato seed pieces of cvs. Sangre, Russet Burbank and Russet Norkotah were inoculated with 10^2, 10^4, 10^6 and 10^9 cfu/tuber of Clavibacter michiganensis subsp. sepedonicus (CMS), the causal agent of bacterial ring rot (BRR), and planted at two locations in Colorado. Cv. Sangre was the most susceptible followed by cv. Russet Norkotah then cv. Russet Burbank. However, susceptibility was relative to the location the cultivars were grown. Regression analyses of BRR symptom expression and environmental data show that as air and soil temperatures increase, the percentage of symptomatic plants increase and the number of symptomless days decrease. High solar radiation also appears to increase the number of symptomless days. Low-level CMS inoculations often resulted in symptomless infections in less susceptible cultivars grown in cool (ave. air temp. = 15.9°C) temperatures.

A911

USE OF PROPORTIONAL HAZARDS MODELS FOR THE PREDICTION OF BACTERIAL RING ROT FOLIAR SYMPTOM DEVELOPMENT IN POTATO. A. A. G. Westra, C. P. Arneson, and S. A. Slack. Plant Pathology Department, Cornell University, Ithaca, New York 14853.

Proportional hazards models were used to estimate hazard and survival functions for time to initial symptom appearance (TIS) and time to maximum disease incidence (TMS) for bacterial ring rot foliar symptoms on potatoes. Models were generated from 1988-1990 data on three cultivars (Russet Burbank, Norland, and Norchip) grown in seven locations (CO, ME, ND, NY, OR, WA, and WI). In general, the models predicted a minimum of approximately 50 and 70 days after planting (DAP) for TIS and TMS respectively. However, the time frame during which TIS and TMS were predicted to occur was found to be significantly affected by location, with relative hazard rates following the general pattern WA, CO > ME, NY, OR, WI > ND. For example, survival probabilities of <0.05 occurred as early as 55 and 70-80 DAP for TIS (ME, 1990) and TMS (WA, 1988), respectively, while TIS and TMS survival probabilities of 0.594 and 0.971 were predicted at 110 DAP for Russet Burbank grown in ND (1988). Current evaluations emphasize the integration of these models with environmental data in order to construct predictive models for bacterial ring rot foliar symptoms.

A912

CORRELATION BETWEEN PATHOGEN FITNESS COMPONENTS AND EPIDEMICS OF WHEAT LEAF RUST. J.S. Lehman and G. Shaner, Department of Botany & Plant Pathology, Purdue University, W. Lafayette, IN 47907

Latent period (LP), infectivity (IF), sporulation (SP), and uredinial size (US) and growth rate (GR) characterize host resistance and pathogen fitness; however, their ability to predict field disease levels is not well established. Our objective was to determine the value of each component in predicting disease development in the field. The components were measured in three greenhouse experiments for three *Puccinia recondita* populations on susceptible and partially resistant wheat cultivars. Area under the disease progress curve (ADPC) was based on three field experiments. Correlation coefficients were calculated for individual components and ADPC. Coefficients of determination were calculated for ADPC regressed on components individually or in combination. Except for IF, all components were highly correlated to ADPC. SP and final US were most

closely correlated to disease, and each explained 72-80% of the pathogen-host variation in ADPC. The addition of LP to SP or US in the model increased r2 only slightly. Adding IF did not improve the model. Results suggest SP and final US are highly correlated to disease epidemics and, individually or in combination with LP, may be used to detect partial resistance in wheat cultivars or fitness in rust populations in monocyclic infection experiments.

A913

TWO ALTERNATIVES TO CLASSICAL EPIDEMIOLOGICAL MODELS FOR PREDICTING DISEASE RESISTANCE IN MAIZE TO SOUTHERN RUST *PUCCINIA POLYSORA* UNDREW. O. Rodriguez-Ballesteros and R. A. Frederiksen, Department of Plant Pathology & Microbiology, Texas A&M University, College Station, Tx. 77843-2132

Two simple and practical alternatives are presented to compare maize cultivars in their reaction to Southern rust (*Puccinia polysora* Undrew.). Values for the Area Under the Disease Progress Curve (AUDPC) have been calculated and compared for each cultivar. It was not possible to fit the same epidemiologic model for all cultivars to compare the rates of change of the disease. Utilizing the rate of increase of the AUDPC, calculated from the partial areas under the curve, it was possible to compare all the cultivars under the same model, solving at the same time the problem that several types of curves can have the same AUDPC. Another effective alternative was to calculate the AUDPC at different distances from the foci, and perform paired *t* tests including all the AUDPC calculated. According to our results, these two alternatives were practical and effective.

A914

SPATIO-TEMPORAL DYNAMICS OF TAR SPOT OF LESPEDEZA CAUSED BY *PHYLLACHORA LESPEDEZAE*. J.D. Mihail, Dept. of Plant Pathology, University of Missouri, Columbia, MO 65211.

Korean lespedeza, *Kummerowia stipulacea*, an annual forage legume, is frequently affected by the tar spot disease which results in extensive, premature defoliation. Spatio-temporal dynamics of tar spot have been studied since 1990 in two field plots in mid-Missouri. Each plot was divided into a grid of eight by eight contiguous, non-overlapping quadrats, each 9-m^2. Biweekly samples of three plants per quadrat were taken from each plot for assessment of disease incidence (proportion of symptomatic leaflets) and disease severity (proportion diseased leaf area). In 1990 disease progress for both sites was described well by the logistic function. Positive autocorrelation of disease incidence levels for quadrats separated by up to 9 m was revealed by significantly positive values of Moran's index. Preliminary spatio-temporal autocorrelation analysis suggested that disease incidence is dependent upon spatially and temporally lagged values of disease incidence (autocorrelation component) and extrinsic factors (moving average component).

A915

DYNAMICS OF OOSPORE GERMINATION IN *PLASMOPARA VITICOLA*. Cortesi, P., and Vercesi, A. Istituto di Patologia Vegetale, Università degli Studi di Milano, Milano, Italy 20133.

Oospore germination of *Plasmopara viticola* was studied during 2 yr in leaves of *Vitis vinifera* 'Croatina' at Oltrepò Pavese and *V. vinifera* 'Corvina' at Verona, Italy. Infected leaves of Croatina were overwintered beneath and on the surface of clay soil, while leaves of Corvina were overwintered beneath and on sand. Oospores were removed from these leaves every 2 weeks from January to June and incubated either at 20 C or within a weather shelter in the field. Oospores in buried leaves germinated sooner in response to incubation than oospores from leaves stored on the soil surface. In Feb, buried oospores germinated within 9 da at 20C, whereas surface-stored oospores required 13 da at 20C, and 30 da at field temperatures. Buried oospores germinated in 4 da at 20C in Apr, while surface-stored oospores required 6 da at 20 C and 9 days at field temperatures. When leaves were stored on the surface of sand, maxima of oospore germination curves occurred later than for buried leaves. No temporal differences in the maxima were observed on clay. The supply of germinable oospores in both sand and clay soils was exhausted before bud break of grapevine. However, the maxima of germination curves for surface-stored oospores, both at 20 C and at field temperatures, were more closely aligned with bud break and initial infection.

A916

Taylor's power law and variance-mean relationship of plant diseases. X.B. Yang and D.O. TeBeest. Dept. of Plant pathology, University of Arkansas, Fayetteville, Arkansas 72701.

Theoretical and experimental analysis showed that Taylor's power law, a linear relationship between the logarithms of variance and the population mean, is appropriate when plant disease means are low but not when disease means are in the mid-range and higher. The variance of a disease variable increases exponentially with mean when means are low, but the variance will decline in middle range of means. Variance of a disease has an upper limit (V_{max}) which follows a quadratic function of disease mean (M) as $V_{max}=CM - M^2$, where C is the maximum of a disease variable. Simulations with a spatial-temporal model indicated that the ceiling point of a variance is affected by dispersal capacity and the spatial distribution of a pathogen. The variance-mean relationship of a disease may change if the disease is measured with different scales, i.e. count or percentage. Examinations of the use of percentages to study spatial pattern revealed that this could lead to misinterpretation. Using the same data set, the variance to mean ratio and Lloyd's index of patchiness calculated from actual count data were different from those calculated from percentage (0-100 scale) data.

A917

A COMPARISON OF SPATIAL PATTERNS IN BACTERIAL BROWN SPOT AND RUST IN SNAP BEANS. A.J. Dik, M.K. Clayton and S.S. Hirano. Dept. of Plant Pathology and Dept. of Statistics, University of Wisconsin, 1630 Linden Drive, Madison, WI 53706.

Non-random spatial patterns in bacterial brown spot incidence in snap beans, caused by *Pseudomonas syringae* pv. *syringae* have been detected consistently in previous years and can adequately be described with Autoregressive Integrated Moving Average (ARIMA) models. In 1991, bacterial brown spot and rust, caused by *Uromyces appendiculatus*, were both found present in a bean field in Wisconsin. All leaflets of every plant in five 5 m row segments were rated for both diseases. Disease incidence and mean severity were calculated for each plant and ARIMA models were used to describe the resulting data. Spatial patterns of incidence for the two diseases were quite similar in four out of the five row segments. For both diseases, ARIMA models describing severity data were as complex as, or more complex than, ARIMA models for incidence. Bivariate ARIMA models can be used to explore the relationship between the patterns for the two diseases.

A918

SENSITIVITY OF SIMULATED DEW DURATION TO MICROCLIMATIC VARIABLES AND CROP PARAMETERS. H. Scherm and A.H.C. van Bruggen. Department of Plant Pathology, University of California, Davis 95616.

The duration of leaf surface wetness (LWD) caused by dew was simulated based on the energy balance of individual leaves in a multi-layer canopy. Rates of condensation and evaporation were inferred from the partitioning of available energy into sensible and latent heat fluxes, and dew durations were calculated based on these rates. A formal sensitivity analysis was performed to evaluate the model over a wide range and different combinations of input variables and parameters. Simulated LWD was most sensitive to the atmospheric variables cloud cover and wind speed and the crop variables leaf area index and the position of the leaf in the canopy. Model outputs were generally least sensitive to changes in aerodynamic and spectral crop parameters. Simulated dew durations showed favorable agreement with real-system data for exposed leaves on a wheat crop. Our simulation results should aid in understanding the integrative effects of weather and canopy conditions on the epidemiology of foliar pathogens that require free moisture for infection.

A919

FRACTAL PATTERNS OF *MACROPHOMINA PHASEOLINA* AND *ARMILLARIA* SPP. J.D. Mihail[1], M. Obert[2], S.J. Taylor[1], and J.N. Bruhn[3], [1]Dept. of Plant Pathology, Univ. of Missouri, Columbia, 65211, [2]Institut f. Biochem. & Endokrin., Justus-Liebeig-Univ., D-6300 Giessen, Germany, and [3]Michigan Technol. Univ., Houghton, MI, 49931.

The concept of fractal geometry and the fractal dimension (D) have been used in the description of the disorder of numerous biological branching patterns. The degree to which a particular value of D is characteristic of an organism and the responses of D to environmental and biological perturbations is largely unexplored. Images of mycelial growth initiated from single sclerotia of *M. phaseolina* were used to explore the stability of D for thalli derived from one isolate, for thalli of different isolates, and for thalli challenged by the presence of incompatible isolates. Images of mycelial and rhizomorph growth of *A. ostoyae* and *A. gallica* were used to examine the variability of D among clones and between species. Images were digitized and D was determined using the box-count method. In preliminary analyses of these data, D was a consistent characteristic of certain isolates and may become a useful taxonomic tool.

A920

COLOR DIGITIZATION OF VIDEO IMAGES OF BEAN LEAVES TO DETERMINE THE INTENSITY OF RUST CAUSED BY UROMYCES APPENDICULATUS. L. M. Bacchi, R. D. Berger, and T. A. Davoli, Plant Pathology Department, University of Florida, Gainesville 32611.

A computerized video system was used to digitize the colors of bean leaves infected with Uromyces appendiculatus prior to the onset of disease flecks until leaf death. The healthy tissue between diseased sites retained its normal green color until 4-5 days after the appearance of rust pustules. As yellow halos developed around the pustules 17-18 days after inoculation, the noninfected tissue between the halos changed to various shades of yellowish green, evidence of physiological stress. About 20-22 days after inoculation, the "green island" symptom appeared around pustules in yellowed leaves. The digitized images were used to develop a photographic scale to assess disease. For accurate assessment of disease intensity, especially for the effect of disease on crop yield, allowances must be made for the reduced photosynthetic efficiency in the unthrifty, but noninfected, leaf area.

A921

MODELING MULTIPLE WHEAT DISEASE LOSSES IN KANSAS. M. G. Eversmeyer and C. L. Kramer. USDA-ARS, Dep. of Plant Pathology; Division of Biology, Kansas State University, Manhattan, Kansas 66506, USA.

Average grain yield reductions for wheat in Kansas from 1976 through 1991 for multiple disease epidemics was 21.1%. A different disease caused the most reduction in yield each year from 1979 through 1983. The most serious diseases were wheat soil-borne mosaic, wheat streak mosaic, leaf rust, speckled leaf blotch, tan spot, and root and crown rots. Multiple regression techniques were used to determine those weather variables that explained the most variation in yield reduction attributed to each disease and multiple disease epidemics. Epidemic year was defined as the period from physiological maturity of one wheat crop to the physiological maturity of the next crop. Daily values for maximum and minimum temperature, precipitation, and snow cover were averaged and/or summed over 2-45 days and used in regression analysis to develop models of wheat yield reductions. Variation in yield reduction explained by multiple disease models ranged from 76 to 99%.

A922

FACTORS INFLUENCING AIRBORNE ASCOSPORE COUNTS OF MYCOSPHAERELLA FIJIENSIS, CAUSE OF BLACK SIGATOKA ON BANANA. Wayne M. Thal[1], Hans P. Sauter[2], Harvey W. Spurr, Jr.[1] and Teresa Arroyo[3]. [1]USDA-ARS, Oxford, NC 27605 and NCSU, Raleigh, NC 27695; [2]POB 4084, [3]POB 1173 [2,3]San Jose, Costa Rica.

Multiple regression was used to analyze ascospore counts collected from June 1984 to August 1991 in a Hirst volumetric spore trap, near a commercial banana plantation in Carmen, Costa Rica. Effects of rain, temperature, relative humidity, wind speed, evaporation, sunshine duration and disease level on spore count variables were studied. Nighttime and daytime counts were analyzed separately. Coefficient of determination (R^2) for 1 to 5 variable models ranged from 0.46 to 0.73 for daytime counts and from 0.21 to 0.48 for nighttime counts. The best single explanatory variables were evaporation and hours high relative humidity, for daytime data. For nighttime data, disease level, wind speed variables and hours of rain were the best single variables.

A923

EFFECT OF MASS INOCULATION OF CEREAL CROPS WITH BYDV-PAV-IL ON THE INFECTIVITY OF APHIDS CAPTURED FLYING AT CANOPY LEVEL. A. D. Hewings, USDA-ARS Crop Protection Research Unit, G. E. Kampmeier, C. E. Eastman, and M.E. Irwin, Illinois Natural History Survey, University of Illinois, Urbana, IL 61801

To determine the effect of within-field inoculum sources on the infectivity of BYDV vectors in cereals, two plots (50 rows of 50 hills) of Don oats were planted each spring from 1989-1991. All hills in one plot were inoculated with BYDV-PAV-IL. Periodically one tiller was randomly selected from each row, rated for aphid colonization, and tested for BYDV-PAV, -MAV, and -RPV by TAS-ELISA. A solar-powered suction trap in each plot captured aphids flying at canopy level. Aphids were collected daily and placed individually on oat test plants for two days. Four weeks later, the test plants were assayed for BYDVs. Aphid catches in 1989-1991 were 400, 173, 138, respectively. Captured vectors that transmitted BYDV-PAV varied from 3.7-15% in the inoculated plots and 12.3-19% in the uninoculated plots. Cardinal wheat plots were planted in October, 1990 and tested in a manner similar to the oat plots. No infective aphids were trapped in the fall. In the spring of 1991, 165 aphids were captured of which 8% were infective for BYDV-PAV in the uninoculated plots and 5.3% in the inoculated plots. In all plots only <5% of the aphids transmitted BYDV-MAV or -RPV. The low and relatively similar rates of transmission in both oats and wheat suggest that infective aphids flying into the suction traps had not acquired BYDV in these fields.

A924

EFFECT OF TOBACCO TRANSPLANT SOURCE ON SPOTTED WILT EPIDEMICS IN THE FIELD. A. K. Culbreath, P. F. Bertrand, A. S. Csinos, Dept. of Plant Pathology, and R. M. McPherson, Dept. of Entomology, Univ. of Georgia, Coastal Plain Expt. Stn., Tifton, GA 31793-0748.

Field-grown tobacco (Nicotiana tabacum) transplants from 16 different sites, representing 9 counties in Georgia were assayed by ELISA for tomato spotted wilt virus (TSWV). Seedlings from the 16 sources were transplanted to tobacco fields in each of six different counties to determine the effect of transplant source on incidence of spotted wilt in the field. Only one of 750 samples assayed tested positive for TSWV. Effects of field location on spotted wilt incidence were significant ($P \leq 0.05$), but no significant effects of seedling source or field location X seedling source interaction occurred. There were no consistent differences in final cumulative incidence of spotted wilt among the transplant sources. Across sources of transplants, disease incidence varied greatly among field sites. Across seedling sources, average incidence for the six fields ranged from 2.3% to 12.9%.

A925

GEOGRAPHIC INFORMATION SYSTEMS AND GEOSTATISTICS AS TOOLS IN THE REGIONAL ANALYSIS AND MANAGEMENT OF PLANT VIRUS EPIDEMICS. M. R. Nelson, R. Felix-Gastelum, T. V. Orum, and L. J. Stowell, Department of Plant Pathology, University of Arizona, Sinalopasta, and Pace Consulting.

Management programs for virus diseases with broad alternate host distribution and dynamic vectors are particularly difficult to design and implement. In desert and semi-tropical climates, the typical crop sequencing results in the presence of virus and vector alternate hosts year round. Some produce crops, such as tomatoes, may be subject to infection by ten or more viruses, and very little disease resistance exists. Virus diseases in tomato fields in a region near Los Mochis, Sinaloa, Mexico were the subject of a management program that uses a computer database keyed to map coordinates assigned with the help of a geographic information system (GIS), ARC/INFO. Moving averages of the spatially referenced data were computed using a kriging program (GeoEAS from USEPA). A method of assigning a level of risk to fields was developed based on nearby infection hazards (weeds, other crops, vectors, and urban areas). The risk assessment process was designed to aid growers in the selection of the fields with the least chance of virus infection for the earliest plantings and to increase awareness among growers of risk factors associated with virus diseases. Risk assessments at the time of planting are compared with follow-up assessments of disease incidence.

A926

STRUCTURE OF PAPAYA RINGSPOT VIRUS EPIDEMICS IN PAPAYA IN MEXICO. Mora-A, G., Nieto-A, D., Téliz, D., and C. Lee Campbell. Fitopat. CP, Montecillo, Méx. 56230, and Plant Pathology Dept. North Carolina St. Univ. Raleigh, NC. 27695

Standardized area under disease progress curve (AUDPC), shape parameter (C) of the Weibull distribution function (WDF), and time between transplant date and first symptoms (Xo) caused by papaya ringspot virus type P were selected by principal component analysis. These variables represent 83.5% of the total variability of nine curve parameters estimated from each of 60 epidemics investigated. The remaining variability was explained by standardized apparent infection rate (r_G), scale parameter of WDF, initial and final disease incidence, total duration of the epidemic, and time to reach a 50% incidence. Cluster analysis performed using AUDPC, Xo and C identified the presence of at least four types of epidemics in each of three sites of papaya plantations located in eastern Veracruz. Late transplant dates (September and November 1988) and high density plantations (4444 and 2500 plants/ha) were associated with epidemics having large values of AUDPC and Xo, respectively.

A927

PRINTING DISEASE SEVERITY GUIDES FROM COMPUTER VIDEO IMAGES. L. J. Francl, Department of Plant Pathology, North Dakota State University, Fargo, ND 58105.

Computer programs such as DISTRAIN and DISEASE PRO can help train individuals to rate disease severity accurately and consistently. One disadvantage of these programs, however, is the lack of printed output that can be used for later referral. DISTRAIN was run under the WINDOWS operating system (version 3.0) and a screen image of a diseased leaf was transferred to an art program (PAINTBRUSH), where the image was edited for printing in black and white. A series of such images representing a full range of disease severities was assembled and printed on a laser printer. Images from DISEASE PRO can be similarly manipulated. Plastic lamination of the paper waterproofs it for field use. DISTRAIN and the printed output are an integral part of the wheat foliar disease assessment program in North Dakota.

A928

TOM-CAST ON TOMATOES IN IOWA: EFFECTS OF MODEL THRESHOLD AND SENSOR LOCATION ON CONTROL OF FUNGAL DISEASES. M. L. Gleason[1], S. E. Taylor[2], H. G. Taber[3], and M. L. Hockmuth[1], Departments of Plant Pathology[1], Agronomy[2], and Horticulture[3], Iowa State University, Ames, IA 50011.

Disease Severity Value (DSV) thresholds and weather sensor locations for the TOM-CAST disease-warning model were compared on processing tomatoes (cv. Heinz 6004) near Ames, IA, in 1991. Unsprayed spreader rows were inoculated in Jul with the causal agents of early blight (Alternaria solani), Septoria leaf spot (Septoria lycopersici), and anthracnose rot (Colletotrichum coccodes). TOM-CAST treatments required 6 to 10 fewer fungicide sprays than a control (weekly sprays). Foliar disease was significantly more severe, and yield was significantly lower, for a DSV threshold of 25 than for the weekly-spray control or DSV thresholds of 15 or 20. Incidence of anthracnose was significantly greater than the weekly- spray control for DSV thresholds of 20 and 25, but not 15. With a DSV threshold of 15, disease control and yield were not significantly different from the weekly-spray control whether temperature and wetness sensors were positioned in the tomato field, on adjacent turfgrass, or 1, 5, or 15 mi away.

A930

STRIGA HERMONTHICA DISTRIBUTION MECHANISMS AND THEIR IMPLICATIONS IN CONTROL. D. K. Berner, K. F. Cardwell, and B. O. Faturoti, International Institute of Tropical Agriculture, Ibadan, Nigeria.

To assay wind distribution of seeds, vaseline coated microscope slides, placed at regular intervals from Striga hermonthica plants and suspended at 1, 2, and 3 m heights from trees within and around heavily infested fields, were checked weekly for parasite seeds. Animal dung in and around these fields and local market supplies of sorghum, millet, maize, and cowpea seed from 6 areas of Nigeria were also assayed for S. hermonthica seed concentration. Results showed that wind distribution of S. hermonthica seed was not extensive, as > 90% of the seed were caught 10-20 cm away from individual plants and no seed were caught at distances greater than 12 m from infested fields. Animal dung and local crop seed contained moderate amounts of S. hermonthica seed, and these mechanisms probably account, respectively, for medium and long distance establishment of the parasite. However, as there is apparently no widespread annual influx of S. hermonthica seed by wind, localized eradication may be feasible.

A938

VARIATION IN ISOZYMES AND PCR-AMPLIFIED rDNA RESTRICTION FRAGMENTS AMONG *Fusarium graminearum* ISOLATES. R.P. Woodward and E.L. Stewart. Dept. of Plant Pathology, University of Minnesota, St. Paul, MN 55108.

To study population variation within the cereal pathogen *Fusarium graminearum*, twenty six isolates of diverse geographic origin were examined for enzyme variation using starch gel electrophoresis. Activity was detected for 11 of 21 enzymes screened. Isolate-specific allozyme polymorphisms were detected with six enzymes, but no correspondence with population type (Group 1 or 2) was observed. A preliminary study on a limited number of isolates was done using restriction analysis of the PCR-amplified 5' end of the 25S rDNA subunit (F63/635). Products were digested singly with 5 restriction endonucleases and electrophoresed in 4% agarose gels. With the exception of a single isolate, no variation in banding pattern was observed. In these studies population variation was more easily detected using isozyme analysis than by PCR restriction analysis on the F63/635 rDNA region.

A946

RECENT FINDINGS IN PHYTOPHTHORA CAPSICI / P. TROPICALIS COMPLEX. M. Aragaki and J. Y. Uchida. Department of Plant Pathology, University of Hawaii, Honolulu, 96822.

The taxonomy and nomenclature of Phytophthora capsici Leonian and P. capsici-like fungi, including 'P. palmivora' MF4, have been studied in several laboratories. Oudemans and Coffey (1991) showed that P. capsici isolates obtained from various sources separate into CAP 1, CAP 2, and CAP 3, based on isozyme analysis. Isolates in CAP 1 appear to be P. capsici Leonian, whereas CAP 2 and CAP 3 isolates appear to be 'P. palmivora' MF4. Morphological studies in our laboratory of Oudemans' CAP 1, CAP 2, and CAP 3 isolates support the proposition that CAP 1 isolates belong to P. capsici Leonian, and that CAP 2 and CAP 3 isolates are readily distinguished from CAP 1.

A962

CHARACTERISATION OF COMPONENTS ON THE SURFACE OF THE FLAGELLA OF *PHYTOPHTHORA CINNAMOMI*. M. Cope and A. R. Hardham, Plant Cell Biology Group, Research School of Biological Sciences, Australian National University, GPO Box 475, Canberra, A.C.T. 2601, Australia.

Monoclonal antibodies (MAbs) have been raised against surface components on the flagella of glutaraldehyde fixed zoospores of *Phytophthora cinnamomi*. One MAb, designated Zf-1, binds to the surface of both the anterior tinsel and posterior whiplash flagella as well as to an intracellular component in permeabilized zoospores. Zt-1 and Zt-2 recognise the surface of the tinsel flagellum only. Zg MAbs bind to the mastigonemes on the tinsel flagellum and to packets of mastigonemes inside zoospores. Dot-blot analysis has shown that binding of the Zt group of antibodies is abolished by pretreatment with pronase or sodium periodate indicating that the antigen is a glycoprotein. These three groups of antibodies have been used to study flagella development during zoosporogenesis using cryomicrotomy and immunofluorescence microscopy.

A968

CONTROL OF CONIDIA GERMINATION AND APPRESSORIA FORMATION OF *MAGNAPORTHE GRISEA IN VITRO*. Y.H. Lee and R.A. Dean. Department of Plant Pathology and Physiology, Clemson University, Clemson, SC 29634.

Conidia germination and appressoria formation of *Magnaporthe grisea* were evaluated *in vitro* on several hydrophobic substrata in the presence of various metabolic inhibitors and nutrients. More than 90% of conidia adhered on all substrata tested within 1 hr. On plastic cover slips 50% of conidia germinated within 1.5 hr and by 3 hr more than 95% had germinated. Of germinated spores 80% formed appressoria on all substrata except on modified polystyrene (27%). The addition of complete medium to conidia had no effect on germination or appressoria formation. Actinomycin D (10,000ppm; 100X usual dose) did not affect the conidia germination, but slightly inhibited appressoria formation if added within 2 hr after incubation. Cycloheximide (3.55 μM) completely prevented conidia germination and appressoria formation if added within 1 and 2 hr, respectively. The data is inconclusive for *de novo* transcription, but suggests that protein synthesis is necessary for infection structure formation. Transcripts may be stored in the spores. However, once a germinated spore has committed to infection structure formation, it is unable to revert to vegetative growth.

A969

MECHANISMS OF TUFT FORMATION IN *RHIZOCTONIA SOLANI* AG 8. H. A. Yang, K. Sivasithamparam and P.A.O'Brien*. Soil Science and Plant Nutrition, School of Agriculture, The University of Western Australia, Western Australia 6009; *Department of Biological Science, Murdoch University, Western Australia 6150

H factors, culture morphology and mycelial pigmentations were used as markers to study the mechanisms of tuft formation in *R. solani* AG 8 which causes bare-patch disease of cereals. Heterokaryotic hyphal tip cultures containing both H factors of the contributing homokaryons were obtained from some tufts, confirming the association of tuft formation with heterokaryon formation. However, the majority of the hyphal tip cultures were homokaryotic resembling one of the contributing cultures. A two-colored tuft was formed when a brown mycelium isolate was paired with a white mycelium isolate. Hyphal tip cultures from the brown side of the tuft were of brown mycelium isolate type, and those from the white side were of white mycelium isolate type. The non-heterokaryotic mycelia in the tufts were attributed to as the result of transient heterokaryon effects which has been reported in *Verticillium dahliae*.

A972

CYTOKININ SENSITIVITY OF GRASS ENDOPHYTES: IN VITRO GROWTH INHIBITION. K.D. Gwinn and D.B. Chalkley, Department of Entomology and Plant Pathology, University of Tennessee, Knoxville, TN 37901-1071.

Interactions between grasses and endophytic fungi of the tribe Balansieae (Clavicipitaceae) vary from mutualistic to pathogenic. Previous experiments have shown that growth of one isolate of Acremonium coenophialum is sensitive to cytokinin levels in growth medium. The objective of this work was to determine if cytokinin inhibits growth of other members of the tribe Balansieae. Radial growth of mycelium on cornmeal-malt extract agar containing 0, 20, 40, 80, or 100 μM kinetin was measured for several members of the tribe. Growth of several isolates of Acremonium coenophialum from tall fescue was inhibited strongly ($\geq$ 75%) by 80 and 100 μM kinetin. Growth of several isolates of Epichloe typhina, A. typhinum, and a closely related epiphytic species, Atkinsonella hypoxylon, was less affected ($<$ 30% inhibition at 100 μM).

A973

COMPARISON OF INTRACELLULAR WITH EXTRACELLULAR PECTINASE ACTIVITY IN ASPERGILLUS FLAVUS. R. L. Brown, T. E. Cleveland and J. Cary. USDA, ARS, Southern Regional Research Center, P.O. Box 19687, New Orleans, LA 70179; R. A. Dean and M. Whitehead, Clemson University, Clemson, SC 29634.

Pectinase activity has been correlated with the higher virulence exhibited by some Aspergillus flavus strains in cotton bolls. In the current study, pectinases were detected both intracellularly and extracellularly when high- and low-virulence strains were grown on pectin. Intracellular pectinase activity was demonstrated by the low-virulence strain even when grown on glucose, although glucose repressed extracellular activity in this strain. SDS-PAGE of all filtrates shows the presence of one band in the 49kD region of all except that from the glucose-incubated, low-virulence culture. IEF/pectin overlay will be used to demonstrate the enzymatic composition of both this putative pectinase band and the intracellular fractions. Results suggest that the relationship of intracellular to extracellular activity may provide insights into fungal pectinase expression during plant infection.

A975

EFFECT OF DIALYZED EXTRACTS AND WASHED PELLETS FROM UNDIFFERENTIATED THALLI GROWN ON A SYNTHETIC MEDIUM AMENDED WITH ETHANOL ON THE ACTIVITY OF ACID PHOSPHATASE FROM AN ISOLATE OF ARMILLARIA. M. O. Garraway, Dept. of Plant Path., The Ohio State University, Columbus, OH 43210.

Seven-day old Armillaria thalli (ATCC # 24648) grew 2-3 times more rapidly after 48 hr of incubation in the dark at 24 C on ethanol-amended (500 ul/l) culture media than on non-amended media. Similarly, the concentrations of amino acids, phenols, phosphate and proteins in cell free extracts were significantly increased in thalli incubated on ethanol-amended media. Acid phosphatase (AP) activity increased after 48 hr in thalli incubated on ethanol-amended media, but it decreased in thalli incubated on media without ethanol. AP activity was unaffected when dialyzed extracts were mixed in a 1:1 ratio with a preparation of the same extract that was boiled to inactivate the enzyme. But AP activity decreased by more than 50% when unboiled dialyzed extract was mixed with a pellet fraction from Armillaria mycelium sonicated in 1M KOH, suspended in 70% ethanol then washed in distilled water. Inhibition of AP activity by the washed pellets was reversed by boiled dialyzed extracts and by certain amino acids, carbohydrates, phenols and proteins. Ethanol stimulation of growth of Armillaria thalli causes accumulation of solutes that prevent inhibition of AP activity.

A976

THE EARLY STEPS OF *USTILAGO HORDEI* MATING. A.D. Martinez-Espinoza, S. A. Gerhardt, and J. E. Sherwood. Dept. of Plant Pathology, Montana State University, Bozeman 59717.

Ustilago hordei is a pathogenic basidiomycete which causes covered smut of barley. Mating of non-pathogenic sporidia results in the development of pathogenic mycelium. The early stages of mating involve recognition of opposite mating-type cells resulting in the formation of conjugation tubes by both cell types. These tubes fuse and a dikaryotic mycelium emerges from this conjugation bridge. Conjugation tube growth is directed toward the tip of conjugation tubes arising from cells of the opposite mating type. Temperature, pH and light affect the timing and frequency of mating. Sporidia separated by a dialysis membrane are capable of inducing conjugation tube formation by cells of the opposite mating type, indicating the involvement of diffusible, small molecular weight mating factors. A critical analysis of the early steps in mating of *U. hordei* will lead to a better understanding of the regulation of the conversion of this fungus from a non-pathogenic to a pathogenic form.

A978

INTRACELLULAR pH ALTERATIONS DURING BACTERIAL HR: A CONFOCAL LASER SCANNING MICROSCOPY STUDY. S. Pike, P. Popham, A. Novacky, and J. Freeman*, Plant Pathology, University of Missouri, Columbia 65211 and *Monsanto Chem. Co., St. Louis, MO 63198.

During the bacterial hypersensitive reaction (HR) suspension-cultured cells (SCC) release K^+ into the surrounding medium and the medium alkalinizes. Atkinson *et al.* (*Plant Physiol.* 79:843, 1985) hypothesized that the interior of the cell also acidifies, but the hypothesis has never been substantiated in living cells. Using a fluorescent pH probe (SNARF-1) and confocal laser scanning microscopy (CLSM) we have demonstrated that the cytosol of SCC does acidify during HR. Cotton SCC (*Gossypium hirsutum*, cv Im 216) were loaded with the pH-probe prior to treatment with the nonhost bacterial pathogen *Pseudomonas syringae* pv. *tabaci*. Acidification (change in fluorescence) was observed within 1 h. By 18 h most bacterially-treated cells exhibited cytosolic acidification in contrast to media-treated controls. Fluorescent probes in conjunction with CLSM will facilitate the study of early cytosolic and vacuolar changes during HR.

A979

ELECTRICAL MEMBRANE POTENTIALS OF COTTON SUSPENSION-CULTURED CELLS EXPOSED TO HYPERSENSITIVE REACTION (HR) -INDUCING BACTERIA. P. Popham, S. Pike, and A. Novacky, Plant Pathology, University of Missouri-Columbia, Columbia 65211.

The membrane potential (E_m) of cotton cotyledon cells begins to depolarize 2 h after inoculation with *Pseudomonas syringae* pv *tabaci*. However, cotyledonary tissue can not be measured at an earlier time because the inoculation and tissue equilibration processes require 2 h. To measure E_m at the time of bacterial-plant cellular contact, cotton (*Gossypium hirsutum* cv IM216) suspension-cultured cells (SCC) were impaled with electrodes prior to exposure with *P. s.* pv *tabaci*. E_m measurements obtained from SCC ranged from -115 to -175 mV prior to treatment with HR-inducing bacteria. The energy-dependant portion of E_m can be stimulated (with fusicoccin) or collapsed (with CCCP). After treatment with *P. s.* pv *tabaci* cell membranes slowly hyperpolarized for 20 to 30 min and then began to depolarize. E_m responses will be compared with those from cells of cotyledonary tissue.

A980

EXPRESSION OF THE BACTERIAL HYPERSENSITIVE REACTION IN COTTON PROTOPLASTS. E. Hoyos, S. Pike, P. Popham, and A. Novacky, Dept. of Plant Pathology, University of Missouri, Columbia MO 65211.

The bacterially-induced hypersensitive reaction (HR) is characterized by several physiological alterations of the plant cell, including membrane depolarization and potassium efflux, parameters which suggest altered membrane channel activity. In order to study channel activity, plant protoplasts must be isolated. However, several laboratories have reported difficulty inducing HR in isolated protoplasts. In our experiments, initial measurements indicate that a non-host bacterial pathogen could induce HR; unfortunately, subsequent experiments were not consistent. Additionally, we also isolated protoplasts from bacterially-inoculated cotton (*Gossypium hirsutum*) cotyledons; these consistently die at a faster rate than protoplasts isolated following water or saprophytic bacterial treatments. We will use the patch clamp technique initially on protoplasts isolated from pre-inoculated tissues to determine the possible role of potassium channels during bacterially-induced HR.

A981

EXPRESSION OF A COWPEA EARLY NODULIN GENE, VUB, WITH HOMOLOGY TO WOUND INDUCED HRGPS, IN INEFFECTIVE AND SENESCENT NODULES. A.T. Trese and D.K. Purdom, Environmental and Plant Biology, Ohio University, Athens, OH 45701.

In developing cowpea nodules the early nodulin gene VuB is induced within 36 hours after inoculation. The 1200 bp VuB cDNA clone has been sequenced and a peculiar stretch of 29 amino acids, consisting entirely of proline and serine, was noted within the open reading frame. A DNA data bank search indicated homology of this region with several HRGP genes from bean, inducible by wounding and fungal infection, but no other significant homology. Although bean nodules contain a VuB homologous transcript of the appropriate size, VuB is not expressed in wounded stems or roots. The mutant cowpea line IC-1 forms nodules that appear as normal until about 14 days, after which they deteriorate rapidly. In these ineffective nodules VuB mRNA remains at elevated levels during nodule senescence, while expression declines in wild type nodules. We are assaying expression of VuB in other incompatible and senescent plant-rhizobium combinations with the intention of assessing the possible role of VuB in symbiosis/pathogenesis.

A982

STIMULATORY VOLATILES FROM GERMINATING CANADA THISTLE SEED AND SYSTEMIC INFECTION FROM SEED INOCULATED WITH TELIOSPORES OF *PUCCINIA PUNCTIFORMIS*. R. C. French and S. E. Nester, FDWSRU, USDA, Ft. Detrick, MD 21702 and R. G. Binder, WRRC, USDA, Albany, CA 94710.

Volatiles collected from germinating Canada thistle (*Circium arvense* L.) seed stimulated germination of teliospores of Canada thistle rust. Extracts of volatiles from Tenax columns increased germination to 6% at day 1, to 37% at day 3, control 0%. Germination on check plates held at the exit port of the germination chamber was 24% at day 1, 48% at day 3, controls 0% (14 days, 18C). Analyses indicate the active material to be related to that described by Binder et al. (Phytopathol. 69:802-5,'79) for teliospores of safflower rust, *Puccinia carthami*. Volatiles from germinating seeds are assumed to be endogenous compounds, not induced by cutting or heat injury. Canada thistle seed inoculated with teliospores were systemically infected after 5 wks. The first shoot appeared normal but was followed a taller, etiolated shoot covered with orange spermagonia. This infection pattern is identical with that from inoculated buds of root cuttings. This may be the first report of systemic infection resulting from the inoculation of seed.

A983

RESISTANCE OF *HORDEUM VULGARE* AND *H. SPONTANEUM* TO ISOLATE 89-3 OF *PUCCINIA HORDEI*. Y. Jin[1], B. J. Steffenson[1], D. M. Wesenberg[2] and H. E. Bockelman[2]. [1]Dept. of Plant Path., North Dakota State Univ., Fargo, ND 58105. [2]USDA-ARS, Aberdeen, ID 83210.

The Moroccan isolate of *Puccinia hordei*, 89-3, possesses a wide range of virulence. This isolate is virulent on nearly all barley genotypes reported to possess resistance to *P. hordei*, except Estate and Aim which possess the *Rph*3 gene and exhibit an intermediate level of resistance (infection types [ITs] 12 and 21). National Small Grains Collection accessions of *Hordeum vulgare* (1,997 entries) and *H. spontaneum* (885 entries), mostly from the Mediterranean region and parts of North Africa, were evaluated for their seedling reaction to isolate 89-3. Fifty-seven accessions of *H. vulgare* from Egypt and 10 accessions from other countries exhibited low ITs. Resistance was common in *H. spontaneum* as 222 accessions exhibited low ITs. Accessions exhibiting low ITs also were evaluated against isolates of *P. hordei* with virulence on *Rph*3. The data suggest that most of the resistant lines of *H. vulgare* possess the *Rph*3 resistance, whereas the resistant lines of *H. spontaneum* possess other unidentified *Rph* genes.

A984

HYDROXAMIC ACIDS, NATURAL DEFENSE CHEMICALS IN CORN AGAINST *FUSARIUM MONILIFORME*. M.D. Richardson, C.W. Bacon, D.M. Hinton, and W.J. Chamberlain. USDA-ARS, P.O. Box 5677, Athens, GA 30613

Corn, (*Zea mays* L.) infected with *Fusarium moniliforme* J. Sheld. is associated with several animal toxicoses and human esophageal cancer. The pathogenicity of *F. moniliforme* to five cultivars of corn was determined using a seedling bioassay and cultivars were scored as susceptible, partially susceptible, or resistant. Cyclic hydroxamic acid (1.4-benzoxazin-3-one) derivatives naturally occur in corn in various amounts and exhibit biological activity against a range of organisms. A test of these compounds on agar media indicated that they are toxic to *F. moniliforme*. Resistant and susceptible corn cultivars were analyzed for two cyclic hydroxamic acids (DIMBOA and DIBOA) during germination and early growth. The susceptible and resistant cultivars contained similar amounts of DIMBOA, but the resistant corn had increased DIBOA levels compared to the susceptible cultivars. These data suggest that hydroxamic acids are potentially natural deterrents to *F. moniliforme* infection in corn and that resistance was related to DIBOA concentrations in the seedlings.

A986

JUGLONE PRECURSOR IS A MAJOR POLYPHENOLIC COMPOUND IN PECAN LEAVES. R.C. Gueldner[1], C.C. Reilly[2], I.E. Yates[1] and M.T. Smith[2], [1]USDA, ARS, P.O. Box 5677, Athens, GA, [2]USDA, ARS, P.O. Box 87, Byron, GA

Leaf extracts of pecan, *Carya illinoensis* (Wangenh.) C. Koch, were analyzed by HPLC to separate mainly polyphenolic components on a C-18 reverse phase column. The chemical nature of the profile components were computer matched by comparing the UV spectra to standard compounds in a library. A major component of leaf samples was the precursor to juglone (4-glucoside of 1,4,5-trihydroxynaphthalene). Juglone precursor in leaves infected with pecan scab, *Cladosporium caryigenum* (Ellis & Langl.) Gottwald, as compared to uninfected leaves was compared; juglone was not detected. Juglone was barely, if at all detectable, in pecan leaves regardless of seasonal maturation. An inverse relationship existed between cultivar resistance to pecan scab and the abundance of the juglone precursor in the 6 cultivars examined. Quercitin glycosides were the major flavonoid components.

A987

INOCULATION WITH *Hypoxylon mammatum* DELAYS WOUND CLOSURE IN *Populus tremuloides*. Bruna Buccirelli[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.

Anthocyanin accumulated in 5 days on tissue cultured plantlets of *Populus tremuloides* whether sites were wounded or wound-inoculated with *Hypoxylon mammatum*. In wounded plantlets, undifferentiated callus grew and completely covered the wound site by day 8. In wound-inoculated plantlets, callus failed to grow. Instead, the tissue on the surface of the wound-inoculated site initially dehydrated, and the mycelium formed a dense mat that completely covered the wound site within 8 days. The mycelium in the vicinity of the wound site failed to spread along the intact stem after 37 days. In wound-inoculated sites, where the mycelium dehydrated and failed to grow, callus was produced but was delayed. In these instances callus began to accumulate by day 8, and by day 37 undifferentiated callus covered the portion of the wound site devoid of mycelium.

A989

MUTATIONAL ANALYSIS OF PATHOGENICITY IN COCHLIOBOLUS HETEROSTROPHUS. P. R. Thorson, L. K. Lyngholm, and C. R. Bronson, Dept. of Plant Pathology, Iowa State University, Ames, IA 50011.

The goal of this research is to identify genes involved in the pathogenicity of *Cochliobolus heterostrophus* to maize. Survivors of ultraviolet light and diepoxybutane mutagenesis are being screened for reduced ability to cause lesions on maize and for altered ability to produce cell wall degrading enzymes that may be involved in pathogenesis. Out of 26,000 survivors, 16 were identified that produce smaller lesions than wild-type on maize seedlings, yet have growth rates and morphologies similar to wild-type on complete and minimal media. These mutants are being crossed to determine whether their phenotypes are under genetic control and how many loci are involved. Thirty-nine survivors have been identified with altered ability to make cell wall degrading enzymes; to date, the phenotypes of 12 of these have been shown to segregate as if controlled by single genes. These mutants are now being tested on maize to determine whether altered enzyme production affects pathogenicity.

A991

LIGNIFICATION OF POTATO TUBER TISSUE IN RESPONSE TO PATHOGENIC AND NONPATHOGENIC STREPTOMYCES SPP. F. Spooner and R. Hammerschmidt. Department of Botany and Plant Pathology, Michigan State University, East Lansing, MI 48824.

Potato scab disease, caused by Streptomyces scabies and other pathogenic Streptomyces spp., is characterized by corky lesions produced when hyphae repeatedly breach layers of a suberin/lignin barrier deposited by the host tissue to restrict the pathogen.

Potato tuber disks were used to determine whether lignification was specific to different isolates of Streptomyces and whether the response would vary between resistant and susceptible varieties of potato. Tuber disks were inoculated with water agar plugs containing spores and incubated in the dark for one to twenty-one days prior to determination of lignin on the inoculated tissue surfaces by the thioglycolic acid method. The amount of lignin deposited in the tissue differed with resistant and susceptible potato varieties and the response was different for pathogenic and nonpathogenic isolates of Streptomyces.

A992

NITRATE AND PLANT EXTRACTS MODULATE CERCOSPORIN ACCUMULATION. M. Ehrenshaft and R.G. Upchurch. ARS\USDA and Dept. of Plant Pathology, NCSU, Raleigh, 27695-7616.

Cercospora kikuchii infects all aerial portions of soybean plants causing lesions and purple staining. The characteristic purple staining is due to accumulation of the pigmented polyketide phytotoxin cercosporin. *C.kikuchii* field isolate strain PR synthesizes high levels of toxin in culture. A pathogenic, spontaneous derivative of PR, designated S2, accumulates high levels of toxin in potato dextrose broth (PDB) but very little toxin in CM broth (a complete medium containing both defined and undefined components). Media studies showed that nitrate is the prime inhibitor of S2 toxin production. Nitrate also down-regulates toxin production in strain PR, but to a lesser degree. Protease and heat-denaturation treatments indicated that a water soluble protein(s) present in both soybean leaves and meal can restore toxin production to S2 cultured in CM broth. To determine if the soybean protein(s) act directly on the media to remove the inhibitory nitrate, CM broth was preincubated with extract and then autoclaved. This preincubated/denatured extract failed to induce toxin production.

A993

BARLEY MILDEW IN EUROPE: POPULATION STRUCTURE BASED ON VIRULENCE AND RAPD VARIATION. McDermott, J.M., K. Müller and M.S. Wolfe. Phytopathology Group, Swiss Federal Institute of Technology, CH-8092, Zürich, Switzerland.

Isolates of the barley mildew pathogen collected from the air spora over Europe were tested for virulence against 12 host resistance alleles and for molecular variation using RAPD-based PCR. The structured sampling scheme was used to select a set of 14 regional samples or metapopulations. A large amount of genetic variation for both virulence and random DNA loci is distributed widely over Europe, and a significant amount of this variation can be partitioned into the effect due to subdivision of the population. Although there was a large range of pathotypes, these were irregularly distributed probably due to heterogeneous use of host resistances. Gametic disequilibrium was readily detected among virulences, distorting pathotype frequencies. The distribution of RAPD variation was more consistent with neutral independent loci.

A994

FUNCTIONAL ANALYSIS OF AVIRULENCE GENES IN *XANTHOMONAS CAMPESTRIS* PV. MALVACEARUM. Y. Yang and D. W. Gabriel. Plant Molecular and Cellular Biology Program, University of Florida, Gainesville, Florida 32611.

Xanthomonas campestris pv. malvacearum strain H (XcmH) contains at least 12 avirulence (*avr*) genes which belong to a multigene family in the genus *Xanthomonas*. To study the possible functions of these *avr* genes, marker exchange-eviction mutagenesis has been carried out in XcmH by use of a suicide vector carrying *X. citri pthA* gene (a member of the same gene family) mutated with a *npt-sacB-sacR* cartridge. More than 120 marker exchange mutants were generated and 40 of them were tested on 10 differential cotton resistance lines. Some mutants were subjected to further marker exchange mutagenesis after eviction of *npt-sacB-sacR* marker. The mutants causing altered cultivar-specific avirulence in comparison to XcmH were chosen for Southern analysis. One of plasmid-borne *avr* genes, *avrb6*, has been previously shown to have pleiotropic avirulence and pathogenicity functions (De Feyter and Gabriel, Molec. Plant-Microbe Interact. 4:423-432). A marker exchange mutation of *avrb6* resulted in a loss of both functions. Surprisingly, a mutation in a different chromosomally encoded *avr* gene resulted in a similar loss of both functions. The avirulence functions of *avrB4* and *avrb7* also appear to have been affected by mutations of other *avr* gene loci. Mutations in some chromosomal *avr* genes resulted in avirulence on the susceptible cotton line Ac44, which contains no known resistance gene. A mutant with at least six *avr* genes evicted not only lost avirulence function on many resistant cotton lines, but also lost much of its pathogenicity. These results suggest possible interactions among these *avr* genes and an essential role of these genes in conditioning pathogenicity in *X. c.* pv. malvacearum.

A995

CONSTRUCTION OF MUTANTS OF Pythium sylvaticum WITH STABLE, DOMINANT ANTIBIOTIC TOLERANCE. F.N. Martin and C.R. Semer, Plant Pathology Department, University of Florida, Ganiesville, FL, 32611.

Sublethal enrichment was an effective means for selection of mutant isolates with stable antibiotic tolerance to the aminoglycoside antibiotics kanamycin and gentamicin. While cross tolerance of isolates to these antibiotics was observed, the kanamycin tolerant isolates were still sensitive to hygromycin B. Analysis of several of the kanamycin tolerant isolates by crossing with wild type isolates of the opposite mating type and back crossing of F_1 progeny indicated that the tolerance was dominant and nuclear encoded. Similar results were obtained for mutants tolerant to tetracycline; however, a low percentage of the F_1 progeny were sensitive to the antibiotic. The usefulness of these markers for investigating protoplast fusion and sexual reproduction will be discussed.

A996

ANALYSIS OF PROTOCOLS FOR DIRECT TRANSFORMATION OF *AGROBACTERIUM TUMEFACIENS*. H. Chen[1], R. S. Nelson[2], and J. L. Sherwood[1]. [1]Department of Plant Pathology, Oklahoma State University, Stillwater, OK 74078-9947, and [2]Plant Biology Division, The Samuel Roberts Noble Foundation, Inc., Ardmore, OK 73402.

Freeze-thaw transformation provides a simple and quick method to directly transform *A. tumefaciens* with plasmid DNA (Holsters, M. et al., Mol. Gen. Genet. 163:181-187). The procedure of An, G. et al. (Plant Mol. Biol. Manual, A3:1-19, Kluwer Academic Publishers) and modifications of this procedure were compared. Each of six different plasmids was used to transform *A. tumefaciens* strains LBA4404, GV3850 and EHA101. A ten-fold increase in transformed colonies per μg DNA was obtained by freezing with liquid nitrogen versus dry ice. Additional modifications increased transformation frequencies 50 to 80 fold. Restriction maps were made of plasmids extracted from 40 transformants obtained from four independent transformations. No rearrangements were found in any of the analyzed plasmids.

A997

INTRODUCTION OF CHITINASE GENES INTO CARROT AND CUCUMBER VIA AGROBACTERIUM-MEDIATED TRANSFORMATION: METHODOLOGY AND APPLICATIONS. S.H.T. Raharjo, M. Hernandez and Z.K. Punja, Dept. of Biological Sciences, Simon Fraser University, Burnaby, British Columbia, Canada V5A 1S6

The role of chitinases in reducing pathogen development in plants can be elucidated through enhanced constitutive expression or by development of transgenic plants. Methods for regeneration and transformation of carrot (*Daucus carota* L.) and cucumber (*Cucumis sativus* L.) with chitinase genes were developed in this study. Embryogenic calli from carrot cotyledons and cucumber petioles on MS medium with 2,4-D (0.5 μM) and 2,4-D/BA (5/5 μM), respectively, were transferred to growth regulator-free media and plantlet development was obtained after 8-12 wk. Disarmed *A. tumefaciens* strains (MOG101, MOG301 and EHA105, courtesy of MOGEN nv), containing a binary plasmid with CaMV 35S promoter, a chitinase gene, and the NPTII gene, were grown to 10^8 cells/ml. Tissues were exposed to bacteria for 3-5 min, cocultivated for 3 days, and transferred to regeneration medium with kanamycin and carbenicillin. Confirmation of transformation is pending analysis of NPTII and Southern blot analysis. The transgenic plantlets will be challenged with filamentous fungi to evaluate whether enhanced resistance to diseases is obtained.

A998

AN INOCULATION TECHNIQUE THAT DETECTS INTERMEDIATE REACTIONS TO SOUTHERN STEM CANKER IN SOYBEAN CULTIVARS. D.V. Phillips and J.B. Manandhar, Department of Plant Pathology, University of Georgia, Griffin, GA 30223

Previous greenhouse inoculation procedures using seedlings could not detect the intermediate levels of resistance to stem canker observed in most cultivars in the field. Procedures using older plants were more sensitive but required nearly three months to complete. The procedure described here detects intermediate reactions and is completed in 6 weeks or less. The stem tip is removed above the first trifoliolate node and a suspension of conidia is injected into the end of the cut stem. Cultivars with known reactions are included as standards. Three to four weeks later, depending on symptom development in the standards, plants are evaluated for damage to the leaves and stems originating at the first trifoliolate node, the unifoliolate node, and the cotyledonary node. Mean ratings (1 to 10 scale) of cultivars correlate well with resistance levels observed under field conditions.

A999

COMPARISON OF WHEAT SEEDLING LEAF REACTIONS TO DEOXYNIVALENOL IN RELATION TO WHEAT SCAB (FUSARIUM GRAMINEARUM) RESISTANCE CLASSES. L. L. Scholbrock, B. K. Fleener, and J. A. Berry, Pioneer Hi-Bred Int'l, Inc., P.O. Box 1004, Johnston, IA 50131.

Fusarium head blight or wheat scab is caused by Fusarium graminearum which produces many secondary metabolites including the mycotoxin deoxynivalenol. Wheat varieties previously classified as resistant, moderately resistant, and susceptible to Fusarium graminearum were grown to the five leaf stage and infiltrated with various concentrations of purified deoxynivalenol using a Hagborg device. Seven days after infiltration, symptoms on the leaf blades varied from moderate to severe according to variety and different susceptibility and resistance to deoxynivalenol. The field resistance class of each wheat variety to Fusarium graminearum was established from previous experiments. A comparison of the two sets of data indicated that the wheat varieties can be sorted into three general wheat scab resistance classes using the described deoxynivalenol infiltration method. The high cost of purified deoxynivalenol may limit the usefulness of this technique.

A1000

TRANSFORMATION OF BEAN APICAL DOME CELLS BY MICROPROJECTILE BOMBARDMENT. Franzine D. Smith[1], Alba Jofre-Garfias[2], and John C. Sanford[1]. [1]Dept. of Hort. Sci., Cornell Univ., NYSAES, Geneva, NY 14456, and [2]Dept. Ingenieria Genetica, Unidad Irapuato, CINVESTAV, IPN, 36500 Irapuato, Gto. México.

Cells of the apical dome of pinto bean (*Phaseolis vulgaris*) were transformed using the helium-driven microprojectile bombardment device. Apical domes were excised from seeds soaked in water, positioned with the dome upward in agar medium and then bombarded with DNA (pBI426 or pUC118) coated particles. Transient expression of β-glucuronidase (GUS) activity was determined five days after bombardment and domes were transferred to 100 μg/ml kanamycin (km) selective medium. Optimal transformation was obtained with a helium pressure of 1200 PSI, a particle flight distance of 6 cm and M10 tungsten particles (mean diameter of 1.07 μm). Stable transformation was confirmed by growth in the presence of km (100 μg/ml), GUS activity in leaves

of rooted plants and passage of the GUS gene to the second generation. Transformation was stable in approximately 1 % of the apical domes which were bombarded. The chimeric nature of these transformants is being determined. Work is underway to use biolistic transformation to incorporate disease resistant genes into bean.

A1001

Robin R.Charlton, Charles W.Carlson, Laure H.Kenyon, Theodore H. Carski, William F.Smith, Wayne J. Steele,II, Frederick A. Liberatore, Kai S.Leung, E.I.Du Pont De Nemours and Company, and Catherine S.Valteris, H.L.Yoh Co.: **USE OF IMMUNOASSAYS TO DETECT CROP PROTECTION CHEMICALS: APPLICATIONS IN SOIL ANALYSIS AND WORKER SAFETY.**

Immunoassays are being developed for a variety of Du Pont crop protection chemicals (CPCs). These high-quality assays are used to detect residues of these CPCs in water, soil, plants or food. Assays designed for use in a laboratory for analyzing large numbers of samples use a plastic ELISA microplate for the solid support. Rapid assays designed for use with individual samples in the field use a porous membrane as the solid support.
Chlorimuron ethyl is a sulfonylurea herbicide used in several Du Pont pre-emergence and post-emergence products for weed control in soybeans. A microplate assay for chlorimuron ethyl in soil has been developed, and prototype kits have been produced and evaluated. The test can quantitate chlorimuron ethyl present in soil extracts or water samples. We are currently studying the relationship between chlorimuron ethyl present in soil extracts and plant growth response in the field.
A rapid membrane test for the insecticide Lannate® (methomyl) will be used to monitor dislodgeable foliar residues (DFR) to ensure worker safety, especially in grape vineyards, where worker exposure to foliage is relatively high. Ongoing field trials will test our ability to correlate on-site monitoring to laboratory monitoring using HPLC.

A1002

STATISTICAL CONSEQUENCES OF COMBINING POPULATION SAMPLES. Linda Kinkel, Plant Pathology, Univ. of Minnesota, St. Paul, MN 55108.

A simulation model was developed to test the influence of sample design on statistical tests and on the estimation of population parameters of skewed distributions, which are common among biological populations. Observations were taken from simulated populations described by a lognormal distribution and having a range of mean-variance combinations. Observations were maintained as individual units in a sample or combined using different bulking strategies, and population means and variances were estimated. Two forms of hypothesis test were performed for each sample: H_o: $\bar{x}$ = true population mean, and H_o: $\bar{x} = \bar{y}$ (in cases where population means and variances were either the same or different). Bulked samples from skewed distributions seriously overestimated population means, underestimated population variances, and resulted in spurious test results. Misestimation was a function of both population variance and bulking strategy. Perceived increases in statistical power with bulked samples are deceptive and conclusions based on test statistics from bulked samples may frequently be incorrect.

A1003

GRAY MOULD OF GREENHOUSE-GROWN DOUGLAS-FIR SEEDLINGS: EFFECT OF SOWING DENSITIES AND CANOPY LEVEL ON NEEDLE SENESCENCE AND DISEASE SEVERITY. S. Skaalid, University of Victoria, Box 1700, Victoria, B.C. and J. R. Sutherland, Pacific Forestry Centre, 506 W. Burnside Road, Victoria, B.C.

Gray mould caused by the fungus Botrytis cinerea is one of the most serious diseases of greenhouse-grown conifers in Canada. Coastal Douglas-fir seedlings were grown in styroblocks at two different densities. Seedling planting times were staggered to produce an even or uneven canopy level within a styroblock. During the growing season samples of foliage were taken every two weeks. Needle senescence rates were determined by tissue assays for chlorophyll, reducing sugars, starch, and protein content. Seedling morphology and gray mould development were monitored. The biochemical and morphological results were correlated with time, styroblock density, canopy level and disease development.

A1004

ELIMINATION OF VERTICILLIUM ALBO-ATRUM INFECTION OF ALFALFA SEED THROUGH SEED SIZING AND HEAT TREATMENT. B. K. Fleener, L. L. Scholbrock, and J. A. Berry. Pioneer Hi-Bred Int'l, Inc., P.O. Box 1004, Johnston, IA 50131.

Verticillium wilt of alfalfa, caused by Verticillium albo-atrum, can be spread by externally and internally infected alfalfa seed. Greenhouse plants of Regan S, a susceptible cultivar, were artificially inoculated with Verticillium. After harvest, the seed was sized into three weight groups. Heat treatments of 75°C and 90°C were tested varying periods of time ranging from 2-24 hours. External and internal infection was determined by isolation from surface sterilized seed. A four day germination test was carried out on heat treated seed to measure heat damage. External and internal Verticillium was isolated from each seed weight group. Infection was greater in lighter seed compared to heavier seed. Heat treatment at 90°C for 24 hours eliminated Verticillium infection both externally and internally in each size category without significant reduction in seed germination.

A1005

BULK-PRODUCTION OF VERTICILLIUM LECANII CONIDIA. L. R. Batra and J. R. Stavely, Microbiology and Plant Pathology Lab., ARS, USDA, Beltsville, MD 20705-2350.

In controlled experiments, effectiveness of seed (whole grain) and grit (split grain) of wheat, oats, rice, barley, rye, pearl millet, and three legumes were compared in various formulations for bulk-producing conidia of the biocontrol fungus V. lecanii. The following was the most productive method. Into a steel or glass 29 x 15 x 6 cm baking dish, spread 125g soft wheat grit (92% retained by 20-mesh, pore 0.84 mm), add 60 ml H_2O, cover with aluminum foil, and autoclave. Spray with 5 ml of a spore suspension (3 x 10^4 propagules/ml) and incubate at 25°C. After 48 hours, mist-irrigate with 2-3 ml sterile water. Punch holes in foil to reduce surface condensation, and cover with cheesecloth. At 1 wk, mix 500 ml sterile water into grits, stir to a slurry, decant, and filter slurry through 4 layers of 16-mesh cheesecloth. This yields 1 x 10^8 to 1.2 x 10^9 conidia.ml.

A1007

IMAGE ANALYSIS OF UREDINIOSPORES WHICH INFECT MENTHA. T. Ball[1], J. S. Gardner[1], D. A. Johnson[2], and W. M. Hess[1]. [1]Department of Botany & Range Science, Brigham Young University, Provo, UT 84602. [2]Irrigated Agriculture Research and Extension Center, Washington State University, Prosser, WN 99350.

Two races of the same species of rust (Puccinia menthae Pers.) infect peppermint (Mentha piperita L.) and spearmint (M. spicata L.) Neither race will infect the other species. The urediniospores of both races of rust have a similar morphological appearance with SEM. A Prism Image Analysis System was used with a Macintosh IIci to quantify morphological differences. Separation was possible with 14 morphological parameters ranging from 92.25% to 99.99% probability of significant difference. The parameters were, area, convex area, perimeter, convex perimeter, length, fiber length, equivalent diameter, form factor, roundness, extent, compactness, aspect ratio, elongation, and curl. Although separation of these urediniospores is not presently tied with economics and marketing, separation of spores of other fungi does have marketing implications.

A1008

THE USE OF PLASTIC GROWTH POUCHES FOR STUDYING INTERACTIONS BETWEEN RHIZOCTONIA SOLANI AND GLIOCLADIUM VIRENS, AND R. SOLANI AND NITROGEN-FIXING BACTERIA ON ROOTS OF LEGUME SEEDLINGS. **P. D. Dery and M. M. Kulik. USDA, ARS, Beltsville, MD 20705.**

Plastic growth pouches that were originally developed for use in seed germinability studies have also proved very useful in investigations of the interactions of various mycorrhizae and the roots of their respective host plants, as well as in other fungus-host plant interactions. We have developed methodology so that these pouches could reliably provide us with suitable material for scanning electron microscope (SEM) studies of the in situ interaction between Rhizoctonia solani and the hyperparasite Gliocladium virens on the roots of legume seedlings. In addition, these pouches have proved useful in our SEM studies of the in situ interaction between R. solani and nitrogen-fixing bacteria on seedling roots of legumes.

A1009

OBSERVING FROZEN, FRACTURED, HYDRATED PLANT PARASITIC NEMATODES WITH A LOW TEMPERATURE FIELD EMISSION SCANNING ELECTRON MICROSCOPE. W. P Wergin[1], R. M. Sayre[2] and E. F. Erbe[1]. [1]Electron Microscopy Laboratory, USDA ARS, Building 177B, BARC-East, Beltsville, MD 20705 and [2]Nematology Laboratory, USDA ARS, Building 011A, BARC-West, Beltsville, MD 20705.

Biological specimens observed with a scanning electron microscope (SEM) are generally chemically fixed, dehydrated, critical point dried and coated. Unfortunately these procedures are frequently associated with artifacts. Our laboratory has recently acquired a field emission (FE) SEM that is equipped with a cryostage. This combination of instrumentation allows us to quickly freeze, fracture, sputter coat and examine frozen hydrated specimens. Results indicate that the cuticle and fractured surfaces of frozen hydrated nematodes can be easily observed at magnifications up to 25,000X. This technique avoids the artifacts associated with the preparation procedures used in conventional SEM and offers the potential to observe nematode fine-structure at magnifications greater than 100,000X with an FESEM .

A1010

NON-DESTRUCTIVE ESTIMATION OF *STRIGA* ABOVE-GROUND BIOMASS. D. E. Hess and W. A. Payne, ICRISAT Sahelian Center, B.P. 12404, Niamey, Niger.

Striga hermonthica (Del.) Benth., a root hemiparasite, seriously reduces cereal yield in the semi-arid tropics. Frequently, counts of emerged *Striga* plants are used to indicate the degree of crop infestation; however, *Striga* biomass is a better indicator. Because destructive sampling is not appropriate for many experiments, we tested simple, nondestructive methods of determining *Striga* biomass. 115 shoots and branches of 28 *Striga* plants of variable size and age were selected from an infested pearl millet (*Pennisetum glaucum* R. Br.) field in Niger, and their length (L), mean diameter (D), and dry mass (M) determined. Our results indicate that M can be estimated from a simple equation of L and D. This would require measurement of diameter as well as length but an equation involving just length is only slightly less precise. Our study therefore shows that *Striga* above-ground biomass can be accurately calculated with simple, non-destructive methods.

A1011

A NEW METHOD FOR DETERMINING THE ANASTOMOSIS GROUP OF ISOLATES OF *RHIZOCTONIA SOLANI* USING THE DNA-BINDING FLUOROCHROME 4'-6-DIAMIDINO-2-PHENYLINDOLE (DAPI).
M. M. Kulik and P. D. Dery. USDA-ARS, Beltsville, Maryland 20705.

Current methods for determining the anastomosis group (AG) of a *Rhizoctonia solani* isolate involve culturing it near an AG tester isolate. The hyphal interaction zone is examined microscopically for the presence of imperfect hyphal fusions (anastomoses), which can only occur between different isolates of the same AG. Although these fusions are usually not frequent, it is thought necessary to detect at least five of them for a valid AG determination. In addition, in order to rule out perfect (self) fusions, it is necessary to trace the fused hyphae back to their respective isolates. This can be time-consuming and is not always possible. By definition, the fused hyphal cells involved in an imperfect fusion will quickly die (the "killing reaction"), which should eliminate the need for hyphal tracing. However, we have found that dead hyphal cells do not always appear different from adjacent, living cells. DAPI, a DNA-binding fluorochrome, rapidly stains nuclear DNA. Using this dye, we can be absolutely certain that a given hyphal fusion is imperfect, since the fused cells are dead and consequently will not react with the DAPI. Thus, it should only be necessary to demonstrate the presence of one imperfect fusion per pair of isolates.

A1013

RULES FOR TEACHERS. D. A. Roberts, Department of Plant Pathology, University of Florida, Gainesville, Florida 32611.

Universities and colleges, once scholastic, seem to have turned materialistic, and the groves of academe have given way to colosseums. Do these perceived changes mark the decline and downfall of American higher education? Certainly not, for there persists a stable and enduring triad that symbolizes continued excellence. The subject, its teachers, and their students comprise the triad, and connecting lines of two-way communication bind its members together. I view the subject as the house of knowledge or truth, teachers as generous and convivial hosts, and students as welcome and honored guests. Good teachers captivate their audiences without sacrificing scholarship for showmanship or pedagogy for popularity. They also obey these rules: 1) like to teach, 2) know and like your subject, 3) know and like your students, 4) teach the principles, 5) have a sense of humor, 6) be honest, 7) be firm but fair, 8) be kind, 9) be ready for anything, and 10) patiently persevere.

A1015

Development of Spiroplasma citri-specific DNA probe and a study of the protein expressed from the recombinant. K. H. Chen and T. A. Chen, Department of Plant Pathology, NJAES, Rutgers University, New Brunswick, NJ 08903.

A genomic library of Spiroplasma citri strain C189 was constructed. Recombinants were screened for expression of S. citri antigens by ELISA using polyclonal antiserum against strain C189. The recombinant plasmid in one of the positive clones contains a 6.7 Kb DNA fragment. Southern blot hybridization, using ^{32}P-labeled DNA as a probe, shows that the fragment only hybridizes with different isolates of S. citri, but not with other spiroplasmas in Serogroup I. The fragment also does not hybridize to DNAs extracted from several MLO-infected plant tissues. Using this fragment as a probe in dot hybridization analysis, S. citri DNA can be detected to 100 pg. When Western blot probed with specific monoclonal antibodies, the protein expressed from the recombinant is serologically related to a major membrane protein of several different isolates of S. citri. However, related protein from S. citri strain Asp-1 is not detected.

A1016

RESISTANCE OF SPIROPLASMA CITRI LINES TO INFECTION BY THE VIRUS SVTS2. Y.-H. Sha, J. Fletcher, and U. Melcher. Departments of Plant Pathology and Biochemistry, Oklahoma State University, Stillwater, OK 74078.

Electroporation of SVTS2 viral DNA in Spiroplasma citri M200H produced 1.5 X 10^5 transfectants/ug DNA, but none in MR2 or MR3. Thus, resistance in MR2 and MR3 may involve failure of the viral DNA to replicate. Western blotting of native viruses from M200H, MR2, and MR3 with anti-SVTS2 serum showed that the four viruses have one major coat protein in common, but different minor coat proteins. Restriction digests of SVM200H, SVMR2, and SVMR3 RF DNA resembled each other, but not those of SVTS2 RF. A SVTS2-specific probe was hybridized with RF DNA of the three lines. All three native viruses shared homology with SVTS2. Two bands absent in SVM200H hybridized strongly in SVMR2 and SVMR3, indicating that a fragment of SVTS2 DNA is present in an extrachromosomal ds DNA in MR2 and MR3, but not M200H. Hybridization also showed a fragment of SVTS2 DNA in the chromosome of MR3. These insertions may provide immunity to superinfection by SVTS2.

A1017

CHARACTERIZATION OF SVBR3, A VIRUS FROM SPIROPLASMA CITRI BR3. Y.-H. Sha and J. Fletcher. Department of Plant Pathology, Oklahoma State University, Stillwater, OK 74078.

A rod-shaped virus was isolated from Spiroplasma citri BR3, the causal agent of horseradish brittle root. Purified SVBR3 measured 6.8-10 X 189-293 nm and had a density of 1.26 g/cm^3 in CsCl. Sensitivity of virus DNA to S1 nuclease indicated a single-stranded form. These features are consistent with characteristics of spiroplasmavirus group I (SpV1). The replicative form of SVBR3 is approximately 8.6 Kb. The virus infected all strains of S. citri tested, but not other spiroplasma species. Many restriction enzymes specific for G+C-rich DNA did not digest SVBR3 RF DNA, suggesting a low G+C ratio. SVBR3 restriction digest patterns differed from those of another SpV1-type virus, SVTS2. Western blotting using anti-SVTS2 serum showed that SVBR3 had two coat proteins in common with SVTS2, but SVTS2 also had others not present in SVBR3. The potential of SVBR3 as a cloning vector for spiroplasmas is under investigation.

A1019

ETIOLOGY OF ARIZONA ASH DECLINE. J. S. Bricker and J. C. Stutz. Department of Botany, Arizona State University, Tempe, Arizona, 85287.

Arizona ash (*Fraxinus velutina*) trees in landscape plantings in the Phoenix, AZ metropolitan area were observed with yellows and dieback symptoms. All of the trees in our study groups, containing 48 and 108 individuals that have been observed since 1985 and 1988, respectively, exhibit some degree of dieback with symptom severity increasing over time. Mycoplasmalike organisms (MLOs) were detected in the phloem of symptomatic plants using the DAPI staining technique. The detection of MLO DNA in diseased trees using the polymerase chain reaction is currently being pursued.

A1020

MYCOPLASMALIKE ORGANISMS IN *FRAXINUS* AT DIVERSE LOCATIONS ARE CLOSELY RELATED TO ONE ANOTHER BUT NOT TO THOSE DETECTED IN ASSOCIATED PLANTS. H. M. Griffiths, W. A. Sinclair, R. E. Davis[1], and I.-M. Lee[1]; Dept. Plant Pathology, Cornell Univ., Ithaca, NY 14853; and [1]USDA-ARS, Beltsville, MD 20705

The genetic relatedness of mycoplasmalike organisms (MLOs) associated with ash yellows (AshY) in *Fraxinus* was addressed by means of dot and Southern hybridizations using AshY-specific DNA probes (Mol. Plant-Microbe Interact. 5:163-169, 1992). DNA from diseased but not healthy *F. americana* or *F. velutina* from five central and eastern states, Utah, and Quebec hybridized with four different AshY-specific probes derived from genomic DNA of MLO strain AshY1. This result supports the concept that AshY is caused by a set of closely related strains of MLOs. MLOs commonly detected in plants associated with diseased ash in New York State were tested for relatedness to AshY MLOs. DNA from diseased *Asclepias* sp. (milkweed) and *Solidago* sp. (goldenrod), but not from healthy controls, hybridized with a probe that detects many MLOs nonspecifically but did not hybridize with the AshY-specific probes. Thus, MLOs in goldenrod and milkweed are distinct from AshY MLOs.

A1021

COMPARISONS OF PROTEIN AND DNA PROFILES OF SPIROPLASMA CITRI BR3 LINES DIFFERING IN TRANSMISSION OR SUBCULTURING HISTORY. G. R. Baker, M. E. Shaw, and J. Fletcher. Dept. of Plant Pathology, Oklahoma State University, Stillwater, OK 74078.

Four lines of Spiroplasma citri strain BR3 differing in transmission or subculturing history were compared with respect to differences in 1) whole protein profiles, 2) expression of four specific surface proteins, 3) extrachromosomal DNAs, and 4) DNA restriction patterns. Proteins were assayed by polyacrylamide gel electrophoresis (PAGE) and Western blotting using anti-whole cell and anti-surface protein sera. Although all four lines had similar profiles, there was differential expression of several proteins. Agarose gels of cesium chloride/ethidium bromide density gradient purified DNA showed differences in extrachromosomal DNAs present. Restriction digests of total DNAs also exhibited differences in banding patterns. It is possible that one or more of the differences detected in our four experimental lines of S. citri is involved in activities related to pathogenicity or differential insect transmissibility.

A1022

ABUNDANCE OF MYCOPLASMALIKE ORGANISMS IN A NON-PHOTOSYNTHETIC HOST. K.L. Klomparens, B.B. Sears, and J.I. Wood. Botany and Plant Pathology and Michigan State University. E. Lansing, MI 48824.

In leaftip cultures of Oenothera elata, a plant-pathogenic MLO reaches high titer. Alterations in the physiology of the plant cultures may allow this proliferation because of reduced photosynthetic activity and, hence, a reduction in O_2. To determine if the O_2 generated by photosynthesis would affect MLO growth, we examined photosynthetic mutants of MLO-infected Oenothera elata tissue culture plants using transmission electron microscopy (TEM) and molecular techniques. By both thin section TEM and Southern blots using MLO-specific plasmid probes, we noted that MLOs were more abundant in white tissue than in green tissue. These data present evidence that a non-photosynthetic host provided an enhanced physiological environment for the multiplication of MLOs.

A1023

USE OF FAB PORTIONS OF SPIROPLASMA CITRI-SPECIFIC ANTIBODIES IN SEROLOGICAL REACTIONS WITH SPIROPLASMAS. J. Fletcher, Department of Plant Pathology, Oklahoma State University, Stillwater, OK 74078.

Purified Spiroplasma citri-specific IgG was digested with papain and evaluated by PAGE to confirm that antibody molecules were split into Fab and Fc fragments. The Fab arms were then purified on a protein A column. Western blots of S. citri cell proteins developed with Fab showed a banding pattern similar to that of blots developed with antiserum. However, in a spiroplasma deformation test, the antiserum caused helical cells to become blebbed, while the Fab preparation caused negligible effects on cell morphology. This difference may be due to the monovalent binding site of the Fab fraction compared to the bivalent site of the whole antibody. The use of Fab preparations will allow serological evaluation of spiroplasmas in biological tests which were previously impossible because of the deforming activity of anti-spiroplasma sera.

A1024

DETECTION AND DIFFERENTIATION OF EUROPEAN GRAPEVINE YELLOWS MLOs USING THE POLYMERASE CHAIN REACTION. J. P. Prince, R. E. Davis, B. D. Mogen, R. W. Hammond, E. L. Dally, I.-M. Lee, A. Caudwell,[1] E. Boudon-Padieu,[1] B. di Terlizzi,[2] V. Savino,[2] M. Barba,[3] R. Osler,[4] L. Carraro,[4] and A. Bertaccini.[5] USDA-ARS Microbiology and Plant Pathology Laboratory, Beltsville, MD 20705, USA. [1]Institut National de la Recherche Agronomique, Dijon, France. [2]Istituto Agronomico Mediterraneo di Valenzano, Italy. [3]Istituto Sperimentale per la Patologia Vegetale, Rome, Italy. [4]Universita di Udine, Italy. [5]Universita degli Studi, Bologna, Italy.

Mycoplasmalike organisms (MLOs) associated with grapevine yellows diseases in Europe can cause severe losses. Attempts to detect these MLOs in grapevine have proven difficult due to their low titer and the inability to isolate them in pure culture. We have used various primer pairs in polymerase chain reactions to detect and differentiate four MLO strains associated with grapevine yellows from northern and southern Italy (in periwinkle host plants) and from France (in broadbean host plants). All four MLOs were detected in diseased plant samples and were differentiated from one another through analysis of PCR products. This approach is being developed as a diagnostic tool.

A1025

INFLUENCE OF VESICULAR-ARBUSCULAR MYCORRHIZAE ON CELERY SEEDLING GROWTH AND DEVELOPMENT. L. E. Datnoff, R. N. Raid, C. A. Sanchez, and M. L. Sommerfeld, University of Florida-EREC and A. Duda & Sons, Inc., Belle Glade, FL.

Allegations that phosphorus (P) enriched drainage water from the Everglades Agricultural Area of South Florida is affecting the trophic status of Lake Okeechobee and the ecology to the Everglades National Park has placed P fertility practices under the scrutiny of environmental regulators. Since vesicular-arbuscular mycorrhizae (VAM) could potentially reduce P inputs in celery production without compromising crop yields, the objective of this study was to determine the influence of Glomus intraradix on celery seedling growth and development. A commercial potting mix was amended with VAM ranging from 0 to 1000 chlamydospores/30 ml of mix and dispensed into containerized flats. Celery was direct-seeded in the flats and maintained under greenhouse conditions. Plants were fertilized daily with a 20-2-20 N-P-K nutrient solution applied by using a rate dispenser. Plants were harvested 14 weeks after planting. Colonization by VAM of celery roots ranged from 82% to 87%. Celery plants infected by VAM had 32% higher dry weights, were 16% taller, had 13% more petioles, and 56% higher total P concentrations in comparison to non-infected plants.

A1033

A GENETIC ANALYSIS OF VARIATION IN ECTOMYCORRHIZAL COLONIZATION BY *LACCARIA BICOLOR*. B. R. Kropp. Department of Biology, Utah State University, Logan, Utah 84322-5305.

Much variation in mycorrhizal ability exists among dikaryotic cultures of the ectomycorrhizal basidiomycete *Laccaria bicolor* which have been synthesized by crossing compatible monokaryons. The present study examined the variation among 32 synthesized dikaryotic cultures of *Laccaria bicolor*. The differences between the cultures were analyzed statistically to determine how easily ectomycorrhizal colonization by this fungus could be improved using classical genetic manipulation. The percent colonization of short roots of *Pinus strobus* after 6 weeks in growth pouches under growth chamber conditions varied from 35 to 84 percent. The analysis of the variation between the cultures showed that between 10 and 23 percent of the total variation was under genetic control. The remaining 76 to 90 percent of the variation was shown to be nonheritable. The remaining heritable variation was primarily due to the nonadditive component. The additive component represented only 3 percent, or less, of the total heritable variation indicating that improvement of ectomycorrhizal colonization through classical genetic manipulation would be slow.

A1035 See page 1180

A1036

INCUBATION CHAMBERS FOR THE STUDY OF MATRIC POTENTIAL AND TEMPERATURE EFFECTS ON MICROBIAL ACTIVITY IN HEATED SOILS. A. B. Filonow, Plant Pathology, Oklahoma State University Stillwater, OK 74078.

Glass Buchner funnels served as hanging-column tensiometers and were wrapped with heating pads which were wired to remote bulb thermostats that regulated line voltage to the pads. Chambers held 100 g soil. Rubber stoppers for sealing chambers were fitted with soil thermistors and with inlet and outlet tubes for the passage of air through the chambers. Some stoppers also held thermostat bulbs, and one thermostat regulated temperature for a pair of chambers. All thermistors were connected to a digital thermometer. CO_2 as a measure of soil microbial activity was collected in $Ba(OH)_2$ solution. Soils can be incubated at matric potentials of 0 to -25kPa and from room temperature to 55 C with low variability. For example, soil in chambers held at -5kPa that was heated to 24.1, 31.3, 43.1 or 52.8 C after 24 h varied 3-5% from these values over the next 4 days. Soil moisture variation from initial values was less than 8% at any temperature.

A1037 See page 1180

A1038

BIOLOGICAL ACTIVITIES INDUCED BY RHIZOBACTERIA AND THEIR INFLUENCE ON SPRING WHEAT YIELD. S. Young, M. S. Reddy, G. Brown and R. Rennie. Esso Chemical Canada - Ag Biologicals, 402 - 15 Innovation Blvd. Saskatoon, SK, Canada S7N 2X8.

A core collection of rhizobacterial strains was screened for production of specific cytokinins, solubilization of calcium phosphate, Togo rock phosphate and production of acid phosphatase under lab conditions. Strains were subsequently screened for emergence promotion of Katepwa wheat and root colonization in field soil under greenhouse conditions. Select strains with biological activities of interest were then field tested for yield of wheat at multiple sites in Saskatchewan and Alberta in 1989, 1990, 1991. Bacteria were applied as in-furrow spray at seeding and normal agronomic practises were followed throughout the growing season. In 1991, field tests were expanded to evaluate the effect of bacterial seed treatment on grain yield of wheat resulting from fields incorporated with various levels of fertilization. Significantly increased yeilds were obtained with strains which had either phosphate solubilizing activity or which over-produced dihydrozeatin riboside in vitro. Bacterially mediated yield increases were limited at sites where the control yield approached genetic maximum. Yield of wheat tested with bacteria in a potential commercial formulation was significantly increased at a range of rates of fertilization. This may indicate improved fertilizer use efficiency. One specific strain with commercial potential increased yield an average of 14% over the untreated control over 3 years of field testing.

A1039

IMPACT OF PEAT DECOMPOSITION LEVEL ON RHIZOSPHERE BACTERIAL POPULATIONS ACTIVE IN SUPPRESSION OF PYTHIUM DAMPING-OFF. M. J. Boehm, L. V. Madden and H. A. J. Hoitink, Dept of Plant Pathology, Ohio State Univ., Wooster, OH 44691.

Rhizosphere bacteria isolated on 10% trypticase soy broth agar from root tips of cucumbers grown in suppressive light and conducive dark peat were identified by GC-FAME analysis. Rarefaction curves revealed the bacterial species diversity for each root tip. The species composition among root tips harvested from light and dark peat differed significantly (P=0.05). *Pseudomonas* spp. were predominant in the suppressive light peat. *Arthrobacter* and *Bacillus* spp. predominated in the more decomposed dark peat. No single species predominated in either of the niches. Although strains capable of inducing suppression were isolated from both peat types, those from the suppressive peat were most effective. Efficacy of strains was significantly (P=0.05) greater in the less decomposed light peat. In conclusion, suppression was a function of peat decomposition level and bacterial species composition.

A1040

ANALYSIS OF SPERMOSPHERE COLONIZATION BY THE BIOCONTROL BACTERIUM ENTEROBACTER CLOACAE USING BIOLUMINESCENCE AND STATISTICAL IMAGE PROCESSING. D. P. Roberts[1], P. Dery[1], and N. M. Short, Jr.[2] [1]USDA, ARS, Beltsville, MD 20705 and [2]NASA-GSFC, Greenbelt, MD 20771.

Location of the biocontrol agent in the spermosphere is potentially of importance to control of seed and seedling diseases. Enterobacter cloacae strain E6(pUCD607), a bioluminescent biocontrol bacterium, was applied to cucumber seeds. Seeds were planted in root boxes and the location of strain E6(pUCD607) was determined by photography as previously described (D. R. Fravel, R. D. Lumsden, and D. P. Roberts, 1990. Plant Soil). Spatial analysis of digitally captured images of E6(pUCD607) in cucumber spermosphere was done using image enhancement and noise filtering, edge detection, and region (blob) analysis algorithms. Spatial analysis allows a statistical representation of the area, size, shape, and location of E6(pUCD607) in cucumber spermosphere.

A1041

FREQUENCY AND VIRULENCE OF *RHIZOCTONIA SOLANI* ANASTOMOSIS GROUPS ISOLATED FROM WHEAT AND SUGAR BEET IN TEXAS. C. M. Rush, Texas Agric. Exp. Sta., Bushland, TX 79012; and D. E. Carling, Univ. of Alaska, Palmer, AK 99645.

Rhizoctonia solani causes disease of wheat and sugar beet in Texas. The anastomosis group (AG) of 46 isolates from sugar beet, 45 from wheat, and an additional 7 from beet seedlings was determined. Eighty-nine percent of the mature beet isolates were AG2-2, 95% of the wheat isolates were AG4, and the isolates obtained from beet seedlings were predominantly AG4 or AG5. Two binucleate isolates were recovered. Selected isolates were used in additional studies. All were capable of saprophytically colonizing wheat, corn, cotton, and sorghum residue and grew best *in vitro* between 25-30C. In pathogenicity studies, only AG2-2 and AG4 isolates reduced emergence or caused postemergence damping off of wheat or sugar beet seedlings. The AG4 isolates from wheat were especially virulent on sugar beet seedlings.

A1042

INHIBITORY EFFECTS OF AQUA-GRO WETTING AGENT ON PHYTOPHTHORA PARASITICA FROM VINCA. S. L. von Broembsen, Dept. Plant Pathology, Oklahoma State University, Stillwater, OK 74078.

Aqua-Gro (AG) is a non-ionic wetting agent used to improve the moisture holding qualities of potting mixes. AG at the recommended rate of 2.5 ml/L halted motility of zoospores produced by P. parasitica isolate 45C1 from vinca instantly and caused >90% lysis of the zoospores. Sporangial production by 45C1 was completely inhibited in mineral salt solution amended with 0.1 ml/L AG. In petri dishes containing soilless potting medium, AG at 1.0 ml/L completely inhibited infection of germinating vinca seedlings by 45C1 motile zoospores. However, AG had little effect on direct germination of encysted 45C1 zoospores. Mycelial growth on corn meal agar amended with 1.0 ml/L AG by 45C1, P. citricola isolate 88A2 (hibiscus), Pythium aphanidermatum isolates 685A (tomato) and 680C (poinsettia), and Rhizoctonia solani isolates 679 (poinsettia) and 335 (petunia) was reduced 0.3, 8.7, 20.3, 25.4, 62.2 and 64.6% respectively. AG also halted motility and caused lysis of zoospores of the P. aphanidermatum isolates. These results suggest that AG might function as an effective control material for certain soilborne pathogens, especially those producing zoospores.

A1043

VIRULENCE, VEGETATIVE COMPATIBILITY, AND mtDNA RFLP ANALYSIS OF FUSARIUM OXYSPORUM ASSOCIATED WITH SPINACH. M. B. Fiely, A. B. Thornton, 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.

Over 200 isolates of Fusarium oxysporum were collected from wilting spinach seedlings from 11 sites in four states (AR, OK, TN, and WA). All isolates were tested for vegetative compatibility (using nitrate non-utilizing mutants) and assigned to a VCG. Over 14 VCGs were identified. Isolates representing all of the VCGs were tested for virulence on spinach

in a greenhouse pathogenicity test and for mtDNA RFLPs. All virulent isolates belonged to one of three VCGs (designated VCG 1, 2, and 3). Approximately 50% of all of the isolates examined belonged to one of these three VCGs. Isolates in VCG 2 were recovered from all four states. All pathogenic isolates tested, representing the three pathogenic VCGs, had an identical mtDNA RFLP phenotype when restricted with several enzymes. Futhermore, the mtDNA RFLP phenotype among virulent isolates was unique and could be used to distinguish pathogenic and non-pathogenic isolates. Multiple mtDNA RFLP phenotypes were identified among the non-pathogenic isolates examined. Non-pathogenic isolates from several different VCGs had similar mtDNA RFLP phenotypes.

A1044

POTENTIAL ROLE OF RHIZOSPHERE COLLEMBOLA (INSECTA) IN SOILBORNE PATHOGEN ECOLOGY. E. A. Curl, R. T. Gudauskas, and B. E. Helms, Dept. of Plant Pathology, Auburn University, AL 36849-5409.

Rhizosphere-inhabiting microarthropods of the order Collembola (Insecta) are predominantly fungus feeders and, therefore, are potential modifiers of fungal activities and biomass; some species are phytophagous, suggesting vector potential. Laboratory tests with *Proisotoma minuta* (Isotomidae) from cotton rhizosphere examined pathogen-insect relationships pursuant to these hypotheses. In dual or multiple cultures (mycelial disks), the insect displayed maximum attraction to and food preference for dark-pigmented fungi (*Thielaviopsis basicola* and *Macrophomina phaseolina*) and made a preferential distinction for *Rhizoctonia solani* AG-4 over AG-1. *Phytophthora parasitica* and *Fusarium oxysporum* f. sp. *vasinfectum* also were suitable food sources. Insect fecundity (egg production) was most favored by *Rhizoctonia* mycelia. High populations (69-78%) of *P. minuta* were attracted to washed, nonsterilized organic particles from field soil but few to sterilized particles. An insect population equivalent to 4000/kg in natural soil reduced the competitive colonization of organic substrate by *R. solani* AG-4 24% below the insect-free control; 2000/kg had no significant effect. In tests of vector potential, the insect did not appear to be involved in transmission of cucumber mosaic, maize dwarf mosaic or tomato spotted wilt viruses.

A1046

COMPARISON OF SELECTIVE MEDIA AND TECHNIQUES FOR QUANTIFYING RHIZOCTONIA POPULATIONS IN SOIL. P. M. Kinney, C. S. Rothrock, and S. A. Winters, Department of Plant Pathology, University of Arkansas, Fayetteville, Arkansas 72701.

Accurately quantifying the low populations of Rhizoctonia spp. that occur in soils is difficult despite the numerous methods and media developed. Soil plating with a multiple-pellet soil sampler onto a modified Ko and Hora medium, with fosetyl-Al instead of etridiazol, or tannic acid benomyl medium (TAB) was compared to a beet-seed baiting technique. Nuclear status and anastomosis group of isolates were determined. The soil-pellet technique gave higher counts on the modified Ko and Hora medium than on TAB for most soils. The baiting technique gave variable results depending on the soil used. These results were confirmed by using several soils artificially infested with known numbers of sclerotia of an isolate of AG 1-1C which was not present in the soils. The efficiency of baiting was influenced by the interaction between the microbial component of a soil and the saprophytic growth of Rhizoctonia, as demonstrated by placing beet seeds at known distances from a sclerotium of AG 1-1C in different soils.

A1047

THE INFLUENCE OF FALL SOIL SOLARIZATION ON SOILBORNE DISEASES OF POTATOES AND BEANS. **C. A. Strausbaugh and R. L. Forster. University of Idaho, Res. and Ext. Center, Kimberly, ID 83341.**

Studies were conducted at Kimberly, ID (42.5° N. latitude, 1200 m elevation) on the effect of solarization of soil after harvest of winter barley. Peak soil temperatures of 67, 32, and 28 C were recorded at 0, 15, and 30 cm depth, respectively, in 6.7 m X 15.2 m plots covered with clear, 4 mil plastic from Aug 14 to Oct 16, 1990. Untarped plots served as controls. The effect of solarization on populations of nematode genera was variable. Potatoes (cvs. Russet Burbank and Russet Norkotah) and beans (cvs. HyStyle and Olathe) were planted in spring 1991. Solarization significantly reduced the number of potato stems with early dying symptoms (caused by Verticillium dahliae) from 32 % to 19 % and increased yield by 16 %. Stand, stem number per hill, and stem canker (Rhizoctonia solani) incidence were not affected. In beans, solarization significantly increased the stand, reduced Fusarium root rot, and increased yield 24 % (672 kg/ha).

A1048

GERMINATION OF *Aphanomyces euteiches* OOSPORES IN THE ROOT ZONES OF VARIOUS PLANTS. J. A. Percich[1], D. K. Malvick[1], and C. R. Grau[2], Dept. of Plant Pathology, Univ. of Minn., St. Paul, 55108[1], and Dept. of Plant Pathology, Univ. of Wisc., Madison, 53706[2].

A technique was used to observe *Aphanomyces euteiches* oospore germination in plant root zones. Nuclepore membranes containing 1×10^5 oospores were placed on a smooth soil surface and overlaid with the roots of 5-day-old seedlings of various plants, covered with nylon mesh and then soil. After varying periods of incubation at 24 C with a 16-hr photoperiod, membranes were observed for oospore germination, and the roots examined microscopically for evidence of infection. There were significant differences in oospore germination among the root zones of alfalfa, canola, peas and oats of 25, 26, 70 and 80%, respectively, at day 4. Root infection and oospores were observed in the root zones of these plant species/hosts. By day 14 maximum oospore germination of 45, 58, 81 and 84% was observed in the root zones of canola, alfalfa, peas and oats, respectively. Oospore germination of 7 and 10% occurred in the root zone of corn and wheat, respectively, with no evidence of root infection after 14 days.

A1049

CROSS PROTECTION AMONG MEXICAN BARLEY YELLOW DWARF VIRUS ISOLATES. R. Ranieri[1], R.M. Lister[2], G. Shaner[2], P.A. Burnett[1], and J. Vallejo[3]. [1] Wheat Program, International Maize and Wheat Improvement Center (CIMMYT), Apdo. Postal 6-641, 00600 Mexico D.F.; [2] Botany and Plant Pathology Department, Purdue University, W. Lafayette, IN 47907, USA; [3] Plant Pathology Institute, Collegio de postgraduados, Montecillo, Texcoco, Mexico.

Cross protection among three Mexican isolates of barley yellow dwarf virus was investigated at the CIMMYT El Batan station in two experiments with barley cultivars Atlas 57 ($Yd2^-$), Atlas 68 ($Yd2^+$), Puebla and Centinela. In each experiment plants were inoculated in the greenhouse with isolates Mex-PAV (PAV serotype), Mex-MAV (MAV serotype), or Mex-RPV (RPV serotype). In the first experiment plants were transplanted in the field. Yield and yield component data from this experiment were consistent with a partial protection by Mex-MAV against Mex-PAV, but not for any other combination of isolates. In the second experiment, plants remained in the greenhouse and protection effects were measured by ELISA. ELISA data confirmed the results of experiment 1, but also suggested that prior infection with Mex-RPV inhibited the establishment of Mex-PAV or Mex-MAV.

A1050

TRANSMISSION STUDIES OF MAIZE CHLOROTIC MOTTLE VIRUS WITH GREENHOUSE COLLECTED THRIPS. X. Q. Jiang, J. E. Campbell, D. R. Wilkinson, R. A. Heiden, and J. A. Berry. Pioneer Hi-Bred Int'l, Inc., P.O. Box 1004, Johnston, IA 50131.

Thrips, collected off chrysanthemums in a greenhouse in York, Nebraska, were used in Maize Chlorotic Mottle Virus (MCMV) transmission studies during January and February in Franklin County, Nebraska where MCMV occurs naturally. Thrips were allowed to feed on healthy maize plants for 30 days. After heavy feeding, both plants and thrips tested ELISA negative for MCMV. The thrips were then transferred to MCMV infected maize plants for a three day feeding. Subsequently, the thrips tested ELISA positive to MCMV. The MCMV positive thrips were moved to healthy caged maize seedlings. Treatments were 10 adults, 90 adults, or 17 larvae thrips per caged pot; three pots with nine plants total for each treatment. ELISA negative thrips were used as controls. Similarly tested were 200 white-flies collected from the greenhouse. After 18 days of feeding, there were no visual virus infection symptoms and all maize plants were ELISA negative for MCMV. Mechanically inoculated plants showed symptoms and were positive for MCMV by ELISA.

A1051

HYPERSENSITIVITY TO PEANUT STUNT VIRUS IN WHITE CLOVER. M. R. McLaughlin and G. A. Pederson. USDA, ARS, Crop Science Research Laboratory, Forage Research Unit, P. O. Box 5367, Mississippi State, MS 39762-5367.

A virus-free plant of white clover (Trifolium repens L. 'Regal') selected from a 3-yr-old pasture with 80% incidence of peanut stunt virus (PSV), was cloned and tested for resistance. The clone (22R) developed local lesions, but no systemic infection. In a field test with heavy PSV pressure in 1989-91, 22R remained free of PSV. In 1990, forage yield of 22R was double that of 'Regal', which had 30% PSV-infected plants in April. Only 53% of 'Regal' plants survived a 4-mo drought in 1990, compared to 89% of 22R plants. In 1991, forage yields of 22R were triple those of 'Regal'. Approximately equal numbers of hypersensitive and nonhypersensitive plants occurred among seedlings grown from 22R open pollinated seed, consistent with inheritance as a single dominant gene. The association of PSV resistance with drought tolerance and hypersensitivity is under study.

A1052

LEVELS OF PRUNUS NECROTIC RINGSPOT VIRUS AND PRUNE DWARF VIRUS INFECTION IN THE NATIONAL GERMPLASM REPOSITORY - DAVIS PRUNUS COLLECTION. L.S. Lile, K.S. Rigert. USDA-ARS, Germplasm Repository, University of California, Davis, CA, 95616.

The National Germplasm Repository - Davis Prunus collection has been tested for Prune Dwarf Virus (PDV) and Prunus Necrotic Ringspot Virus (PNRSV) using enzyme-linked immunosorbent assay (ELISA) methods. For PDV-ELISA, tissue extracts were plated directly into uncoated wells and samples were probed with PDV-11, a polyclonal rabbit antiserum, at 2ug/ml concentration. Alkaline phosphatase labelled goat anti-rabbit IgG was used at 0.5ug/ml concentration. The presence of PNRSV was tested by precoating the plates with a PNRSV-polyclonal antiserum (#1414, 1ug/ml concentration). The samples were probed with ascites fluid (Na70C9, Agdia) at a 1:5000 dilution. Alkaline phosphatase labelled goat anti-mouse IgG was used at 0.5ug/ml concentration. Well volume was 200ul. Of the approximately 1600 plants in the collection (708 clonal accessions and 892 seedlings representing 160 accessions), 345 plants tested positive. Two hundred plants were infected with PNRSV, 33 with PDV, and 112 with both viruses. Fifty of the virus-positive plants were seedlings. To facilitate budwood distribution and field evaluation, the collection must be virus free. Therefore, accessions testing positive are undergoing heat therapy in an attempt to obtain virus free clones. Every year, the collection will be tested using ELISA to assure the viral status of the plants. As other virus antisera become available, the collection will be screened for additional viruses.

A1053

DISTRIBUTION OF WATERMELON MOSAIC VIRUS 2 IN WATERMELON AND ACQUISITION OF VIRUS BY APHIDS: EFFECTS OF PLANT AGE AT INOCULATION. Susan E. Webb. Central Florida Research and Education Center, University of Florida, Leesburg, FL 34748.

Watermelon was planted in field cages at three intervals, 3 wk apart, and inoculated with watermelon mosaic virus 2, using aphids, when the youngest plants were at the first true leaf stage. After one week, ELISA absorbance values averaged 0.26 for plants that were 7.5 wk old at inoculation, 0.86 for plants 4.5 wk old, and 1.00 for those 1.5 wk old(n=19). At one week, symptoms on new growth were mild or absent on older plants and aphids were unable to acquire virus from them. Leaves were tested at weekly intervals at different positions along runners. Virus was distributed unevenly in the oldest plants and ELISA values were lower than those from plants infected at 1.5 or 4.5 wk. Aphid transmission was related to virus concentration but presence of symptoms was not. Symptomless leaves occurring on runners showing mosaic on new growth were often positive (ELISA) but were negative if from runners that had no symptoms of infection.

A1054

STUDY OF GENE EXPRESSION RELATED TO BYDV RESISTANCE IN OATS BY TWO-DIMENSIONAL PROTEIN GEL ELECTROPHORESIS. Xuejun Shen, Program of Physiology and Plant Molecular Biology and Leslie L. Domier, USDA-ARS, Dept. of Plant Pathology, University of Illinois at Champaign-Urbana, Urbana, IL 61801

To identify proteins preferentially expressed in oats resistant to BYDV infection, the two-dimensional protein profiles of one susceptible line (Clintland 64) and four resistant lines (Ogle, IL86-1156, IL86-5698, IL86-6404) were analyzed. Total proteins were extracted from one-month old healthy plants and plants infected with BYDV-PAV. The protein patterns obtained by normal two-dimensional gel electrophoresis and NEPHGE were compared. No difference were detected in major protein spots. However, some minor protein spots were different among the oat lines. More detailed analysis of the patterns is in progress. Work is also in progress for correlate these differences with resistance to BYDV infection.

A1055

NONISOTOPIC DETECTION OF APPLE SCAR SKIN VIROID BY DIRECT TISSUE BLOT AND DOT BLOT HYBIDIZATION. E. V. Podleckis[1], R. W. Hammond[2], A. Hadidi[1] and S. S. Hurtt[1], USDA, ARS, PSI, [1]National Germplasm Resources Laboratory and [2]Microbiology and Plant Pathology Laboratory, Beltsville, Maryland 20705

A chemiluminescent molecular hybridization protocol was compared to ^{32}P autoradiography for detecting apple scar skin group viroids (ASSVd). Labeled cRNA probes for ASSVd were generated by SP6 RNA polymerase transcription using digoxigenin-11-UTP or α-[^{32}P] UTP. Both probes were equally sensitive for detecting ASSVd in dot blot hybridizations with purified viroid or sap and total nucleic acid extracts from graft-inoculated apple trees (*Malus pumila* cv. 'Stark's Earliest'). The digoxigenin-labeled probe detected a minimum of 2.5 pg of purified ASSVd and could detect viroid in as little as 0.4 ng of total nucleic acid extract. ASSVd was detected in sap extracts diluted as much as 1/2000 with healthy apple extracts. Direct tissue blots of infected Stark's Earliest, but not healthy apple, gave positive reactions with leaves, stems and petioles when hybridized with the digoxigenin probe. Chemiluminescent detection of digoxigenin-labeled cRNA probes is a sensitive, specific, safe and easy alternative to radioisotopes in assaying for ASSVd.

A1056

EVALUATION OF BARLEY GENOTYPES FOR RESISTANCE TO BARLEY YELLOW STREAK MOSAIC. S. K. Z. Brumfield and T. W. Carroll. Montana State University, Dept. of Plant Pathology, Bozeman, MT 59717.

A special core collection of 1000 genetically diverse barley genotypes from the USDA-ARS national small grains collection was evaluated in the greenhouse for possible resistance (tolerance) to barley yellow streak mosaic disease. About 5000 nymphs and adults of the brown wheat mite, *Petrobia latens* Mueller, some of which were viruliferous for the causal agent barley yellow streak mosaic virus (BaYSMV), were transferred on pieces of paper to caged barley seedlings. After 5 wk, the plants were removed from the cages, fumigated, returned to the greenhouse uncaged, and evaluated an additional month for symptom expression. No barley genotype showed complete resistance. However, two genotypes, CIho 734, Haua and CIho 1032, Skinless had only 5.5% and 27.4% diseased plants, respectively, in comparison to 81.4% and 71.0% diseased plants for Alpine barley, CIho 9578, the susceptible check. These genotypes could provide sources of resistance for future development of commercial barley cultivars.

A1057

PHYSIOLOGICAL CHARACTERIZATION OF MULTIPLE POTYVIRUS RESISTANCE IN THE CUCUMBER LINE TMG-1. T. Wai and R. Grumet, Department of Horticulture, Michigan State Univ., East Lansing, MI 48824.

The inbred Chinese cucumber line TMG-1 is resistant to three related potyviruses: ZYMV, WMV-2, and PRV-W. The genetics of resistance to the three viruses is different: ZYMV is due to a single recessive gene, WMV-2 to two recessive genes, and PRV-W to a single dominant gene. We sought to determine if the resistances also differ in their effect on systemic spread. The kinetics of virus accumulation were studied in the resistant TMG-1 genotype and compared with the pattern in WI-2757, an inbred line that is susceptible to all three viruses. While the spread of PRV-W is initially retarded, eventually levels of virus detected by ELISA are comparable to those in WI-2757. ZYMV, however, spreads more slowly and does not attain as high a titer in TMG-1 as in WI-2757. In contrast, WMV-2 does not appear to move systemically in TMG-1. Mixed infection with PRV-W suggests possible facilitation of spread of WMV-2, but not of ZYMV.

A1058

DETECTION OF BYDV-RMV IN SWEET CORN FROM ILLINOIS, MINNESOTA, AND WISCONSIN. C. J. D'Arcy, Department of Plant Pathology, A. D. Hewings, USDA-ARS, University of Illinois, L. E. Sweets, The Pillsbury Co., LeSueur, MN, and W. L. Pedersen, Department of Plant Pathology, University of Illinois, 1102 S. Goodwin Ave., Urbana, IL 61801.

Sweet corn (Zea maize L.) with leaves reddened from the margins inward was found in Illinois, Minnesota, and Wisconsin fields in August 1991. BYDV-RMV serotypes were detected in some maize samples by DAS-ELISA with antibodies to RMV-NY. PCR analyses and DAS-ELISA for PAV, MAV, and RPV serotypes were negative. Aphids (Rhopalosiphum maidis Fitch; R. padi L.) were used to transfer isolates from maize to oat seedlings (Avena sativa L.). Oats developed leaf tip necrosis and reddening in 2-5 wk. All symptomatic oats tested were positive for RMV-like isolates of BYDV by PCR and by DAS-ELISA. No MAV, PAV, or RPV serotypes were detected in oat samples by DAS-ELISA or TAS-ELISA. The monoclonal antibody RPV1 detected some of the Illinois and Minnesota RMV-like isolates.

A1059

ISOLATE-SPECIFIC SYNERGY IN SYMPTOM PRODUCTION BETWEEN A CAULIMO- AND A TOBAMOVIRUS. U. Melcher, R. T. Lartey, R. E. Pennington, Department of Biochemistry, Oklahoma State University, Stillwater, OK 74078

Turnip (*Brassica rapa*) is a host for cauliflower mosaic virus (CaMV) and for a recently characterized tobamovirus, turnip vein-clearing virus (TVCV). TVCV causes vein-clearing but only slight stunting of leaves. The Cabbage S isolate of CaMV causes mild stunting of leaves. Stunting, significantly more severe than seen with Cabbage S inoculation, was observed in turnips inoculated with a mixture of the Cabbage S isolate and TVCV. The mixed inoculum also severely reduced the yield of swollen hypocotyls. In contrast, severe yield reduction and extreme stunting were not observed when the inoculum mixture contained TVCV and the CM4-184 isolate of CaMV. Individually, CM4-184 and TVCV caused a slight stunting of turnip leaves. CaMV nucleotide sequences responsible for the CaMV isolate-specific synergy with TVCV are being identified using CaMV's whose DNA's are chimeras of Cabbage S and CM4-184 DNA's. Whether synergy between

CaMV and TVCV is also observed in other hosts of the viruses is also under study. Research supported by the Oklahoma Health Research Program and the Oklahoma Agricultural Experiment Station.

A1060

SEROLOGICAL RELATIONSHIPS AMONG ISOLATES OF MAIZE CHLOROTIC MOTTLE VIRUS FROM NEBRASKA, HAWAII, AND PERU. E. M. Ball and S. G. Jensen. University of Nebraska, Lincoln and USDA-ARS Lincoln, NE 68583

Antisera to maize chlorotic mottle virus (MCMV) from Nebraska, Hawaii, and Peru were raised in rabbits. Several virus isolates from each location were compared using these antisera with several serological techniques. Gel double diffusion and intragel specific adsorption demonstrated much homogenity among locations but Nebraska isolates also carried antigenic groups in addition to those possessed by the Hawaiian and Peruvian viruses. Indirect ELISA did not demonstrate significant differences between isolates or locations. Double antibody sandwich ELISA showed titration end point differences between Nebraskan viruses and those from other locations. Competition ELISA supported that observation. In no case with any test were distinct and consistent differences observed between isolates from the same location. In these tests there appear to be two serotypes of MCMV, a Nebraska serotype and a second serotype found in Peru and Hawaii. No biological differences have been observed among any of these MCMV isolates.

A1061

EXPRESSION OF A CHIMERIC BCTV COAT PROTEIN GENE IN TRANSGENIC TOBACCO. G. J. Vandemark and P. E. Thomas, USDA-Agricultural Research Service, Route 2 Box 2953A, Prosser, WA 99350-9687.

Beet curly top virus (BCTV), a member of the geminivirus group, causes severe diseases in sugarbeet (*Beta vulgaris* L.) and over 300 other species in 44 families. Coat protein mediated resistance has not been demonstrated in transgenic plants against BCTV. A recombinant vector was produced by cloning a chimeric BCTV coat protein gene into the binary vector pBI101. The recombinant vector was introduced into tobacco (*Nicotiana tabacum* cv. Xanthi-nc) by *A. tumefaciens* mediated gene transfer. Southern blot analysis identified six transgenic lines which contained this chimeric construct. Northern blot analysis indicated that four lines produced an RNA transcript of approximately 950 bases in length that hybridizes to the chimeric BCTV coat protein gene construct. The production of endogenous BCTV coat protein subunits in these transgenic lines has not been confirmed by ELISA. Controlled greenhouse experiments are being conducted to determine if these transgenic lines are resistant to BCTV.

A1062

HOST RANGE OF TOMATO MOTTLE GEMINIVIRUS. J.E.POLSTON, University of Florida, IFAS, Bradenton, FL 34203, E. HIEBERT, University of Florida, IFAS, Gainesville, FL 32611, R. J. McGOVERN, University of Florida, IFAS, Immokolee, FL 33934, P. A. STANSLY, University of Florida, IFAS, Immokolee, FL 33934, and D. J. SCHUSTER, University of Florida, IFAS, Bradenton, FL 34203.

Since the fall of 1989 tomato crops in the central and southwest regions of Florida, and later all production areas of the state, have been infected by a whitefly-transmitted geminivirus, recently named tomato mottle geminivirus (TMoV). A study of the host range of this virus was undertaken. Thirty six species of plants representing eight families which have been reported to be infected by other whitefly-transmitted viruses were selected for the study. Test plants were inoculated with TMoV by viruliferous whiteflies (Bemisia tabaci) using 48 hr acquisition and inoculation access periods or by mechanical inoculation. Plants which were inoculated and became infected belonged to four genera, three in the Solanaceae and one in the Fabaceae. Several species of Lycopersicon, Nicotiana, and Physalis were infected by TMoV. Only one species in the Fabaceae, Phaseolus vulgaris, became infected. This virus appears to have a unique host range compared to other whitefly-transmitted geminiviruses reported to infect tomato.

A1063

VIRUS DISEASE OF YELLOW-POPLAR. O. W. Barnett, V. B. Shelburne, Junmei Yao, and F. H. Tainter. Dept. of Plant Pathology, Dept. of Forest Resources, Clemson University, Clemson, SC 29634-0377.

Virus-like symptoms were observed on leaves of yellow-poplar (Liriodendron tulipifera L.) near the New River in Ashe County, NC in June 1987 and in Pickens County, SC in 1991. Tissue from the affected trees was grafted to healthy yellow-poplar seedlings and leaf symptoms similar to those on the naturally infected trees developed in the greenhouse. Many branches on the original tree in Ashe County had small leaves and shortened internodes while other branches appeared normal. Smaller leaves and short internodes were not seen on other trees, including graft-inoculated seedlings. Leaves exhibited distinctive chlorotic vein banding, blotches, and ringspots. Spherical virus-like particles were observed in cytoplasm in thin sections of leaf tissue from yellow-poplar infected by grafting. This is the first known report of a virus-like disease of yellow-poplar in the United States.

A1064

cDNA CLONING OF PRUNE DWARF VIRUS RNAs 1,2 AND 3, DETECTION OF VIRAL RNA IN PEACH. E. J. Bachman, S. W. Scott*, and V. B. Vance. Dept. of Biological Science, Univ. of S. Carolina, Columbia SC, 29208. * Dept. of Plant Pathology & Physiology, Sandhill Research & Education Center, Columbia 29224-3205

We have isolated cDNA clones to the three genomic RNAs of prune dwarf ilarvirus (PDV). The partial clone for RNA 1, pPDV1, is 1.5 kb and hybridizes to RNAs 1,3 and 4. pPDV2, the partial clone to RNA 2, is 1.8 kb and hybridizes to RNA 2 only. pPDV3 is nearly full length clone to RNA 3, is 2.1 kb. Hybridization and immunological assays have confirmed that PDV RNA 3 is organized in a similar manner as other llarviruses. A 0.9 kb EcoRV fragment from the 5' end hybridizes to RNA 3 only. In contrast, the 1kb HincII fragment from the 3' half of the virus hybridizes to RNAs 3 and 4. When the pPDV3 insert is expressed in E. coli, coat protein of PDV is detected by western analysis. The HincII fragment that hybridizes to RNAs 3 and 4 also expresses coat protein while the 5' fragment does not. This data suggests that PDV RNA 3 encodes the coat protein gene in the 3' 1 kb, an arrangement in accordance with the other tripartite viruses studied to date. The complete nucleotide sequence of PDV RNA 3 is also given. pPDV3 was used as a probe to demonstrate the presence of high concentrations of viral RNAs in various flower parts, buds, and leaves of flowering and dormant peach.

A1065

NUCLEOTIDE SEQUENCE OF APPLE MOSAIC VIRUS (ApMV) RNA4. Rudaina H. Alrefai, Department of Physiology and Plant Molecular Biology, L.L. Domier, USDA-ARS, C.J. D'Arcy, Department of Plant Pathology, and S.S. Korban, Department of Horticulture, University of Illinois at Champaign-Urbana, 1201 W. Gregory Drive, Urbana, IL 61801.

ApMV (Idaho strain) was purified from infected cucumber cotyledons. Virions 3 and 4 (top components in sucrose gradient) were isolated and RNA was extracted. A double stranded cDNA copy of RNA4 was synthesized by random priming and cloned into pUC118. A set of overlapping cDNA clones derived from RNA4 was used to determine the nucleotide sequence of RNA4. A mixture of RNA3 and RNA4 was polyadenylated *in vitro*, used as a template for cDNA synthesis by polymerase chain reaction, and cloned into a pBluescript-T vector. A set of overlapping clones was sequenced. ApMV coat protein was extracted from virions and further purified from SDS-polyacrylamide gels using the chromophore staining technique (Promega). The coat protein sequence was used to confirm the nucleotide sequence of RNA4.

A1066

COMPLEMENTARY DNA CLONING, SEQUENCING, AND RIBOPROBE DETECTION OF SWEETPOTATO LATENT VIRUS (SPLV). J. A. Abad and J. W. Moyer. Dept. of Plant Pathology, North Carolina State University, Raleigh, NC 27695-7616.

RNA of sweetpotato latent virus (SPLV) has been purified and cDNA clones have been made for this putative potyvirus. Several cDNA clones ranging in size from 0.9 to 4 kb were selected for analysis. A 4 kb clone containing the polyadenylated tail, 3'non-coding region and cistrons for capsid protein and NI_b was used for sequencing analysis. A 900 b, Eco RI subclone containing the coding region for the C- terminus of the capsid protein and part of the 3' untranslated region was selected to generate a riboprobe in an *in vitro* transcription system. Riboprobe hybridization allowed the SPLV detection from grafted and mechanically inoculated sweetpotato plants (cv. Jewel) in either single or mixed infections with sweetpotato feathery mottle virus.

A1067

PEANUT MOTTLE (PMV) OR PEANUT STRIPE (PStV)? U.B. Gunasinghe, J. Sherwood[1], R.S. Nelson and B.G. Cassidy, Noble Foundation, P.O. Box 2180, Ardmore, OK 73402; [1]Oklahoma State University, Stillwater, OK 74078.

Inconsistency and ambiguity of the application of serological tests to characterize potyviruses has been reported (Shukla and Ward, 1988). We have produced cDNA clones representing coat protein genes of an Oklahoma isolate of PMV and a blotch isolate of PStV. The deduced amino acid sequences obtained by sequencing these clones indicated that the coat proteins of these two viruses are 97% identical. The non-translated 3'-end regions of these two viruses show about 99% sequence similarity. Evidence from sequence analysis and Western blot analysis suggest that PMV and PStV are two strains of a potyvirus that produce distinguishable symptoms on the same host. Further cloning of other PMV isolates is being pursued to determine the range of this similarity.

A1068

SEQUENCE ANALYSIS OF THE PUTATIVE 3' READ-THROUGH PROTEIN OF SOYBEAN DWARF LUTEOVIRUS (STRAIN Y). O. P. Smith[1], V. D. Damsteegt[1], K. F. Harris[2], B. H. Taylor[2], and R. Vonder Haar[2]. [1]USDA-ARS, Ft. Detrick, Frederick, Maryland 21702 and [2]Texas A&M University, College Station, Texas 77843.

The 3' portion of the RNA genome of soybean dwarf virus strain Y (SDV-Y) has been studied by sequence analysis of cloned complementary DNA (cDNA). A large in-frame open reading frame (ORF)(517 amino acids) has been identified immediately downstream of the coat protein ORF (200 amino acids). By analogy to other luteoviruses and by reference to a recently defined consensus sequence for "leaky" UAG stop codons for several plant viruses (J. Mol. Biol. 218:365), this 3' ORF is likely expressed by read-through translation of the coat protein UAG stop codon. Northern-blot hybridization analysis of total RNA from virus-infected plants shows the presence of a 3' 3.1 kb ssRNA, supporting the hypothesis that this putative protein is expressed by the transcription and subsequent read-through translation of a coat protein-encoding subgenomic RNA. Sequence comparisons of this putative 3' read-through protein to other luteoviruses will be presented.

A1069

BIOLOGICALLY ACTIVE cDNA CLONES OF PANICUM MOSAIC VIRUS SATELLITES. J. Monis, D. S. Sopher, and A. O. Jackson. University of California, Berkeley, CA 94720.

Two strains of panicum mosaic virus (PMV) naturally found infecting centipede and Saint Augustine grass, PMV-C and PMV-S, respectively were investigated. These PMV strains are associated with two distinct classes of satellite agents: a satellite virus (826 nt) that encodes its own capsid protein and satellite RNAs (ca. 380 - 440 nt). We have initiated studies to determine the structure-relationships between the helper, the satellite virus, and the satellite RNAs. For these investigations, the satellite RNAs from the PMV-S strain (ca. 380 nt) and the PMV-C strain (ca. 380 nt and 440 nt) have been cloned and sequenced. The sequence data indicates that the satellite RNAs have extensive relatedness at the 5' end of their genomes but no significant relatedness with the satellite virus. Full length transcription cDNA clones of PMV-C (ca. 440 nt), PMV-S (ca. 380 nt), and SPMV (ca. 826 nt) RNAs were constructed. RNA transcripts derived from these clones were of the size expected of the respective satellite RNAs and were infectious when inoculated onto pearl millet plants in combination with helper virus inoculum free of satellite RNAs. Results of the ongoing mutational analyses of the satellites indicates that minor perturbations impede the ability to establish systemic infection.

A1070

VARIABILITY AMONG ISOLATES OF BYDV-RMV. L. L. Domier, USDA-ARS, L. I. Lukasheva, and C. J. D'Arcy, Department of Plant Pathology, University of Illinois, 1102 S. Goodwin Ave., Urbana, IL 61801.

Illinois (IL) and Minnesota (MN) RMV-like isolates of BYDV were transferred from maize with red leaf symptoms to oats by aphids (Rhopalosiphum maidis Fitch; R. padi L.) and analyzed by PCR and ELISA. Restriction enzyme analysis of luteovirus-specific PCR products yielded banding patterns similar to, but not identical to, those from BYDV-RMV-NY infected oats. IL and MN isolates produced identical banding patterns with five restriction enzymes. The pattern for the NY isolate was different for two of the five enzymes. IL and MN isolates were detected with polyclonal antiserum to the NY isolate in DAS-ELISA. Some IL and MN isolates could be detected with the monoclonal antibody RPV1 in TAS-ELISA; RPV1 did not detect BYDV-RMV-NY. DNA sequence analysis of the coat protein genes confirmed that IL and MN RMV-like isolates were distinct from BYDV-RPV-NY.

A1071

Hemagglutination of swine blood by tomato bushy stunt tombusvirus. M. H. Walter, L. A. Heaton, Department of Plant Pathology, Kansas State University, Manhattan, KS 66506.

Tomato bushy stunt virus (TBSV) coat protein (CP) and the lectin concanavalin A are structurally similar (Argos, P., Tsukihara, T. and Rossmann, M.G. 1980. J. Mol. Evol. 15:169-179). Hemagglutination (HA) assays were used to investigate possible saccharide binding by TBSV. Virus purified from TBSV-infected *Nicotiana benthamiana* plants agglutinated swine RBCs in HA assays with various animal red blood cells. Hemagglutination occurred at virus concentrations as low as 11 micrograms/ml (HA titre of 512) in PBS, pH 6.8. TBSV HA titres differed from titres of mock-inoculated controls by 8 to 512 fold, depending on virus purification procedure. Other controls consisted of hemagglutinating animal viruses and various plant lectins. Similar HA assays are underway with turnip crinkle carmovirus.

A1072

INFLUENCE OF ENVIRONMENT AND GENETIC COMPOSITION ON ABILITY OF CAULIFLOWER MOSAIC VIRUS TO INFECT SOLANACEOUS HOSTS. S.G. Qiu and J.E. Schoelz. Dept. of Plant Pathology, University of Missouri, Columbia, MO 65211.

We have identified regions of CaMV strain W260 involved in systemic infection of Nicotiana bigelovii and Datura stramonium by constructing chimeric viruses between W260 and strain CM1841, a strain that is unable to systemically infect either solanaceous host. All of the chimeric viruses constructed between W260 and CM1841 systemically infected turnips, demonstrating the viability of the chimeric viruses in a host that is susceptible to both viruses. Three regions of W260, containing primarily genes I, IV, and VI influenced the ability of that virus to induce systemic symptoms in the solanacsous hosts. Additionally, the involvement of regions containing genes I and IV was affected by environmental conditions. When infected plants were grown under conditions of low light, low temperatures (18 C), and short days (9.5 hr day), the source of genes I and IV no longer influenced whether a chimeric virus moved systemically. As light intensity and day length were increased, the genetic requirements became more stringent and genes I and IV, as well as gene VI, had to be derived from W260. In contrast to genes I and IV, the involvement of gene VI was insensitive to environmental conditions.

A1073

EXPRESSION OF DISCRETE POTYVIRUS GENE PRODUCTS BY A NON-OCCLUDED RECOMBINANT BACULOVIRUS FED TO *TRICHOPLUSIA NI* LARVAE. J.F.J.M. VAN DEN HEUVEL, D.W. THORNBURY, J. A. LESNAW, AND T. P. PIRONE. UNIVERSITY OF KENTUCKY, LEXINGTON, KY 40546.

A gene construct consisting of the first three cistrons (34K, HC-Pro, 42K) of tobacco vein mottling virus was engineered into the nuclear polyhedrosis virus of *Autographa californica* in lieu of the polyhedrin gene, using a plasmid transfer vector containing the beta-galactosidase gene. Non-occluded recombinant baculovirus was used to inoculate *Trichoplusia ni* larvae *per os* (oral feeding) and infected larvae were identified by an X-gal assay of the hemolymph. Western blot analysis of the infected larvae demonstrated the presence of mature-sized 34 kDa, HC-Pro and 42 kDa polypeptides, indicating the biological activity of the viral proteases. However, the possibility of proteolytic cleavage by insect proteases cannot be ruled out at this time. The aphid transmission-mediating activity of the baculovirus-produced HC-Pro in virus transmission by aphids is currently being investigated.

A1074

DELINEATION OF MONOCLONAL ANTIBODY-DEFINED VIRUS-SPECIFIC AND POTYVIRUS GROUP-COMMON EPITOPES USING SYNTHETIC PEPTIDES, BACTERIALLY EXPRESSED COAT PROTEIN (CP) GENE PRODUCTS AND CP AMINO ACID SEQUENCE COMPARISONS. Ramon Jordan and John Hammond, USDA-ARS, Florist & Nursery Crops Lab, Beltsville, MD.

Antigenic determinants defined by bean yellow mosaic virus-(BYMV) specific (VS) and potyvirus group cross-reactive (GR) PTY monoclonal antibodies (McAbs) were mapped to within 8 to 20 amino acids using a variety of complementary techniques. ELISA tests using 92 synthetic overlapping octapeptides encompassing the 273 aa BYMV GDD coat protein (CP) sequence confirmed and further identified the N-terminal sequence location of the VS epitopes. Using carboxyterminal deletion mutants prepared by *Ba*l31 deletions of a BYMV GDD cDNA, the VS epitopes were mapped to an N-terminal domain while the GR epitopes were mapped to at least five distinct C-terminal or CP-core domains. Comparative sequence alignments of the CP amino acids of immunoreactive and non-immunoreactive potyviruses also allowed further delineation of VS and GR McAb-defined epitopes.

A1075

THE COAT PROTEIN GENE OF A BARLEY YELLOW DWARF VIRUS SGV SEROTYPE FROM TEXAS. C.-H. Lei, R. M. Lister, and J. R. Vincent, Dept. Botany and Plant Pathology, Purdue University, W. Lafayette, IN 47907-1155.

MAV, PAV, SGV and RPV are serotypes of barley yellow dwarf virus (BYDV). Published nucleotide sequences group MAV and PAV together (in BYDV Group 1), separately from RPV (in BYDV Group 2). Here we report that the sequence encoding the putative coat protein (CP) of a Texan SGV isolate (TX-SGV) places it in Group 1. The sequence was determined from cDNA clones from a TX-SGV library and PCR fragments made by using primers containing MAV and PAV consensus sequences. The CP gene and a putative VPg gene embedded therein contain 591 and 441 nucleotides, respectively, encoding proteins with predicted Mr of 21.7 and 16.4 kD, respectively. The CP gene shows 69.5%, 70.2%, and 52.4% homology with those of MAV, PAV and RPV respectively, and cross-hybridized strongly with an Idaho SGV and weakly with NY-SGV. Although TX-SGV reacts like NY-SGV with polyclonal antisera, unlike NY-SGV it is readily transmitted not only by Schizaphis graminis but also by other aphids.

A1076

REACTIONS OF NICOTIANA TABACUM CV. 'XANTHI' CONTAINING THE SMV-CP GENE TO INOCULATION WITH SEVERAL POTYVIRUSES. T.A. Thompson, R.N. Beachy and B.B. Reddick, Department of Entomology and Plant Pathology, University of Tennessee, Knoxville, TN 37996.

Coat protein-mediated protection results in delay of symptoms and lower virus concentration within tobacco plants. In this study, plants of two transgenic 'Xanthi' tobacco lines expressing the soybean mosaic virus coat protein were inoculated with four potyviruses: potato virus Y (PVY), tobacco vein mottling virus (TVMV), tobacco vein banding mosaic virus (TVBMV) and tobacco etch virus (TEV). Protection varied from no delay in disease development to cross-protection for up to 20 days. With PVY and TVMV there was either no delay or a two-day delay depending on the transgenic tobacco line. Symptom expression and TVBMV accumulation were delayed for four days in both tobacco lines. Cross protection was observed in TEV for 20 days.

A1077

PVY CAPSID PROTEIN SEQUENCE COMPARISONS AND EXPRESSION IN TRANSGENIC TOBACCO. S. L. Woloshuk, Sudarsono, G. M. Hellmann, S. A. Lommel, and A. K. Weissinger. Departments of Plant Pathology, Crop Science, and R. J. Reynolds Inc. Box 7620, North Carolina State University, Raleigh NC 27695-7620.

PCR amplified cDNAs of the capsid protein cistron from 6 PVY strains were sequenced. Amino acid sequence alignments revealed greater than 91% identity, establishing them as strains of PVY. Transcripts of each cDNA, directed the synthesis of a polypeptide comigrating with PVY capsid protein and were immunoprecipitable with PVY capsid protein antibodies. The severely necrotic 'Chilean' strain was ligated into the plant transformation vector pBI121, and transformed into several commercial burley and flue-cured tobacco cultivars. Capsid protein expression in transgenic plants was confirmed by Southern and Northern analysis and ELISA. Selfed transgenic capsid protein expressing lines are being assayed for protection against virus infection.

A1078

COMPARATIVE SEQUENCE ANALYSIS OF THE REUNION ISOLATE OF ZUCCHINI YELLOW MOSAIC VIRUS. Baker, C.A., Hiebert, E., Marlow G.C., and Wisler, G.C. University of Florida, Gainesville 32611-0680.

Sequences of specific regions of the Reunion isolate of zucchini yellow mosaic were compared to similar regions of a California isolate of ZYMV (R.Balint, personal comm.). Except for the leader sequence and the P1 coding region, the nucleotide (NT) similarity between corresponding regions of the two isolates ranged from 81-85%. The majority (75-84%) of NT changes were silent, and the predicted amino acid (AA) similarity ranged from 91-93%. Approximately 4% of the NT changes were transversions. For the P1 gene, the NT similarity was 63%; 15% of the NT changes were transversions and the predicted amino acid similarity was only 60%. For the leader sequence, the NT similarity was 30% and 40% of the NT changes were transversions. Gaps (1-2 codons long) were seen only in the P1 sequences and an insertion of 36 bases was found in the non-coding region at the 3' end of the Reunion isolate. While the overall AA similarity of the capsid protein was 91%, the N-terminal 41 AA of the capsid protein had a similarity of only 51%. The N-terminus of the capsid protein, the leader sequence, and the P1 protein were the most divergent regions of the Reunion isolate of ZYMV.

A1079

TRANSGENIC PLANTS EXPRESSING ZYMV COAT PROTEIN OR ANTISENSE RNA ARE PROTECTED AGAINST POTYVIRUS INFECTION. G. Fang and R. Grumet. Horticulture Dept., Michigan St. Univ., E. Lansing, MI 48824.

Three versions of the zucchini yellow mosaic virus (ZYMV) coat protein (CP) were engineered for expression in plants: the full length CP sequence (CP); the conserved core portion of the gene (Core) and an antisense version (AS). These constructs were introduced into muskmelon and tobacco; gene expression was verified by northern and western analysis. Transgenic R_0 muskmelon plants expressing CP or Core and inoculated with ZYMV show a lack or delay of systemic symptoms, and little or no virus accumulation as determined by ELISA. Furthermore, transgenic R_0 and R_1 tobacco plants expressing the CP, Core or AS constructs of ZYMV, a non-pathogen of tobacco, show a delay in symptom development and reduced virus titer when inoculated with the heterologous potyvirus, PVY. The transgenic tobacco were not protected against the non-potyvirus, TMV.

A1080

ANALYSIS OF THE ZUCCHINI YELLOW MOSAIC VIRUS (ZYMV) POTYVIRAL HELPER COMPONENT, POSSIBLE IDENTIFICATION OF AN APHID-INTERACTION DOMAIN. R. Grumet, R. Bada, S. Hammar. Horticulture Dept., Michigan St. Univ., E. Lansing, MI 48824.

Potyviruses are transmitted by aphids in a stylet borne manner that requires the presence of a viral-encoded protein, the helper component (HC). In order to better understand the molecular basis for HC-aphid and HC-virion interactions, the HC gene of the non-aphid transmissible Connecticut (Ct) strain of ZYMV was cloned and sequenced. Aphid transmission assays indicate that the failure to transmit virus is due to a lesion in the HC protein rather than the coat protein. The ZYMV HC sequence differs from other sequenced HCs in 3 regions that are conserved among the Solanaceous potyviruses and PPV. Among the changes is a K→E substitution that was associated with loss of transmissibility in PVY. Sequential feeding tests comparing the effect of a transmissible and non-transmissible (Ct) strain on subsequent PVY transmission, indicate that the Ct strain is defective in interaction with the aphid rather than the virion.

A1081

AN ANTIBODY THAT BINDS TO A COAT PROTEIN REGION INVOLVED IN APHID TRANSMISSION OF SOYBEAN MOSAIC VIRUS. Ch. Jayaram, J.H. Hill, A.W. Schwabacher[1] and R. Van Deusen, Departments of Plant Pathology and [1]Chemistry, Iowa State University, Ames, Iowa 50011.

Potyviruses, which include soybean mosaic virus (SMV), are transmitted both mechanically and by aphids. It has been proposed that the helper component, in association with the coat protein, is involved in attachment to maxillary stylets of aphids for transmission of potyviruses. According to several studies, a conserved tripeptide: aspartate--alanine-glycine (DAG), near the N-terminus of the coat protein may be involved in binding to the helper component. In addition, experiments with monoclonal antibodies specific to the N-terminus epitopes of the coat protein are often conformation-sensitive. In order to further characterize this region, antibodies were generated against four synthetic deca-peptides containing the amino acid sequences of the DAG region. These distinguish between aphid and non-aphid transmissible strains of SMV. Further studies that reveal whether this epitope is sequence or conformation specific will be reported.

A1082

CLASSIFICATION OF POACEOUS POTYVIRUSES USING POLYMERASE CHAIN REACTION. S. G. Jensen and J. S. Hall, USDA-ARS Lincoln, NE and University of Nebraska, Lincoln, NE 68583

Twenty one isolates of four poaceous potyviruses, maize dwarf mosaic virus, sugarcane mosaic virus, johnsongrass mosaic virus, and sorghum mosaic virus were purified and characterized by PCR technology. Coat proteins were removed from the viruses with proteinase K and the RNA converted to c-DNA with reverse transcriptase. Polymerase chain reaction (PCR) primers were a poly-T oligonucleotide for the 3' terminus and a conserved sequence from the NI_b segment (RNA polymerase) of the viral genome for the 5' terminus. A segment of approximately 2500 bp was amplified and visualized by gel electrophoresis. Secondary bands of DNA smaller than 2500 bp were also found with all isolates. Although there was some variation, the pattern of bands was consistent within each of the four viruses and allowed the classification of isolates to the proper virus. Unidentified virus isolates from diverse geographic locations were first identified by PCR and then their tentative classification was confirmed by determining their biological properties.

A1083

THE COAT PROTEIN INSERT OF SUGARCANE MOSAIC VIRUS STRAIN MD-B MAY NOT CORRELATE WITH THE LACK OF INFECTIVITY TO SUGARCANE. S. G. Jensen, J. S. Hall, M. J. Frenkel, and J. M. Jilka. USDA-ARS Lincoln, NE, University of Nebraska, Lincoln, NE, CSIRO Parkville, Victoria, Australia, and Monsanto Corp, St. Louis, MO,68583.

Nineteen strains of potyvirus were examined for the presence of the previously reported (Frenkel et al J. Gen. Virol. 72) coat protein peptide insert suspected of blocking infectivity to sugarcane. DNA probes to a sugarcane infecting virus, sugarcane mosaic virus (SCMV)-A, and to a non-infecting strain, SCMV-MD-B, were used in Northern blots to identify the presence or absence of the insert. PCR amplification of the c-DNA of a coat protein encoding segment of the viral genome gave a 300 bp DNA product from SCMV-A and a 350 bp segment from the insert containing SCMV-MD-B. SCMV-D had only a 300 bp segment but it reacted with both probes and it infected sugarcane. SCMV-J had a 300 bp segment, did not react with either probe and did not infect sugarcane. SCMV-E reacted with the SCMV-MD-B probe but it had a 320 bp segment and did not infect sugarcane. Two isolates from Hawaii had the insert and did not infect sugarcane. Sorghum mosaic virus strain M-La reacted with the SCMV-MD-B probe and also infected sugarcane. Correlation between the presence of the insert and the inability to infect sugarcane was inadaquate to confirm a relationship.

A1084

TURNIP CRINKLE VIRUS COAT PROTEIN IS TRANSLATED IN VITRO FROM SWOLLEN VIRIONS. M. M. Laakso, and L. A. Heaton. Department of Plant Pathology, Kansas State University, Manhattan, KS 66506.

When full-length TCV transcripts are translated in vitro, there are 6 major polypeptide products of 88, 56, 38, 36, 30, 27 kDa. As part of an ongoing study of the early events of infection by small icosahedral viruses, TC virions were translated in vitro. Particles were pretreated at pH 5.5 and 8.5, pelleted through a 20% sucrose pad to isolate intact virions, and translated in a rabbit reticulocyte system. The two major polypeptide products appeared within 20 minutes. These proteins co-migrated with p27 and p38, which are encoded by the 5'- and 3'-most open reading frames, respectively. We suggest that upon swelling, RNA is pulled from the virion as a complex between the N-terminal domains of a few coat protein subunits and the high affinity binding sites on the RNA described by Wei et al. (1990. J. Mol. Biol. 214, 85-95).

A1085

DEFECTIVE MOVEMENT OF TOBACCO MOSAIC VIRUS PREVENTS SYSTEMIC INFECTION OF TURNIP R. T. Lartey, S. Hartson and U. Melcher, Dept. Biochem., Oklahoma State Univ., Stillwater, OK 74078

The relation between systemic movement of infection and species specificity of viruses was explored with tobacco mosaic virus (TMV) and turnips. Turnip protoplasts inoculated with TMV supported TMV replication, as detected by dot blot hybridization. TMV-inoculated turnip plants remained symptomless and contained no TMV detectable by infectivity assays on tobacco, by TMV-specific hybridization using the 30 kDa gene as probe, or by a TMV-specific assay for PCR amplification of the TMV 30 kDa gene. Thus, a failure in systemic movement may limit TMV infection of turnips. Since the 30 kDa gene of TMV is required for systemic movement in tobacco, the sequence of the 30 kDa gene of turnip vein-clearing virus (TVCV), a recently isolated tobamovirus capable of systemic movement in turnip, is being compared to that of TMV. Coinoculation of turnip plants with TVCV and TMV failed to result in systemic infection by TMV, as determined by hybridization and PCR assays. The failure of TVCV to serve as helper virus for TMV infection of turnips suggests that factors other than or in addition to inability of the TMV 30 kDa protein to recognize a turnip component are involved in TMV species specificity. Research supported by the Okla. Health Res. Prog. and the Okla. Agric. Expt. Sta.

A1086

DIRECT NUCLEOTIDE SEQUENCING OF PCR-AMPLIFIED cDNAs OF THE FLORIDA ISOLATES OF CITRUS VIROIDS IIa AND IIb (CACHEXIA). L. Levy, A. Hadidi, and S.M. Garnsey*. USDA-ARS, NGRL, Beltsville, MD, 20705, and *USDA-ARS, USHRL, Orlando, FL, 32803.

Citrus viroids IIa (CVIIa) and IIb (CVIIb), members of the hop stunt viroid (HSV) group, were distinguished based on their biological properties in 'Etrog' citron. CVIIa and CVIIb isolates from Florida were reverse transcribed and subsequently amplified in vitro from total nucleic acid extracts of infected citrus tissue using a reverse transcription-polymerase chain reaction assay (RT-PCR). The nucleotide sequences of CVIIa and CVIIb were determined by direct sequencing of purified RT-PCR products without the need for cloning or generation of asymmetric PCR products. CVIIa is 95% and 96% homologous when compared to the published sequence of HSV citrus variants 1 and 2, respectively. CVIIb is 94% and 95% homologous to HSV citrus variants 1 and 2, respectively. The sequence of CVIIb is 99% homologous to the sequence of CVIIa. Diagrams of the rod-like secondary structures of citrus viroids IIa and IIb will also be presented.

A1087

MARKING RHIZOBACTERIA WITH THE LUCIFERASE OPERON FOR MONITORING SEED AND ROOT COLONIZATION. W. F. Mahaffee[1], J. J. Shaw[2], P. A. Backman[1], and J. W. Kloepper[1], [1]Dept. of Plant Pathology, and [2]Dept. of Botany and Microbiology, Auburn University, AL 36849.

Recovery of rhizobacteria from soil or rhizosphere traditionally has been accomplished with antibiotic-resistant mutants or selective media paired with colony morphology to distinguish introduced bacteria from indigenous soil and rhizosphere bacteria. Insertion of foreign DNA encoding novel phenotypes offers an alternative approach for marking. The luciferase operon from *Vibrio fisherii*, which encodes bioluminescence, was evaluated as a genetic marker for several genera of rhizobacteria. Seed and root colonization patterns of bioluminescent rhizobacterial mutants can be monitored *in planta*, without destructive sampling, using a CCD (charge-coupled device) camera. With a model strain (LT2-139), metabolic activity of the introduced bacterial strain was greatest at the point of radicle emergence in the spermosphere and at the root hair zone in the rhizosphere. Mutants can also be detected and quantified using a "metabolic boosting" procedure and a luminometer, with a log 3 cfu/g tissue detection limit. With standard plating procedures and enumeration of bioluminescence in the dark, detection limits have been lowered to log 1. Advantages and disadvantages of the luciferase marker will be discussed.

A1088

USE OF A BIOLUMINESCENT TRANSCONJUGANT OF *XANTHOMONAS CAMPESTRIS* PV. *CAMPESTRIS* TO MONITOR DISEASE PROGRESS IN CABBAGE SEEDLINGS UNDER DIFFERENT NUTRITIONAL REGIMES. R. McElhaney, A.M. Alvarez, and C.I. Kado. University of Hawaii, Honolulu, HI 96822 and University of California Davis, CA 95616.

A bioluminescent transconjugant of *Xanthomonas campestris* pv. *campestris* (Xcc), 171 LIIH-7, was isolated and compared to the wild type for growth *in vitro* and *in planta*. The strain remained stable with respect to bioluminescence and virulence over six serial passages through cabbage seedlings. Host nutrition and its relation to black rot of cabbage was studied in *Brassica oleracea* cv. *capitata* (C-G cross) seedlings using this transconjugant. In seedlings grown with different levels of NH_4NO_3, $(NH_4)_2SO_4$, KNO_3, K, and P for four weeks prior to inoculation, insufficient amounts of nitrogen in all forms increased disease, whereas NH_4NO_3 and $(NH_4)_2SO_4$ at twice the level required for optimum growth reduced the invasiveness of the pathogen. Movement of the bacteria in the vascular system of the leaf was confirmed with X-ray film. Visual lesions were reduced with high levels of KNO_3, but leaf blots revealed that bacteria had extensively invaded the petiole. Higher levels of K and P fertilization had no apparent effect; however, when P was omitted, disease development was greatly inhibited. The bioluminescent pathogen proved to be a valuable tool in nutritional studies because visual symptoms did not reflect the extent to which the pathogen had progressed in cabbage leaves.

A1089

EVIDENCE FOR POSSIBLE MASKING OF RIFAMPICIN-RESISTANCE PHENOTYPE OF MARKED BACTERIA *IN PLANTA*. J. A. McInroy, G. Wei, G. Musson, and J. W. Kloepper, Department of Plant Pathology, Auburn University, AL 36849-5409.

During studies on population dynamics of internal plant colonization by rhizobacteria and endophytic bacteria, we frequently observed lack of growth of rifampicin-resistant bacteria (rif+) on tryptic soy agar (TSA) amended with rifampicin (rif-TSA). Following seed treatment of cucumber with 6 rif+ rhizobacteria, all strains were recoverable on rif-TSA when external root colonization was monitored. However, when internal root colonization was assessed, bacteria grew upon primary isolation on TSA but not rif-TSA. When bacteria from TSA were transferred to rif-TSA, growth occurred within 18 h. Similar results were obtained in stems and roots of sweet corn and cotton with 7 rif+ endophytic bacterial strains. Comparative fatty acid analysis of introduced and recovered rif+ mutants of all strains indicated no differences. Collectively, these results suggest that expression of rifampicin resistance may be altered *in planta* and, therefore, methods for quantifying internal plant colonization by rif+ bacteria should account for this possibility. Investigations are underway to determine if results are similar with other antibiotics, if inhibitors are involved, and if disinfestation agents affect growth on rif-TSA.

A1090

ENHANCED DETECTION OF *XANTHOMONAS CAMPESTRIS* PV. *DIEFFENBACHIAE* USING A "MINIPLATE SYSTEM". D. Norman, R. Lipp, A. Benedict and A. Alvarez. University of Hawaii, Honolulu, HI 96822.

Detecting, identifying and monitoring *Xanthomonas campestris* pv. *dieffenbachiae (Xcd)* in anthurium fields is laborious and complicated by the pathogen's ability to remain latent for months. Recently, we have developed a "miniplate system" for detecting infections in large numbers of samples. Tissue exudates are applied to individual wells of 96-well tissue-culture plates filled with 150 ul of esculin-trehalose medium, which promotes growth of *Xcd* and turns brown, indicative of esculin hydrolysis. Identity of *Xcd* is confirmed by an ELISA using an *Xcd*-specific monoclonal antibody (Xcd108). Sensitivity and specificity were evaluated by applying 10 ul subsamples containing end-point dilutions of *Xcd* or a mixed suspension containing various ratios of competitive bacteria to *Xcd*. *Erwinia herbicola*, *Pseudomonas fluorescens*, *Agrobacterium tumefaciens* and two common unidentified epiphytes from anthurium were used as competitive strains. All wells were positive for esculin hydrolysis and ELISA through dilutions calculated to contain 1cfu/well. Positive reactions were obtained at all ratios of *Xcd* to competitive bacteria except for the highest ratio of *A. tumefaciens*:*Xcd* (5587:1 cfu/well). The system was evaluated on 142 leaf samples with and without symptoms. The miniplate system gave 3.3% false positives compared to the standard isolation method with 0% false negatives. The predictive value of a positive result for the miniplate system was 97.9%.

A1091

TRACKING A *lac*ZY-MARKED STRAIN OF *PSEUDOMONAS FLUORESCENS* IN A WISCONSIN FIELD TRIAL. J. L. Parke[1], R. M. Zablotowicz[2] and R. E. Rand[1]. [1]Dept. of Plant Pathology, University of Wisconsin-Madison, Madison, WI 53706 and [2]USDA-ARS Southern Weed Science Laboratory, Stoneville, MS 38776

This study addresses environmental implications of field release of a genetically engineered pseudomonad. We compared the potential for movement, colonization of weed species and persistence in soil of a *lac*ZY-marked strain and a rifampicin-resistant mutant of *Pseudomonas fluorescens* PRA25 introduced as pea seed inoculants. Both strains were found up to 20 cm from inoculated seeds indicating their potential for lateral movement in soil. Low population densities of both strains were detected on roots of two non-target weed species, redroot pigweed and lambsquarters. The population density of both strains was log 4 cfu per g soil at 25 days

after pea harvest. At 50 days after pea harvest, populations of both strains had declined significantly to less than log 3 cfu per g soil. These data provide evidence for ecological similarity between the rifampicin-resistant strain and the *lac*ZY marked strain in movement and persistence in soil. We are currently monitoring overwintering survival and potential for recolonization of peas.

A1092

PLEIOTROPIC MUTATIONS ASSOCIATED WITH SPONTANEOUS ANTIBIOTIC-RESISTANT MUTANTS OF RHIZOBACTERIA. C. M. Press, J. W. Kloepper, and J. A. McInroy, Department of Plant Pathology, Auburn University, AL 36849.

Pseudomonas cepacia strain PC90-1 provided statistically significant protection against cotton seedling damping-off, caused by *Rhizoctonia solani*, in several growth chamber and field trials in 1990 and 1991. Spontaneous rifampicin-resistant mutants (rif+) of PC90-1 were generated to investigate its root colonization capacity. Seven rif+ mutants were compared to wild-type PC90-1 for growth rates, fatty acid analysis, utilization of 95 carbon sources, and biological control activity against *R. solani* in a growth chamber assay. Changes in growth rates, fatty acid analysis, and C-source utilization were generally associated with rif+ mutants. Treatment of cotton seed with all 7 rif+ mutants and wild-type PC90-1 resulted in significant stand increases in the presence of *R. solani*, compared to controls, indicating that the pleiotropic mutations did not affect biological control activity under the specific assay conditions. Resistance to rifampicin may be due to an alteration in the ability of DNA-dependent RNA polymerase to bind rifampicin or in cell permeability which could account for the observed pleiotropic changes. The association of frequent pleiotropic mutations with spontaneous mutagenesis suggests that mutants should be compared to wild-type strains for biological responses on plants.

A1093

THE MICROECOLOGY OF PLANT-ASSOCIATED BACTERIA GENETICALLY ENGINEERED TO BIOLUMINESCE. F. Dane[1], J. J. Shaw[2], A. Temann[2] and W. Klingmueller[3]. [1]Dept. of Horticulture and [2]Dept. of Botany and Microbiology, Auburn University, AL 36849, and [3]Lehrstuhl fuer Genetik der Universitaet Bayreuth, W-8580 Bayreuth, Germany.

Bacteria may colonize plant leaves endophytically or epiphytically. This microecology is of interest in predicting the development of plant disease. *Xanthomonas campestris* pv. *campestris* (XCC), the causal agent of black rot of crucifers, was genetically engineered with the *lux* transposon, Tn*4431*, to bioluminesce and used in field introductions to study the persistence and movement of bacteria on resistant and susceptible host plants. The bioluminescence of the bacteria was tracked with a sensitive electronic camera, providing remote and nondestructive sensing of bacterial location *in planta*. Bacteria were able to epiphytically colonize both resistant and susceptible hosts at similar levels, but were only able to invade the susceptible host. To explore the use of bioluminescence to monitor horizontal gene transfer in soil, *Enterobacter agglomerans* 339, a nitrogen fixing soil bacterium that associates with wheat roots, was also marked with Tn*4431*.

A1094

SEROLOGICAL MARKERS FOR MONITORING *XANTHOMONAS CAMPESTRIS* PV. *DIEFFENBACHIAE* IN AEROSOLS. J. Venette, A. Alvarez, and D. Norman. Depts. of Plant Pathology North Dakota State University, Fargo, ND, 58105 and University of Hawaii, Honlulu, HI 96822.

Bacterial blight of anthurium caused by *Xanthomonas campestris* pv. *dieffenbachiae* (*Xcd*) has limited anthurium production in many tropical areas. Exclusion of the pathogen through a clean stock system is currently a major endeavor of the anthurium industry in Hawaii. We have found that the pathogen is readily transported as aerosols. Airborne bacteria were collected in Andersen viable particle samplers on esculin-trehalose medium in commercial production shadehouses. Suspect colonies were streaked onto tetrazolium medium and transferred to ELISA plates and tested with Xcd108, a monoclonal antibody (mAb) specific for anthurium strains of *Xcd*. Representative strains were tested for pathogenicity by injecting a 10^4 cfu/ml suspension into leaves or by misting 10^6 cfu/ml on *Anthurium* plants. These strains were serotyped with a panel of mAbs that distinguish serogroups of *Xcd*. Strains which reacted with Xcd108 were pathogenic and strains which did not react with Xcd108 were non-pathogenic to weakly pathogenic. Serotypes of *Xcd* in aerosols were identical to those previously found on the farms. The population density of Xanthomonads in aerosols was greater during periods of rain or under sprinkler irrigation then under dry conditions.

A1095

EXPRESSION OF THE ß-GLUCURONIDASE (GUS) GENE SYSTEM ALLOWS TRACKING OF *AUREOBASIDIUM PULLULANS IN SITU* ON LEAF SURFACES. L.F. Yourman, J.H. Andrews, and S. Leong, Department of Plant Pathology, University of Wisconsin, Madison, WI 53706.

Our objective is to develop an accurate and reliable system to quantify, identify, and determine morphotypes of an introduced fungus. The epiphyte *A. pullulans* was co-transformed with plasmids encoding the genes for GUS (*uidA*) and hygromycin resistance (*hygB*). GUS was expressed at significant levels in co-transformants. No activity was detected in extracts of the untransformed isolate. Southern hybridization analysis indicated integration of the plasmids into the genome. Extracts were made from leaves spot-inoculated with a dilution series of GUS strain B3. The specific activity of GUS in the extracts was used as a measure of the amount of fungus present. Apple leaves and microbes associated with apple leaves were assayed for endogenous GUS activity and either no, or insignificant, activity was observed. Apple leaves in growth chamber experiments were sprayed with blastospores of strain B3. Disks were removed from these leaves at 8 time-points during a 5 week period and microscopically examined after staining with the GUS-specific substrate x-gluc. Cells of the GUS$^+$ strain stained blue on all disks sampled, indicating that the GUS marker was stable *in situ*. Four distinct cell types were identified on leaf surfaces: blastospores, swollen cells, chlamydospores, and hyphae.

A1096

COMPARISON OF MARKING SYSTEMS TO MONITOR ROOT COLONIZATION BY *PSEUDOMONAS FLUORESCENS* STRAIN PRA25. R. M. Zablotowicz[1] and J. L. Parke[2]. [1]USDA-ARS Southern Weed Science Laboratory, Stoneville, MS 38776 and [2]Dept. of Plant Pathology, University of Wisconsin-Madison, Madison WI 53706

Root colonization of peas by seed inoculants of a *lac*ZY construct (PRA25L1), a rifampicin resistant mutant (PRA25rif) and the wild-type strain PRA25 was addressed in a Wisconsin field trial. Initial root colonization by PRA25L1 was less than for PRA25rif but both strains maintained populations of log 6 to log 7 cfu per cm root on the upper taproot section for the remainder of the growing season. Midseason patterns of lateral root and taproot colonization were similar for PRA25L1 and PRA25rif. All PRA25 strains have intrinsic resistance to 1000 μg ml^{-1} ampicillin, a useful marker for some root colonization studies. PRA25L1, however, was unable to express both this level of resistance and lactose utilization upon direct recovery from the rhizosphere. Selective recovery of PRA25L1 was improved when M-9 medium was amended with 100 μg ml^{-1} ampicillin to suppress growth of indigenous lactose-utilizing soil bacteria. The *lac*ZY marker has utility in root colonization studies but may result in an underestimate of propagule density.

A1097

USE OF NATURALLY OCCURRING CHITINASE ACTIVITY AND COLONY MORPHOLOGY FOR MONITORING EPIPHYTIC POPULATIONS OF APPLIED ANTAGONISTS. P. A. Backman and L. D. Ploper, Dept. of Plant Pathology, Auburn University, AL 36849-5409.

For the past three years we have conducted many field experiments to determine the survival and growth of numerous applied antagonists on leaf surfaces. It was impractical to introduce markers for the large numbers of isolates tested. Since bacteria were applied to leaves amended with chitin, the ability to hydrolyze chitin as a food source was a primary selection criterion. We selected *Bacillus* spp. preferentially over numerous other species because of their environmental tolerance. Populations were monitored on leaf surfaces by plating leaf washates from a stomacher blender on chitin-nutrient agar and enumerating chitinolytic colonies with *Bacillus* colony morphology and color. Counts of colonies with these characteristics were virtually nonexistant on untreated control plots. Heating the washate for 15 min at 80 C was an easy means of determining that chitinolytic colonies were indeed *Bacillus* spp. Similar tests have indicated that nonspore forming, chitinolytic *Serratia* and *Curtobacterium* can also be monitored by this procedure.

A1098

Measuring the Persistence and Survival of PGPR on Canola and Soybean Overwinter Using Spontaneous Antibiotic Markers. EM Tipping[1], GL Brown[1], RM Zablotowitcz[2], and JW Kloepper[3], [1]ESSO Ag Biologicals (formerly Allelix), 15 Innovation Blvd, Saskatoon, Canada S7N 2X8, [2]USDA/ARS, Southern Weed Sciences Lab, Stoneville, MS 38776, [3]Department of Plant Pathology, Auburn University, AL 36849.

Multiple year, multiple crop root colonization measurements were done on canola and soybean using spontaneous rifampicin-resistant mutants of PGPR strains. Assessments were taken after emergence, at flowering, at harvest, over-winter, at replant, and at emergence of a second crop. All strains colonized at, or above Log 6 CFU/g root during early vegetative growth and generally, populations declined as the crop matured. Strains which persisted at high levels through the growing season survived over-wintering and were able to colonize, though at reduced levels (Log 4-5 CFU/g root), crops after replanted. Most strains present only at low levels at the end of the first growing season (<Log 2/g root), were not able to re-establish high population levels in the subsequent year.

A1099

Oligonucleotide probes to detect bacteria introduced into the environment. S Gill[1], JP Mercier[2], C Lemieux[2], S Gagné[3], GL Brown[4] and P Dion[1]. [1]Département de phytologie and [2]département de biochimie, Université Laval, Québec, Qc, Canada G1K 7P4; [3]Centre de recherche Premier, Rivière-du-Loup, Qc, Canada G5R 4C9; [4]ESSO Ag Biologicals, 15 Innovation Blvd., Suite 402, Saskatoon, SK, Canada S7N 2X8.

The well-conserved ribosomal RNA (rRNA) genes of bacteria are interspersed with sequences showing variability at the genus, species or even strain level. This mixture of common and unique sequences provides a basis for a general approach for the design of strain-specific DNA probes. The 600 bp intergenic region, defined by primers specific for conserved 16S and 23S rRNA gene regions, was amplified using the polymerase chain reaction. Cloning, sequencing and comparison of the amplified products from different root-colonizing pseudomonads allowed identification of strain-specific sequences. Oligonucleotide probes constructed from these sequences were highly specific in colony hybridizations for bacterial isolates collected from environmental samples.

A1100

ICE-NUCLEATION ACTIVITY AS A REPORTER OF *IN SITU* GENE EXPRESSION BY RHIZOSPHERE PSEUDOMONADS. J. E. Loper and M. D. Henkels, USDA-ARS, HCRL, 3420 N.W. Orchard Ave., Corvallis, OR 97330.

Pseudomonas spp. that contained an ice nucleation gene transcribed from its native promoter (*iceC*) expressed similar ice nucleation activity (INA) in the rhizosphere and spermosphere of several plant species and in culture media, indicating that the physical or chemical properties of the plant-soil interface and standard methods for retrieving bacteria from roots or seeds did not interfere with INA. INA of cells in the rhizosphere was stable for at least 7 days and was similar in soils that varied in pH and other properties. In contrast, INA of *Pseudomonas* spp. containing a *pvd-inaZ* construct (an ice-nucleation reporter gene devoid of its native promoter cloned downstream of an iron-regulated promoter for pyoverdine biosynthesis) was responsive to iron availability in culture media and soil and was expressed differentially in the rhizosphere and spermosphere of several plant species.

A1101

BIOLUMINESCENCE AS A MARKING SYSTEM TO MONITOR THE FATE OF PSEUDOMONAS PUTIDA, STRAIN 61.9A.3L, RELEASED IN THE SOYBEAN RHIZOSPHERE. C. J. Beauchamp[1], J. W. Kloepper[1], J. J. Shaw[2], and P. A. Lemke[2]. [1]Department of Plant Pathology, and [2]Department of Botany and Microbiology, Auburn University, AL 36849.

In 1991, a courtesy permit was obtained from APHIS to perform a small field release of *Pseudomonas putida* strain, 61.9A.3L, genetically engineered for bioluminescence. In May, soybean seed were inoculated with the antibiotic-resistant strain 61.9A.Rif+, the Tn*5-luxAB* engineered strain 61.9A.3L, and a 1:1 mixture of both strains. Recovery and survival of the strains during the growing season were monitored. In the soybean rhizosphere, strain 61.9A.3L was recovered using dilution-plating, root prints, and broth enrichment. Seed inoculation resulted in mean rhizosphere populations of log 6 (cfu + 1) per root system 11 days after planting (DAP), and populations decreased to nondetectable levels by 70 DAP. In mixtures of the two strains, strain 61.9A.Rif+ displaced strain 61.9A.3L during the season. Total bacterial and fungal populations were not affected by treatment with either strain. These results indicate that bioluminescence can be used in field conditions to detect and monitor rhizosphere populations of engineered bacteria, and that engineered bacteria may have reduced competitive rhizosphere colonization capacities.

A1102

USE OF THE TN7::*LAC* ZY CONSTRUCT IN STUDYING BACTERIA IN THE ENVIRONMENT. D. Kluepfel[1], D. Tonkyn[2], J. Lawrence[1], and T. Lamb[2]. Departments of [1]Plant Pathology and Physiology, and [2]Biological Sciences, Clemson University, Clemson, SC 29634.

The release of microorganisms into the environment to perform specific utilitarian functions presents several interesting challenges. When studying the basic ecology of these microbes one such challenge is the selection of an unambiguous marking system. Although no single system is appropriate for all bacteria in all environments, we have found the Tn7::*lac* ZY insertion extremely valuable in the study of fluorescent pseudomonads in both soil and plant tissue. The use of the *lac* ZY construct permits the use of lactose as a sole carbon source, a trait not exhibited by native fluorescent pseudomonads. This characteristic allows us to reduce our reliance on antibiotics as selective markers for detection and quantification of released microbes. Secondly, the terminal sequences of Tn7 facilitate direct detection. By coupling DNA extraction from soil with specific amplification accomplished with the polymerase chain reaction, extremely sensitive quantitative and qualitative analysis of microbes released into the environment is possible.

A1103

CHROMOSOMAL INSERTIONS OF *xylE* IN *PSEUDOMONAS SYRINGAE* AS IDENTIFIABLE MARKERS FOR ECOLOGICAL STUDIES. S.E. Lindow, E. Clark, and M. Wilson, Department of Plant Pathology, University of California, Berkeley, CA 94720.

The *xylE* structural gene encoding the production of 2,3-catechol dioxygenase, which converts catechol (a colorless substrate) to 2-hydroxymuconic semialdehyde (an intensely yellow compound), was cloned into the *ice*C gene which confers ice nucleation activity in *Pseudomonas syringae* strain Cit7. This gene fusion construct was used to incorporate *xylE* into the chromosome of strain Cit7 by marker-exchange mutagenesis, producing the isogenic Ice^{-} Xyl^{+} strain denoted Cit7xylE. Colonies or colony lifts of Cit7 remained colorless while Cit7xylE turned yellow within 1 min after being sprayed with a 0.1M solution of catechol. No discernable differences in growth rate between Cit7 and Cit7xylE were noted in culture. When Cit7 and Cit7xylE were inoculated onto bean leaves in different proportions but at a constant total cell concentration, the ratio of these two strains recovered from plants after growth for more than 10 generations was similar to the ratio of the two strains in the inoculum for a given mixture. The constitutive expression of the *xylE* gene in *P. syringae* thus does not significantly reduce the ability of host strains to compete for limiting resources on leaf surfaces.

A1104

SYSTEMIC RESISTANCE AGAINST NORTHERN LEAF BLIGHT AND COMMON RUST IN MAIZE INDUCED BY FOLIAR SPRAY OF PHOSPHATES. R. Reuveni*, M. Reuveni** and V. Agapov**. Div. of Plant Path., ARO, Newe Ya'ar Res. Stn.* Haifa 31-999, Israel and Golan Res. Inst.**, Univ. of Haifa, PO Box 97, Kazrin 12900, Israel.

One spray of phosphate salts on the upper sides of maize leaves 1, 2 and 3, two hours before inoculation, induced systemic protection up to 83% reduction in the number of lesions per plant of Northern Leaf Blight caused by Exserohilum turcicum, on leaves 4, 5 and 6. Similarly, remarkable systemic protection of 98% reduction in the number of pustules of common rust caused by Puccinia sorghi was observed on leaves 4, 5 and 6 of maize plants in which leaves 1, 2 and 3 were sprayed with K_2HPO_4, 2h before inoculation. Induced systemic protection, accompanied with remarkable stimulation in plant fresh weight, was evident in both host-pathogen interactions regardless of the lack or appearance of traces of chlorotic stipplings, resulted by the phosphate spray. The possible dual use of phosphate salts as foliar fertilizers and as agents for induced systemic resistance is discussed.

A1105

LOCAL AND SYSTEMIC PROTECTION AGAINST POWDERY MILDEW AND GROWTH INCREASE IN CUCUMBER PLANTS INDUCED BY PHOSPHATE SALTS. M. Reuveni*, V. Agapov* and R. Reuveni**. Golan Res. Inst.*, Univ. of Haifa, PO Box 97, Kazrin 12900, Israel and Div. of Plant Path., ARO, Newe Ya'ar Res. Stn.** Haifa 31-999, Israel.

A single spray of 0.1M solutions of K_2HPO_4, KH_2PO_4, $Na_4P_2O_7$, Na_2HPO_4 and Na_3PO_4 on the upper surface of the first true leaf of cucumber before inoculation with Sphaerotheca fuliginea, inhibited disease development on this leaf and induced a significant systemic protection on leaf 2. Spraying of K_2HPO_4 on leaf 1 at 96, 48 and 2h before inoculation induced 74, 76 and 96%, respectively, of systemic protection in the number of pustules per plant as compared to water spray. However, removal of the induced leaf at various intervals after induction revealed that at least 72h were needed in order to establish a significant protection (86%) on leaf 2. One spray of K_2HPO_4 on leaf 1, two or four days before inoculation stimulated plant growth, regardless to inoculation. The possible dual use of these compounds as foliar fertilizers and induced resistance agents is considered.

A1106

EFFECT OF LEAF AGE ON COMPONENTS OF PARTIAL RESISTANCE TO *Stagonospora nodorum* ON WHEAT. Jennifer A. Yocum and Barry M. Cunfer, Ciba-Geigy Corp., RD 2 Box 92, Hudson, NY 12534, and Department of Plant Pathology, University of Georgia, Griffin, GA 30223

Resistance components varied with leaf age under differing environmental conditions for *Stagonospora nodorum* on adult wheat in both field and greenhouse tests. There was an inverse relationship between leaf age and the components incubation period (IP) and latent period (LP). IP and LP were longest on the youngest plant part. IP and LP were successively longer on the flag-1, flag leaf, and glumes in four field trials during two years with differing environmental conditions. In the greenhouse, where microclimate differences within the plant canopy were not a factor, IP and LP also were longer on the flag leaf than the flag-1 leaf. Results were consistent among 10 cultivars which ranged from very susceptible to moderately resistant.

A1109

Inorganic Nitrogen Nutrition of Germinated Embryos of the Giant Witchweed (*Striga hermonthica* Benth.) Cultured *In Vitro*.

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ABSTRACT

Striga spp., commonly called witchweeds, are flowering hemiparasitic weeds causing severe yield losses to grain cereals and legumes in the tropics. None of the control methods so far used in Africa, including catch and trap cropping, breeding for resistant varieties and handpulling offer complete control of *Striga* in infested soils. Recently, it was observed that nitrogen fertilisers often reduced the severity of *Striga* attack and increased yield of infested crops. However, the mechanism by which nitrogen brings about its effect, or the forms that are most effective have not been clarified. In axenic medium, all available inorganic nitrogen compounds were screened to elucidate their effect on the growth and development of *Striga*. Results show nitrogen is essential for shoot development from germinated *Striga* embryos, as there was no shoot development in culture without nitrogen. Nitrogen compounds, including KNO_2, $NaNO_2$, $(NH_4)_2MoO_4$, and $Fe(NO_3)_2$, completely inhibited *Striga* development beyond radicle emergence at all concentrations tested, while $(NH_4)_2HPO_4$, NH_4F, NH_4Cl, NH_4NO_3, $(NH_4)_2(CO)_2$, $(NH_4)_2SO_3$, $(NH_4)_2SO_4$, $NaNO_3$, KNO_3 and $Ca(NO_3)_2$ did not. Ammonium compounds suppressed *Striga* development at lower concentrations than at higher concentrations, while the reverse was the case with nitrate compounds. A combination of NH_4^+ and NO_3^- as in NH_4NO_3 stimulates high *Striga* shoot development, therefore would not be the recommended source of fertilizer for *Striga* infested soils. Our experiments therefore provides data to show that nitrogen reduces the severity of *Striga* attack through suppressd growth and development of the parasite.

A1110

GLYCEOLLIN ACCUMULATION IN ISOLINES OF SOYBEAN CV WILLIAMS INFECTED WITH PHYTOPHTHORA SOJAE. R. E. Wagner, J. D. Paxton, and P. H. Nass. Dept. of Plant Pathology, University of Illinois at Urbana-Champaign, Urbana, IL 61801.

Resistance to Phytophthora sojae in soybean (Glycine max) is conferred by Rps genes. Two types of lesions form on the taproot of aeroponically-grown resistant plants following incompatible interactions with the fungus. A type 1 lesion is characterized by brown, necrotic flecks that form at the site of penetration. A type 2 lesion is characterized by a discontinuous, brown, water-soaked lesion that encircles the taproot and extends to the root tip. Following inoculation with 1000 zoospores of P. sojae race 3, glyceollin accumulation was monitored by HPLC in roots of cv Williams (rps; compatible), and two isolines, Williams 82 (Rps1-k; incompatible, type 1 lesion) and L83-570 (Rps 3; incompatible, type 2 lesion). At 4, 8, 12 and 16 hr after inoculation, glyceollin concentration was 0, 0, 0 and 127 ug/g in Williams; 0, 496, 1476 and 3818 ug/g in Williams 82; and 0, 94, 685, and 1108 ug/g in L83-570.

A1035

EFFECTS OF FUNGICIDES ON THE IN VITRO GROWTH OF STRAINS OF ACTINOPLANES spp. L. L. Singleton, A. B. Filonow, C. S. Anderson, and N. I. Khan. Oklahoma State University, Stillwater, OK 74078.

Some Actinoplanes spp. are parasites of oospores. Fosetyl-Al, Metalaxyl, Benomyl, Captan, PCNB, and Carboxin (@ at 1 and 10 ppm[000] a.i.) were tested for in vitro growth suppression of 45 strains of Actinoplanes species. Fungicides were suspended in reagent grade acetone, and 20 μl of each were added to 6.35mm paper disks. Cultures were grown in Emerson's YpSs broth with agitation (RT dark; 13 d.). Aliquots of cultures (0.1ml) were spread on YpSs agar (1/5X) dishes followed by the addition of 2 treated disks/dish (2 dishes @/rate), and incubated (RT; 12h light). Zones of inhibition were measured after 7 days (slow growth strains at 14 days). All 45 strains were sensitive to both rates of Captan, and high rates of Carboxin. No stains were sensitive to either rate of Fosetyl-Al or low rates of Metalaxyl and Benomyl. Some strains were sensitive to the high rate of Metalaxyl and Benomyl. The strains were variable in their sensitivity to both PCNB rates and low rates of Carboxin.

A1037

CHEMOTAXIS OF ZOOSPORES OF ACTINOPLANES SPP. TO OOSPORES OF PYTHIUM SPP. N. I. Khan, A. Filonow and L.L. Singleton, Dept. of Plant Pathology, Oklahoma State Univ., Stillwater, OK 74078

Three strains of Actinoplanes spp. were grown on Czapek's sucrose-nitrate agar for 1-4 weeks. Plates were flooded with phosphate buffer to release zoospores from sporangia. Cultures of P. ultimum or P. arrhenomanes were grown in V-8 broth for 1-2 months and macerated in buffer. Oospores were sieved from hyphal debris and washed 3X with buffer. Open-ended capillaries (1 ul, 3-cm long) were filled with buffer, one end was inserted into a chamber containing a suspension of oospores (ca. 10^4) or buffer, and the other end into a suspension of zoospores (ca. 2×10^6). Numbers of zoospores in capillaries were assessed by dilution plating. Zoospores of all strains readily accumulated in capillaries inserted in oospore suspensions, but not into buffer. For example, zoospore attraction to P. ultimum oospores after 2 h was 211-fold greater than attraction to buffer. After overnight incubation, oospores in chambers were parasitized by Actinoplanes spp.