Abstracts of Presentations at the 1990 Annual Meeting of The American Phytopathological Society and The Canadian Phytopathological Society

August 4–8, 1990
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The number above an abstract corresponds to its designation in the program of the 1990 APS Annual Meeting in Grand Rapids, MI, August 4-8. If a presentation was not given at the meeting or was published in the Canadian Journal of Plant Pathology, the abstract is not printed among the following pages.

The index to authors begins on page 1073.

A1
DETECTION AND COMPARISON OF ASTER YELLOW STRAINS BY MONOCOCCAL ANTIBODIES. K. D. Yang and T. A. Chen. Department of Plant Pathology, Rutgers University, New Brunswick, NJ 08903.

Monococal antibodies against the aster yellow mycoplasma-like organism New Jersey strain (AY-NJ) and the aster yellow Eastern strain (AY-E) have been used to detect disease in AY infected plants. Five isolates partially purified from AY-NJ, AY-E, AY-Western (AY-W), Minnesota, and AY-Wisconsin infected lettuce have been compared with each other using AY-NJ monococal antibodies (Mabs). AY-E Mabs and Maize Bushy Stunt Mabs. In enzyme-linked immunoassay (ELISA) and immuno-fluorescent assay (IFA), AY-NJ Mabs strongly react with AY-NJ, AY-E, AY-Minnesota, and AY-Wisconsin but not with AY-Western. AY-E Mabs react with all five isolates. However, Maize Bushy Stunt Mabs only react with AY-Western strain but not with other AY strains. This suggests that the AY-Western strain has different antigenic determinants from other AY strains and also suggests a relationship between Maize Bushy Stunt and AY-E.

A2

Monococal antibodies (McAbs) raised against tomato big bud MBLO were employed in dot immunobinding assays. The McAb reacted only with strains in the aster yellow (AY) MBLO strain cluster, and not with any of several other MBLOs (including elm yellows, ash yellows, western X, clover proliferation, and potato witches' broom MBLOs). However, reactions with AY strain cluster MBLOs varied with strain of MBLO. None of the McAb reacted with certain well-characterised strains of AY MBLO, including strains termed MAV (Maryland aster yellows), DAV, MA, OKJ, NOY, and TIAK. However, all reacted with any of several other MBLO strains previously termed "aster yellows", including strains OKJ, NAV, NOJ, and AY.27. These results are consistent with nucleic acid hybridizations in which two distinct groups were identified within the AY MBLO strain cluster (1988. Mol. Plant Microbe Interact. 1:303-310).

A3

Molecular hybridization probes containing cloned DNA fragments from beet leafhopper transmitted virescence (VR) MLO or from clover proliferation (CP) MLO were prepared. The probes were employed in dot hybridizations against nucleic acid extracted from plants of Catharanthus roseus infected by VR or CP MLO or by one or another of ten different MLOs. Results from the hybridizations indicated that VR and CP MLOs are distinct from one another and are only distantly related to other MLOs, including aster yellows (AY), tomato big bud (BB), perikinKle little leaf (P-O), elm yellows (EY), and western X disease MLOs. The results are consistent with results from hybridizations with probes containing cloned DNA fragments from AY, BB, O-P, and EY MLOs, and with the concept that VR and CP MLOs are representatives of new MLO strain clusters.

A4
ISOLATION AND MOLECULAR CLONING OF DNA FROM A MYCOPLASMA-LIKE ORGANISM (MBLO) ASSOCIATED WITH WALNUT HITCHES' BROOM (WHB) DISEASE. J. Chen, C. J. Chang, R. Jarrett,2 and N. Gawe1.
1Department of Plant Pathology and 2USDA-ARS, Plant Introduction Station, University of Georgia, Griffin, GA 30223.

Total DNA was extracted with CTAB buffer from freeze-dried petioles and leaves of walnut (Juglans nigra L.) showing severe symptoms of WHB disease. DNA of WHB MLO was separated from host DNA by centrifugation in CsCl. WHB MLO DNA and pUC18 were digested with Eco RI and Hind III. ligated and used to transform E. coli JMB3. WHB MLO DNA clones were screened using [32P]-labeled DNA from both healthy and diseased trees. MLO DNA inserts were verified by agarose gel electrophoresis and dot blots of healthy and diseased walnut DNA with [32P]-labeled cloned plasmids. Using dot hybridization, at least one WHB MLO DNA clone (pWBA1 - 1.9 kb) hybridized strongly to DNA extracted from tissues of WHB infected trees, but only weakly to that from pecan bunch MLO, and not at all to blots of periwinkle infected with Western X, BLAT, eastern and severe western aster yellows MLs, and Spiroplasma citri.

A5
DISTRIBUTION AND MULTIPLICATION OF WESTERN ASTER YELLOW MLs IN CATHARANTHUS ROSEUS. C. R. Kuken and B. C. Kirkpatrick.
Department of Plant Pathology, University of California, Davis, CA 95616.

The distribution and multiplication of two AY-MLO strains in C. roseus plants were monitored over a ten week period. Plants were graft-inoculated with either the severe (SAY-MLO) or dwarf strain of AY-MLO. DNA was isolated from plant tissues and hybridised with [32P]-labelled DNA probes derived from chromosomal or plasmid DNA of SAY-MLO. Relative concentration of MLOs in different regions was quantified by scintillation counting of hybridized DNA. Colonization patterns of the two AY-MLO strains were similar. MLs were first detected in grafted shoots about two weeks before symptoms appeared. The MLOs moved from grafted shoots into ungrafted shoots, and then systemically throughout the plant. Distribution and concentration of MLOs correlated directly with expression of virescence and proliferation symptoms. MLO concentrations were highest in symptomatic, actively growing shoots and lowest in roots.

A6
GIBBERELLINS AND THEIR POSSIBLE INVOLVEMENT IN THE HOST INDUCTION RESPONSE OF THE BEST LEAFHOPPER TRANSMITTED VIRESCENCE
Research has been undertaken to determine whether changes in gibberellin (GA) levels are involved in the heat induction response (HIR), the precocious flowering brought about by infection with a mycoplasma-like organism, the beet leafhopper transmitted virose agent (BLVTA). Quantitative and qualitative analysis of endogenous GAs in rosette healthy, bolting healthy and bolting BLVTA-infected Raphanus sativus indicated that GAs are elevated in the infected plants. Infection of GA deficient dwarf mutants (GA-1) of Arabidopsis thaliana with BLVTA did not restore the normal phenotype. This mutant is reported to be deficient in ent-kaurene synthase. Thus, although elevation of GA levels may be involved in the HIR, these experiments indicate that BLVTA can not compensate deficiencies in the synthesis of ent-kaurene, an important regulated step in GA synthesis.

A11
DETERMINATION OF WALL-DEGRADING ENZYME PRODUCTION BY Fusarium moniliforme AND F. graminearum USING ELISA AND IMMUNOGOLD LABELING. L. R. Todd, Department of Plant Pathology, University of Minnesota, St. Paul.

The production of wall-degrading enzymes of two corn-infecting Fusarium species were assayed. When two isolates each of F. moniliforme and F. graminearum were grown in cellulose nutrient broth (CNB) and in glucose ammonium nitrate asparagine medium (GANS) for 4 and 6 days, ELISA testing of culture fluids of CNB-grown isolates indicated that endo-1,4-β-glucanase and 1,4-β-D-glucan cellobiohydrolase were present in all isolates. Lignin peroxidase activity was not detected in culture fluids from isolates grown in GANS; however, endo-1,4-β-xylanase was present in all isolates. Immunogold labeling of hyphae indicated that in all experiments the enzymes were associated with the cell wall and with the plasmalemma. In young, actively growing hyphae, gold particles were also observed within the cytoplasm. These results confirm that wall-degrading enzymes are produced by Fusarium species and suggest that these enzymes may be involved in infection of corn.

A12
BACTERIAL PROMOTER-LIKE SEQUENCE IN CLONED CHROMOSOMAL DNA OF MYCOPLASMA-LIKE ORGANISM (MLO) ASSOCIATED WITH CLOVER PROLIFERATION. S. J. Dong and C. Hiroki, Dept. of Plant Science, Univ. of Alberta, Edmonton, Alta. Canada T6G 2P5.

Recombinant plasmids containing chromosomal DNA segments of MLO associated with clover proliferation (CP) were identified by dot- and Southern-blot hybridizations. The sequence of the cloned CP MLO DNA in pUC19 was determined by the dideoxynucleotide chain termination method using modified T7 DNA polymerase and the M13 DNA polymerase. A DNA segment of about 1.8 kb was sequenced. The sequence encoded a 36 amino acid polypeptide with 8.5% identity to the Pseudomonas syringae pv. tomato HrpL protein. A highly conserved sequence was identified that is similar to the consensus sequence for the hrcC gene of Agrobacterium tumefaciens. This sequence corresponds to the leader sequence of the CP MLO. The results suggest that the CP MLO promoter may be involved in gene regulation.

A13

HMG-CoA reductase (HMGR) catalyzes the first reaction committed to isoprenoid biosynthesis and is involved in the coordinated regulation of sesquiterpenoid phytoalexin accumulation in potato. HMGR cDNA derived from the total RNA of avocado and arachidonic acid elicited Kennebec tubers was amplified via the polymerase chain reaction and cloned into pSP72. Restriction digests and dyeoxy sequencing defined 3 classes of cloned inserts 443, 295 and 155 base pairs long, each with a deduced amino acid sequence that is highly similar to Arabidopsis HMGR. Probing genomic Southern blots with the labeled cDNA inserts indicated the presence of a small gene family in potato. RNA probe protection experiments showed maximum HMGR expression in tuber disks at 18 hours after elicitation. Work is in progress to characterize HMGR genomic clones.

A14
ISOLATION AND CHARACTERIZATION OF cDNA CLONES FOR PHENYLALANINE AMMONIA LYASE (PAL) FROM ALPalfa CELL SUSPENSION CULTURES. G. Goverri, K. Dalkin and R.A. Dixon, The Noble Foundation, Plant Biology Division, P.O. Box 2180, Ardmore, OK 73402.

L-phenylalanine ammonia-lyase (PAL) catalyzes the first step of the phenylpropanoid pathway in plants. PAL activity has been
shown to be induced dramatically in response to fungal elicitor in alfalfa. In order to elucidate the mechanism of this induction, we have recently isolated a near full-length cDNA clone (PAL-1) from an expression library using anti-
alalfa PAL antibody. Northern analysis using PAL-1 showed a maximum induction of the PAL mRNA in suspension cultures four hours after application of the fungal cell wall elicitor. The deduced amino acid sequence of this clone is highly similar to that of PAL clones from other species. Northern analysis of alfalfa genomic DNA with PAL-1 revealed the existence of a multigene family. Further, we have isolated several different PAL cDNA clones by rescreeing the library with PAL-1, and characterization of these clones is in progress.

A15

DERIVATIVES OF CERCOSPORIN SHOW ALTERED TOXICITY. G. B. Leisman and M. E. Daub, Dept. of Plant Pathology, North Carolina State Univ., Raleigh, NC 27695-7616

We have synthesized four derivatives of cercosporin (CR) in order to understand structural requirements for toxicity. Dithionite-reduced cercosporin and its acetylated derivative were both unstable and could not be tested for toxicity. Nonanhydro-
cercosporin (NRC) and its methylated reduced derivative (TMNC) were tested for toxicity in the light to Neurospora crassa. Growth inhibition of crassa by CR, NRC and TMNC was 72, 98 and 73% respectively. There was no growth inhibition in the dark. A lipid peroxidation assay with methyl linolate showed that NRC is twice as active as TMNC; CR was intermediate. Thus, NRC is more toxic than its reduced derivative and both require light for toxicity.

A16

CELL SURFACE REDOX POTENTIAL AS A POSSIBLE MECHANISM FOR CERCOSPORIN RESISTANCE. C. Cooperman, A. R. Jenns, and M. R. Daub, Dept. of Plant Pathology, North Carolina State University, Raleigh, NC 27665.

The phototoxin cercosporin is toxic to plant cells and to many fungi, yet the producing fungus Cercospora is resistant. We hypothesized that resistance to cercosporin may result from a reducing environment at the cell surface which could reduce the tautom molecule or prevent generation of singlet oxygen. Toxicity of cercosporin has been shown to be directly related to light absorption. Dithionite reduction decreased light absorption by cercosporin approximately two-fold. Further, the addition of the reducing agents cysteine and sodium of ascorbate to growth medium significantly decreased cercosporin toxicity to sensitive fungi. Ninetener, tetrazolium dyes were tested as possible indicators of cell surface redox potential of five fungi differing in resistance to cercosporin. Dye reduction was media-dependent, and could not always be correlated with cercosporin resistance in different fungi.

A17

CHARACTERIZATION OF Rhizoctonia solani BY GAS-LIQUID CHROMATOGRAPHY OF CELLULAR FATTY ACIDS. J. Stevens, J. Johnk, and R. K. Jones, Dept. of Plant Pathology, University of Minnesota, St. Paul 55108.

Methyl esters of cellular fatty acids were analyzed by gas liquid chromatography using a fused silica capillary column (HP-5, 0.2 mm, 25 m). Preparation of the fatty acids from mycelia was accomplished by base saponification and organic solvent extraction. The major fatty acid found in six intraspecific groups (ISGs) of AG-1 and AG-2 was 18:2 cis 9,12 which constituted 68-80% of the whole-cell fatty acid content. This fatty acid along with 16:0 and 18:1 cis9 account for greater than 93-97% of the C9-C20 fatty acids present. Smaller amounts of ten other fatty acids were consistently identified. Isolates within an ISG can be differentiated using principal component analysis of percent composition of the thirteen fatty acids identified.
and in its ability to penetrate intact cucumber tissue. A cosmids library was constructed using wild-type genomic DNA from C. lagenarium and the cosHgl vector. Transformation of the mutant with the cosmids library resulted in recovery of approximately 1000 hygromycin resistant colonies. The putative transformants were screened for the ability to utilize cellulose as the sole carbon source and for the ability to penetrate and cause disease in intact cucumber tissue. Several of the transformants were found to have regained the wild-type phenotype for these characteristics. All of the transformants contain vector DNA and appear to be identical as shown by Southern hybridization analysis. Attempts are being made to recover the transforming DNA by cosmid "rescue".

A27

Six X. campestris pv. malvacearum (Xcm)avr genes have been cloned and characterized by reverse transcription-PCR (RT-PCR) and DNA sequence analyses. All six genes were found on a single 50 kb plasmid, all are larger than 3 kb in size, and, similarly organized. XcmavrB3 and XcmavrB6 upregulate anavr resistant gene from pepper and to a pathogenicity gene from X. citri. Two of the genes appear to enhance symptom development on compatible hosts, and thus, like the X. citri gene, may play a role in virulence. The other four do not enhance symptom expression. Despite their similar structure, XcmavrB3 and XcmavrB6 are not cross-compatible.

A28
AN AVIRULENCE FUNCTION FROM PSEUDOMONAS SYRINGAE PV. TOMATO IS LOCATED WITHIN A HRP CLUSTER. J. M. Lorang, G. A. Boucher, S. Dahlbeck, B. Staskawicz, and N. T. Keen. Dep of Plant Pathology, University of California, Riverside 92521, and University of California, Berkeley 94720.

Cosmid clone pTL105, previously cloned from Pseudomonas syringae pv. tomato P127 (Kobayashi et al., PMAS 86:157, 1989), carried the avr5 gene. gyrB, gyrB6 gives a strong HR on cotton lines with the B4 resistance gene. Similarly, Xcm gene gyrB6 gives a strong HR on cotton lines with the B65 gene. However, both gyrB4 and gyrB6 alone elicited a weak HR on cotton lines with B1 or B2 (and without B4 or B6). Spontaneous mutations of the cloned genes have been obtained such that the null mutants are in Xcm, the strains elicit a strong HR on all cotton lines tested, including lines not known to carry resistance genes. The conservation of this class ofavr gene in Xanthomonas implies a highly conserved phenotypic function, but their "recognition" of resistance genes appears to be a gratuitous phenotype, and not as specific as presented in the gene-for-gene hypothesis.

A29
CHARACTERIZATION OF POLYCLONAL ANTIBODIES TO THE HOST-SPECIFIC TOXIN VICTORIN. K. Akimoto, L. F. Hart, and J. D. Walton. Dep of Botany & Plant Pathology and DOE-Plant Research Laboratory, Michigan State University, East Lansing, MI 48824.

Polycholal antibodies against victorin, the host-specific toxin of Cochliobolus victoriae, were produced in rabbits immunized with victorin-BSA conjugates. The anti-victorin antibodies were purified from the serum by protein A columns. Specificity and characterized by indirect ELISA with goat anti-rabbit IgG alkaline phosphatase conjugate as a second antibody. The concentration of victorin inhibiting anti-victorin antibody also elicited by 80% in the indirect ELISA was 10 ng/ml. The lowest concentration of victorin detectable in the indirect ELISA was 10 pg/ml. About 60 ng of tritium-labelled victorin (5.3 mCi/mg) was bound to 1 mg of anti-victorin in a binding assay. Using these antibodies, victorin binding to leaf tissue proteins of oats was detectable in western blots.

A30
DEVELOPMENT OF A HYPERSUSCEPTIBLE PHENOTYPE IN TRANSGENIC PLANTS EXPRESSING ELICITOR COAT PROTEINS OF TOBACCO MOSAIC VIRUS. J. N. Culver and W. O. Dawson, Department of Plant Pathology, University of California, Riverside, CA 92521.

Recently, the induction of the N. hypertensive resistance gene in Nicotiana sylvestris has been shown to be elicited by the coat protein of specific tobacco mosaic virus (TMV) mutants. In this study, both elicitor and non-elicitor coat protein open reading frames (ORF) were placed behind the CaMV 35S promoter and moved into the genome of N. sylvestris. Southern and Western blot techniques were used to show that transformed plants contained and expressed the coat protein ORFs. Plants
expressing a non-elicitor coat protein were slightly stunted but otherwise unchanged from the healthy, non-transformed phenotype of \textit{N. sylvestris}. Plants expressing elicitor coat proteins showed mild to severe stunting and developed necrotic spots that eventually coalesced, collapsing entire leaves. This demonstrates that TMV elicitor coat proteins are singly responsible for the induction of the hypersensitive reaction in \textit{N. sylvestris}.

A31
MONOCYTOLEANTIBIOTICS SPECIFIC TO PHYTOXIN ULTIMUM AND PHYTOXIN SPP.
G. Y. Yuen and M. L. Craig. Department of Plant Pathology, University of Nebraska, Lincoln, NE 68503

Monocyclic antibiotics were produced for the identification and quantification of Phytoxyn ulimum (PU). A crude preparation of PU cell wall material from Csmekal Dox broth culture was used as the antigen. Initial selection of hybridomas was based on positive reactivity in indirect-ELISA with a culture of PU and negative reactions with a strain of \textit{P. irregulare} and of Phytophthora cinnamomi. Four antibodies were selected for further screening against 23 strains of PU, 35 strains of 16 other species of Phytophthora and 27 strains of other genera of soil fungi. One antibody (ES) was found to be specific to PU and to have the highest binding affinity. The remaining three exhibited some cross-reactivity to various species of Phytophthora. An antigen competition assay using ES was developed that can detect PU specifically in soybean roots to 2.5 \textmu g of PU protein per mg of root protein. Comparison of this protocol with root culture methods to measure root infections by PU will be discussed.

A32
SEUDODIAGNOSIS OF FUNGUS OF THE DIAPORTHE/PHOMOPSIS COMPLEX OF SOYBEANS, L. M. Brill and J. B. Sinclair, Dept. of Plant Pathology, University of Illinois at Urbana-Champaign, Urbana 61801-4709.

ELISAs were developed for detection and quantification of fungi of the \textit{Diaporthe}/\textit{Phomopsis} complex of soybeans. A culture filtrate of \textit{Phomopsis longicolla} was used an immunogen to produce rabbit polyclonal antibodies. With cultured fungi, antibodies reacted strongly to isolates of the complex with limited cross-reactivity to other soybean fungi. Cross reactivity was lower and sensitivity 1000x higher in antigen capture ELISA than in antigen coat ELISA. The diagnostic capability of antigen capture ELISA was tested with soybean seeds. Extracts from pathogen-free seed were negative controls; extracts from seeds inoculated with members of the complex were positive controls. Detection of fungal antigens in seed tissues was successful, based on comparisons to agar plate bioassays. Monocyclic antibiotics are being developed using BALB/c mice. The mice responded poorly to culture filtrate immunogens, but strongly to mycelial extracts of \textit{P. longicolla}.

A33
PREDICTION YIELD LOSS FROM PHYTOPHTHORA MEGASPERMA F. SP. GLYCINEA IN SOYBEAN WITH A QUANTITATIVE IMMUNOASSAY OF PHYTOPHthora IN SOYBEAN. F. Scheithauer, Dept. of Plant Pathology, The Ohio State Univ., Wooster, OH 44691.

Soil from 20 fields was assayed for Phytophthora antigen in April and August 1989 using an ELISA immunosassay developed by Agri-Diagnostics Assoc., Cinnaminson, NJ. Eight soil subsamples were pooled from 4 quadrats in each field and compared to a single bulked sample composed of 25% of each pooled sample. In addition, Phytophthora inoculum potential was bioassayed in each soil by evaluating seedling disease of soybean cv. Sloan. Yield of four alternate strips of metalaxyl treated and untreated soybeans was compared in each field. To assess yield loss from Phytophthora, ELISA and bioassay Phytophthora values were higher in August soil than in April soil. Mean ELISA and bioassay values from the four quadrats collected in the April significantly predicted yield loss, while data from the August samplings did not, based on regression analysis. ELISA and bioassay values from the single bulked samples were poor predictors of yield loss.

A34

Sensitive laboratory and on-site enzyme-linked immunosorbent assays (ELISAs), based on a mixture of two monoclonal antibodies, have been developed to detect \textit{Phytophthora} spp. in plant tissues. The two monoclonal antibodies provide a broad spectrum of reactivity within the genus. The laboratory assay, utilizing a 96-well microtiter plate format, has a lower limit of sensitivity of 25 ng protein/ml \textit{P. megatrichia} f. sp. \textit{glycinea} mycelial extract. The laboratory assay can be completed in less than one hour. A rapid, on-site kit was developed utilizing a flow-through immunooassay format. Sensitivity of the on-site assay is similar to that of the laboratory assay. Testing requires no special equipment, and can be completed in 10 minutes. In a experimental system of azaleas, rhododendrons and junipers inoculated with \textit{P. cinnamomi}, \textit{P. cactorum} and/or \textit{P. citrophthora}, both assays detected the pathogen in roots well before symptoms were visible above ground.

A35

Phytophthora specific ELISA immunosassay kits (Kit E, Agri-Diagnostics, Dept. of Plant Pathology, Oregon State University) were used in the diagnosis of plant specimens expressing root or crown rots, sent to OSU’s Plant Disease Clinic. The following data were collected: field history, symptoms, fungi isolated on selective media, and color reaction of ELISA kits. Clinics samples with typical symptoms of Phytophthora root rot produced a positive reaction with the immunosassay and did pure cultures of \textit{Phytophthora} sp. one of some PU sp. isolated from these samples. An alfalfa leaf sample with downy mildew (\textit{Peronospora parasitica}) also produced a positive reaction. Other samples without typical \textit{Phytophthora} symptoms and associated with a variety of other pathogens did not produce a positive reaction with the immunosassay. Cross reactivity with some PU sp. makes interpretation difficult, but when kit results are combined with field histories and symptomology, the immunosassays have proven to be a useful tool in the Plant Disease Clinic.

A36

Healthy azalea rooted cuttings were inoculated with a zoospor suspension (400 zoospores/plant) of \textit{Phytophthora parasitica}. ELISA (Agri-Diagnostics Associates, Puyallup, Washington) was used to screen inoculated and non-inoculated control plants for the presence of \textit{P. parasitica} in field soil. All inoculated cuttings were positive, while none of the control plants were positive. All 3 techniques detected \textit{P. parasitica} soon after inoculation. ELISA consistently gave positive results throughout the study, but culture and bait techniques gave variable results after fungicide treatment. With all 3 techniques, non-inoculated control plants tested negative for \textit{P. parasitica}, and inoculated, non-fungicide treated plants gave consistently positive results except for the first few days after inoculation.

A37
AN IMPROVED MEDIUM FOR THE ASSAY OF SEPTORIA NODORUM FROM WHEAT SEED. J.N. B. Kanangah and Barry M. Cunfer. Department of Plant Pathology, University of Georgia, Georgia Station, Griffin, GA 30223.

An agar medium was developed to improve the recovery of \textit{Septoria nodorum} from wheat seed compared to the current best medium, oxgall agar. \textit{S. nodorum} fluoresces under near UV light on oxgall agar, but it does not sporulate, and growth is strongly partially suppressed. The new medium contains 10 g potato dextrose agar, 15 g agar, 1 g peptone, 1.5 g oxgall, 5 mg chloroben, 5 mg dicloran, and 5 mg CuCl2 per liter. Antibiotic (2 mg penicillin, 1 mg streptomycin, and 12.5 mg tetracycline HCl per L) to control bacteria are added after autoclaving. This medium retains the \textit{S. nodorum} and permits moderate sporulation within 7 days. Recovery of \textit{S. nodorum} from seed is improved 15-35% and growth of other fungi is reduced >30% compared to oxgall agar.

A38
A DIAGNOSTIC IMMUNOASSAY TO DETECT CEREAL EYESPOOT (FOOT ROT) IN WHEAT. D. M. Saunders, D. M. Feinlnd, L. P. Alston, C. M. Vanstone, Du Pont, P. O. Box 6101, Newark, DE 19714-6101.

A diagnostic immunosassay (ELISA) has been developed which detects presymptomatic infection of wheat by the fungal pathogen \textit{Pseudocercosporella herpotrichoides} (Ph), the
causative agent of cereal eyespot disease. Rabbits were immunized with extracts of Ph. purpureo-antiph. The Ph was rendered monospecific by absorption with cross-reacting fungi. The monospecific antibodies formed the basis of an immunodiagnostic assay. Strains of Ph. tested in this assay react positively. No cross-reactions are observed with other common fungi, soil, or plant components. The ELISA detects 20 pg of a characterized Ph antigen preparation. An extract of symptomless wheat stems diluted 10,000-fold reacts positively. In field trials, the ELISA detected the presence of Ph in inoculated fields prior to the appearance of symptoms. This detection occurred earlier and was more consistent than microbiological culture isolation methods.

A43
ULTRASTRUCTURAL KARYOTYPE FOR Fusarium graminis f. sp. tritici, E.W.A. Boehm, W.B. Bushnell, D.J. McLaughlin, A.P. Roelfs, and L.J. Szabo. 1) USDA/ARS Cereal Rust Laboratory, Dept. Plant Pathology and 2) Dept. of Plant Biology, University of Minnesota, St. Paul, MN 55105.

Accurate cytological determination of the karyotype (chromosome number and morphology) for many fungi is hindered by the cell wall, the small size of the nucleus and the presence, generally, of a large number of chromosomes. Past estimates of chromosome number in F. graminis f. sp. tritici have yielded n = 6. We report here on the karyotype of F. graminis resulting from fusion nuclei in meiotic pachytene, using three-dimensional reconstructions from TEM serial sections of synaptonemal complexes. Pachytenes occurred shortly after karyogamy in young, thin-walled, slightly melanized teloplasms. Cells in diplotene were selected prior to mass screening by epifluorescence microscopy of fixed, DAPI-stained teloplasma protoplasts from which walls were mechanically removed. Three-dimensional reconstructions from TEM serial sections of synapsed homologues in teloplasms from a single isolate (CSL7-45-1801-3) indicated a chromosome number of eighteen.

A44
RELATEDNESS OF THREE DISTINCT POPULATIONS OF Fusarium graminearum studied by ALLOZYME AND RIBOSOMAL DNA POLYMORPHISM. S. Brooks, T. D'Souza and G.C. Adams. Dept. of Botany and Plant Pathology, Michigan State University, E. Lansing, MI 48824.

Allozyme polymorphisms and RFLPs of the nuclear ribosomal DNA were compared to investigate molecular evolution among three conspecific populations of F. graminearum. These populations are known to be genetically differentiated, each with a unique profile of perithecia and mycotoxins, colony growth and morphology. Two populations, Groups 1 and 2, coexist in Australia and California and two, Types A (Group 2) and B, coexist in the north central United States. Eleven enzymes showed strain specific allozyme polymorphisms among 13 isolates but no correspondence to Group or Type was evident. Similarly, differences among Group and Type were not evident in an analysis of cDNA cut with nine restriction enzymes. However, allozyme and mitochondrial DNA polymorphisms were useful in distinguishing parental factors inherited by hybrids formed by protoplast fusions between type A and type B strains.

A45
FERTILITY OF ISOLATES BELONGING TO Fusarium section Leisea FROM CORN AND SORGHUM. J. F. Leslie, C. J. R. Kiltich, and C. Chalsisook. Dept. of Plant Pathology, Throckmorton Hall, Kansas State University, Manhattan, Kansas 66506-5502.

Within Fusarium section Leisea four mating populations were previously recognized (A-D). We have crossed more than 440 wild-collected isolates from the central and eastern United States with mating type testers representing these four populations. Isolates belonging to all four populations have been identified and accounted for approximately 50% of the isolates examined. Members of the A and D populations are recovered most commonly. Additionally, we have detected two more mating populations, E and F, that account for a further 20% of the population. Within the remaining 30% of the population there is preliminary evidence for three additional mating populations for which reliable testers are not yet available. Some isolates appear to be sterile regardless of conditions used for crosses. All fertile strains are competent as males, but many male fertile strains are female sterile. Isolates from the A and F populations are all morphologically F. moniliforme; isolates from the B and E populations are all morphologically F. subglutinans; and most isolates from the D population are morphologically F. proliferatum.

A46
Immunoelectrophoretic techniques, including crossed immunoelectrophoresis (CIE), CIE with an intermediate gel, and CIE following antibody absorption in situ, were used to differentiate among 21 isolates of *Fusarium oxysporum* f. sp. *lycopersici* representing three vegetative compatibility groups (VCGs) and several single member VCGs (SMVCs). Each of the isolates in multiple member VCGs had specific antigens that were detected within but not among VCGs, and three antigens were associated with pathogenicity and vegetative compatibility and were found in all multiple member VCGs. There were six, five, and five antigens that were specific for vegetative compatibility and unique to VCGs 0030, 0031, and 0032, respectively. No VCG-specific antigens were found for the SMVCs. These results suggest that antigens responsible for heterokaryosis were lost in SMVCs.

### A47

**CHILL-INDUCED FORMATION OF SCLEROTIA IN *SCLEROTIORUM ROLFSSII***

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On glucose-yeast extract agar (GYE) a *Sclerotium rolfsii* isolate grows from center to edge (40 mm) in 3 days and then forms sclerotia (i) in a central cluster opposed to the agar and (ii) scattered in the aerial mycelium. In colonies chilled briefly (5, 4 hr) at 2 days (23 mm radius) and then returned to 25 C, the pattern is different. Fewer, or none, form in the center; a ring of sclerotia forms later just inside where the colony edge was at the time of the chill. A colony chilled twice before it reaches the dish wall forms the sclerotic ring just inside where the colony edge was at the time of the second chill, and few form in response to the first chill. Colonies chill when the dish wall reaches the dish temperature but not when mycelia sclerotia at the wall, where many typically do not form. On a glucose-NaNO₃, agar (GN) most sclerotia form at the dish edge, unlike the pattern on GYE. Yet chilled colonies form sclerotic rings as on GYE, except that twice-chilled colonies on GN form the sclerotic ring before lead hyphae reach the wall. Chilling temporarily arrests growth and redirects sclerotial initiation.

### A48


Cluster analysis of isozyme data showed that some *P. sytium* spp., such as *P. carlostrum*, and *P. grandincola* or *P. irrorulare* and *P. graminis*, are closely related. Several isolates of the species were selected for studies of ribosomal DNA. Polymerase chain reaction was employed to amplify regions of nuclear DNA that code for the 18s unit and internal transcribed spacers (ITS). Variations were observed in ITS size. ITS for *P. carlostrum* and *P. grandincola*, *P. irrorulare* and *P. graminis*, and *P. ultimum* were about 850, 1000, and 920 bp, respectively. Each of the three ITS sizes showed a distinct banding pattern after restriction analysis. Present data show identical DNA sequences for a limited region of the 18s unit, but restriction analysis revealed 18s differences between the first and last two ITS size groups. ITS size and ITS DNA sequences are being determined to compare these morphologically and isozymatically similar species.

### A49

**EFFECT OF POLARITY ON IN VITRO TUMOR FORMATION BY *AGROBACTERIUM TUMEFACIENS* AND NECROTIC RESPONSE OF GRAPE CULTIVARS.** M. D. Green and R. W. Goodman, Department of Plant Pathology, University of Missouri, Columbia, MO 65211

Tumor formation by *Agrobacterium tumefaciens* (AT) was significantly increased when the grape cultivars were grown in isolated and inoculated basal end upwards in agar medium as compared with apex upwards. Catacomb and Chancellors stem pieces inoculated basal end upwards exhibited a 40% increase in tumor occurrence and a 1.5 times increase in tumor size. The enhanced tumor production reflected basal petal auxin accumulation in the grape stem explants. This in vitro inoculation procedure provided an efficient method to study pathogenesis of AT strains on a large scale in grape cultivars.

Some necrosis accompanied tumor formation following in vitro inoculation of grape stem explants. The necrotic response was more profound when the shoots were inoculated in upward direction. In some cases, tumors produced in early stages but became necrotic later. It might be possible that higher auxin production in transformed cells leads to higher auxin oxidase activity and that might be responsible for this necrosis.

### A50

**CLASSIFICATION OF *PHIZOMONAS SUBRFICIENSI*, THE CAUSAL AGENT OF CORK ROOT OF LETTUCE, IN *SUPERFAMILY IV*.** Kenneth R. Nuchiaen and Arleena H.C. van Bruggen, Department of Plant Pathology, University of California, Davis, CA 95616.

The causal agent of cork root of lettuce was recently identified as *Phizomonas subrficien*is, a new gram-negative genus and species, with a characteristic fatty acid profile (including 2-EN-14:0 fatty acid), ubiquinone Q10, and a G+C content ranging from 58.2 to 59.5 mol%. The presence of ubiquinone Q10 indicated potential placement of the genus *Phizomonas* in superfamliy IV which contains the family *Rhizobiaceae*. This hypothesis was tested by determining the thermal melting profiles of hybrids from 35-labeled ribosomal RNA of *R. subrficien*is strain C1A and chromosomal DNA of 15 species of gram-negative bacteria. The melting temperature for members of superfamliy IV ranged from 55 to 80°C whereas that for *Phizomonas fluorescens* was about 60°C. The data indicate that *R. subrficien*is belongs to superfamliy IV.

### A52

**DETECTION OF *XANTHOMONAS CAMPESTRIS* PV. PRUNI BY OUTER MEMBRANE PROTEIN PROFILES AND MONOCLONAL ANTIBODIES.** C.A. Goodman and M.J. Hattingh, Department of Plant Pathology, University of Stellenbosch, Stellenbosch 7600, South Africa

Bacterial spot of stone fruit caused by *Xanthomonas campestris* pv. pruni (Xcp) sporadically causes heavy crop losses in certain fruit-growing areas of the south-western Cape Province of South Africa. Outer membrane proteins of five strains of Xcp. 15 reference strains of different pathovars of *X. campestris*, and common bacterial ephiobiles isolated from stone fruit trees were separated by SDS-polyacrylamide gel electrophoresis. Two proteins from a strain of Xcp were injected singly into BALB/cice. Monoclonal antibodies developed against Xcp were used to probe gels for homologous proteins.

### A53

**THE INFLUENCE OF TI PLASMID UPON ATTACHMENT OF AGROBACTERIA TO GROUND-ALLUS CELLS.** R. P. and R. N. Gromberg, Department of Plant Pathology, University of Missouri, Columbia, Missouri 65211.

Low-speed centrifugation, 500 rpm for 2 minutes, provided better recovery of bacteria than filtration through Miracloth and Whatman #1 filter paper. It allowed the separation of bacterial cells attached to grape callus cells from unattached ones. This technique, various strains of *Agrobacterium tumefaciens* and *R. radiobacter* were tested for their attachment efficiency to grape tissue culture cells. Our data indicated that the presence of Ti plasmid in *agrobacter*ia is critical for early attachment to grape cells. In addition, the strains carrying octopine type Ti plasmids are more aggressive than nopaline types, attaching to grape cells more rapidly and in greater numbers. On the other hand, bacteriocin-producing strains, *R. radiobacter* R84, which fails to suppress grape isolates, did not show any adhesion to grape callus cells during 5 hours incubation with grape cells. However, *R. radiobacter* MLB-2, which is capable of inhibiting grape isolates in vitro and in plants, exhibited a certain amount of adherence to grape cells. Its attachment performance trends to explain the biological control activity of *R. radiobacter* MLB-2 against grape isolates.

### A54

**SEROLOGICAL RELATIONSHIPS AMONG *XANTHOMONAS CAMPESTRIS* STRAINS ASSOCIATED WITH CITRUS BACTERIAL SPOT.** A. B. Alvarez, A. A. Benedict, and T. R. Gottwald, University of Hawaii, Honolulu, HI 96822; USDA, Orlando, FL 32803.

In contrast to *A. citrulli* and *A. eucalypti* strains that share a common epitope as revealed with a monoclonal antibody (mAb) A1, *xanthomonads* associated with citrus bacterial spot (CBS) in Florida were antigenically heterogeneous and shared epitopes with other *X. campestris* pathovars. Aggressive CBS strains reacted with a mAb designated CBS1, whereas moderately aggressive and nonaggressive strains reacted with mAb A1 and other mAbs generated to a moderately aggressive CBS strain, an A. A.
A55

GENETIC CHARACTERIZATION OF PATHOVARs OF XANTHOMONAS CAMPESTRIS CAUSING DISEASES ON CITRUS.

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Citrus bacterial spot, caused by Xanthomonas campestris citrulina, is a new disease of citrus in Florida. The relationship between X. c. citrulina and X. c. citri, causal agent of citrus canker, is uncertain. The SI nuclelease technique was used for DNA-DNA hybridizations between strains of the above pathovars and the type strain of X. c. campestris. Strain F1, an aggressive strain of X. c. citrulina, was 56% similar to X. c. citri strain 9771, and 34% similar to X. c. campestris. Strain 9771 was 27% similar to X. c. campestris. In both DNA-DNA hybridization and pulsed field gel electrophoresis with rare-cutting restriction enzymes, strains of X. c. citrulina were diverse. Although X. c. citri and X. c. citrulina cause similar diseases on citrus, they are not closely related based on genetic analyses.

A56

OPTIMIZATION OF ELECTRICAL PARAMETERS FOR EFFICIENT ELECTROPHORESIS OF PLASMID DNA INTO XANTHOMONAS CAMPESTRIS PV. ORYZAE.

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Electrophoresis provides an effective transformation system for US strains of Xanthomonas campestris pv. oryzae (Xco). Initial attempts to introduce a plasmid pUF19 into Xco strain X37-2 with electroporation yielded moderate transformation efficiencies (10^4 transformations/µg DNA). Optimization of the two most critical parameters, electric field strength and pulse duration, indicated a large window of transformability for X37-2. A range of electric field strengths was investigated with pulse durations of 1, 5, and 10 msec. Using a 5 msec pulse, electric fields from 8.5 - 17.1 kV/cm resulted in efficiencies of 10^6 to 10^7 transformations/µg DNA, with percent survival ranging from 6 to 92%. Frequency of transformation for the applied electric fields averaged 2 X 10^8 transformations/µg DNA. When optimized conditions for X37-2 were tested on four different strains of Xco, lower efficiencies were observed and survivability was strain dependent. Results indicate optimization of electric field strength and pulse duration may be strain dependent for Xco. Effects of cell density and DNA concentration on transformation efficiencies for Xco were also investigated.

A57

EFFECT OF NUTRIENTS ON SURVIVAL OF ANTAGONISTIC PSEUDOMONAS SP. ON ALFALFA LEAVES.

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Supplemental nutrients (peptone, yeast extract, dextrose, glycerol, King's B broth) were used in bacterial suspensions that were applied by an immersion technique onto alfalfa plants with different densities of leaf hairs. Plants were kept in a dew chamber for 48 hours and then transferred to the greenhouse. Samples were taken at 0, 1, 2, 3, and 7 day intervals. Leaflets were washed with agitation 1/2 h in buffer and then plated on a selective medium in order to recover the antagonist. Bacterial survival was enhanced by King's B broth + dextrose, but no significant increase was observed with the other treatments. More bacteria were recovered from varieties with dense leaf hairs. Scanning Electron-microscopy studies showed that the bacteria were more numerous on the abaxial surface, in the crevices between cells, at the base of and directly on trichomes, and on main veins and stomatal surfaces.

A58

ARYL & GLUCOSIDASE ACTIVITY OF ENTEROBACTER CLOACAE ECCT-501.

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The rhizosphere bacterium, Enterobacter cloacae strain ECCT-501, exhibits at least two distinct aryl glucosidase activities in culture. The β-glucosidase activities of ECCT-501 grown in MS basal salts plus 0.5% glycerol were separated spatially into extracellular and cell-bound fractions and biochemically by carbohydrate inhibition assays. The β-glucosidase activity associated with the cell-bound fraction was inhibited 51% by 2, 6-

A59

GENETICS OF RESISTANCE TO PHYMATOTRICHUM OMNIVORUM IN UPLAND COTTON.

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Seven cotton genotypes were crossed in a diallel mating scheme with no reciprocals. Parents, F1, and F2 progenies were field evaluated for resistance to Phymatotrichum omnivorum at Temple, TX for two yrs. Data were analyzed using Hayman's diallel and Griffing's combining ability procedures. Significant differences in number of plants killed were obtained among the parents, F1's and F2's four and five wks after appearance of first symptoms. Additive and non-additive (dominance and epistatic) effects were significant. Dominance was more important than additive effects in the variation for resistance to P. omnivorum. Only general and not specific combining ability was significant. Transgressive segregation was observed in the F2 population for resistance to P. omnivorum. Broad sense heritability ranged from 0.31 to 0.46. Environment (year), growth stage, and maturity of the host had a significant effect on the contribution of the genetic components.

A60

DNA FINGERPRINTING OF SEPTORIA TRITICI ISOLATES REVEALS A HIGH LEVEL OF CLONAL DIVERSITY DISTRIBUTED ON A FINE SCALE IN A CALIFORNIA POPULATION.

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Genetic diversity in a California population of the wheat pathogen Septoria tritici was measured using variation at RFLP loci. A sample of 93 isolates collected from a single wheat field was assayed for restriction fragment length variation in nuclear DNA using 10 plasmid probes containing anonymous S. tritici DNA sequences. Nine of the probes hybridized to sequences present in only one or two copies in the genome and one probe (pstTL40) hybridized to highly variable sequences present in 4-13 copies in the genome. Isolates with identical multilocus haplotypes based on the nine single-copy probes usually had identical pstTL40 RFLP banding patterns, indicating that these isolates were clones and that pstTL40 is useful for DNA fingerprinting. pstTL40 identified 25 different clones among the 93 isolates, with identical clones clustered in the same location in the field. Possible mechanisms contributing to the high levels of RFLP variation associated with pstTL40 will be discussed.

A61

CHANGES IN THE GENETIC DIVERSITY OF PHYTOPHTHORA INFESTANS DURING AN EPIDEMIC IN CENTRAL MEXICO AS DETERMINED BY DNA FINGERPRINTS.


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Central Mexico is the center of diversity of Phytophthora infestans and is an area where it is known to reproduce sexually. We determined the identity of over 300 isolates of Phytophthora infestans, collected at regular intervals during an epidemic, from three sites near Tulua, Mexico. Two moderately repetitive DNA clones were used as fingerprints to determine the genetic diversity of the populations within fields, and how that diversity changed during an epidemic. At two of the sites isolates were collected from blocks of uniform cultivars the third site consisted of a mixture of cultivars and isolates were collected from two cultivars. A large number of clones were identified at each site and sampling date.

A62

REASSESSMENT OF VEGETATIVE COMPATIBILITY GROUPS OF VERTICILLIUM DAULAE AND THE COMPATIBILITY OF ISOLATES FROM CALIFORNIA POTATOES.

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Strains of Verticillium dahliae assigned to 16 vegetative compatibility groups (VCGs) by Puhalla and Hame using micro-

Vol. 80, No. 10, 1990 965
sclerotal color mutants (Phytopathology 73:1305-1308) were reassessed using nitrate-nonutilizing mutants. The strains they assigned to VCG 1 were vegetatively compatible only with themselves. A second compatibility group was formed from strains they included in VCGs 2, 3, 5, 7, 8, F, 13, and 14. The VCGs assigned to groups 4, 6, 12, 15, and 16 constituted a third group. The one strain assigned to VCG 10 was only self-compatible. The mutant obtained from VCG 11 was not compatible with strains from the other VCGs. Three isolates from potato-growing in the Bakersfield region of California were compatible with strains from VCG 1. Twenty-seven isolates from Potatoes growing in the Tulalake region, however, were vegetatively compatible only with strains from the group containing VCG 4.

A63
INHERITANCE AND A POSSIBLE MECHANISM OF RESISTANCE IN LETTUCE TO PLASMOPOREA LACTUCAE-RADICIS. M. E. Mangelli, R. W. Michelmore, S. L. Rasmussen, and G. J. Vandemark. First, second, and fourth authors, Dept. of Plant Pathology, University of Arizona, Tucson, Ariz. Final author, Dept. of Vegetable Crops, University of California, Davis, Calif. 95616.

The inheritance of resistance in lettuce to Plasmodina lactucae-radicis, a recently described root pathogen, was studied by segregation analysis. In the F2 progeny derived from crossing a resistant (Cobham Green) and a susceptible (Calmar) cultivar segregate at approximately 3 resistant : 1 resistant ratio. These ratios suggest that the resistant phenotype is due to a homoeozygous recessive genotype at a single locus. Fusarium colonisation but no sporulation occurred on the roots of resistant plants. Microscopic observation of roots revealed a differential callus deposition between resistant and susceptible plants. Callose is deposited more abundantly around haustoria in resistant plants. Callose deposition may be a mechanism of resistance in lettuce to P. lactucae-radicis.

A64

Restriction fragment length polymorphisms (RFLPs) among different geographic isolates of Ustilago violacea were used as markers for studying mitochondrial inheritance. Haploid cultures of opposite mating type from isolates that exhibit RFLPs were mated on artificial medium and induced to form dikaryotic hyphae by treatment with vitamin E. The hyphae grow until the vitamin E is exhausted and then revert to haploid unicellular budding. The haploid progeny of these crosses were analyzed for nuclear and mitochondrial genotypes. All possible nuclear and mitochondrial combinations were recovered from most crosses. Most (70%) of the haploid progeny, however, had the mitochondrial genotype donated by the a2 parent. Possible explanations for this bias will be discussed.

A65
GENETIC CHARACTERIZATION OF RESISTANCE TO TOMATO MOSAIC VIRUS (ToMV) in TOMATO SOMACONES. S. S. Smith, Dept. of Biochemistry, University of Maine, Orono, ME 04469 and R. H. Nakash, Dept. of Botany and Plant Pathology, Michigan State University, East Lansing, MI 48824-1312.

Six tomato somacones, regenerated from a fully ToMV-susceptible line (GCR1-26), were selected for resistance to ToMV. The inheritance of this resistance appears to involve an incomplete-dominant nuclear gene with a maternal effect, for each of the somaconal lines. In crosses with isogenic tomato lines expressing known ToMV resistance genes, it was determined that the somaconal resistance was additive with ToM-1 but not with ToM-2. The type of resistance seen in the somaconal lines was similar to that of the gene ToM-1 in that it suppressed symptom formation, limited virus multiplication, was not temperature sensitive and had similar virus strain responses. That the viral resistances generated through somaconal variation resemble that of a viral resistance gene found in a wild tomato species (L. hirsutum) is notable.

A67
GENETIC DIVERSITY IN Fusarium oxysporum f.sp. Lycopersici USING RESTRICTION FRAGMENT LENGTH POLYMORPHISM. D. D. Pope, Department of Plant Pathology, University of Georgia, Athens, GA 30602.

Random fragments of a PstI library made from Fusarium oxysporum f.sp. lycopersici (FOL) were used to probe Southern blots of genomic DNA extracted from 15 FOL isolates that represented three known races, three vegetative compatibility groups, and nonpathogens. Restriction fragment length polymorphisms were detected using unique and repeat sequence probes. A binary system was adopted to code isolates with respect to the presence (1) or absence (0) of specific fragments. Genetic diversity values calculated from these binary codes indicated the degree of genetic similarity among isolates. Genetic diversity values were analyzed with UPGMA. Results indicated that the three races probably underwent adaptive adaptations to newly introduced resistance genes such that race 2 arose from race 1 and race 3 from race 2.

A68

Fifty-two isolates of Diaporthe phaseoli var. caulivora (DPC) originating from Louisiana (11), Mississippi (11), Florida (11), Georgia (11), Arkansas (2), Tennessee (1), Iowa (2), and Ohio (3) were collected and grown on potato dextrose broth for 2-3 weeks. Double-stranded RNA (dsRNA) was extracted by two cycles of cell wall (CF-11) chromatography and electrophoresed on 6% polyacrylamide gel. Among all isolates, 29 (56%) contained at least one molecule of dsRNA. Molecular weights of dsRNA from DPC ranged from 0.23 to 3.8 x 106 daltons, and eight different band patterns were observed. Southern isolates exhibited all eight, whereas only one band pattern was observed in northern isolates. Cytoplasmic fractions from two isolates showed the same dsRNA band patterns as those from the total cell extracts. This suggested that dsRNA of DPC might be located in the cytoplasm of the cell. The dsRNA of DPC was not associated with virulence, toxin production, growth rate, or the activity of phenol oxidase. Isometric virus-like particles of about 30 nm in diameter were detected from one of the virulent isolates.

A69
EPIDEMIOLOGY OF SEEDBORNE XANTHONONAS CAMPESTRIS PV. TRANSLUCENS in Winter Wheat. R. A. Milus and T. L. Kirkpatrick, Dept. of Plant Pathology, University of Arkansas, Fayetteville, AR 72701 and SWRC, Rm. 3, Box 258, Hope, AR 71810.

Bacterial stripe (black chaff) of wheat, caused by Xanthomonas campestris pv translucens (Xct), is an important disease in Arkansas. Research was conducted to evaluate seed treatments for reducing seedborne inoculum and to determine the relationship between seedborne inoculum and epiphytic populations of Xct, disease severity, and yield loss. A rifampicin-resistant isolate of Xct was applied to seed of cultivar Florida 302. Infested seed was not treated or was treated with 1% acidified cupric acetate (ACA), Kocide SD (2.5 g/kg), or heat (70 °C for 10 days), resulting in different levels of seedborne inoculum. The treated seed lots and a noninfested, nontreated check were planted at Foreman, AR, in October 1989. After 3 mo, plants from infested seed averaged 6 x 102 cfu/g of leaf tissue. Epiphytic populations of Xct averaged 10-, 100-, or 1000-fold lower on plants from infested seed treated with Kocide, ACA, or heat, respectively.

A70
RECEPTIVITY OF WINTER WHEAT LEAVES TO COLONIZATION BY A STRAIN OF PSEUDOMONAS FLORESCENS. F. J. Gough and H. M. El-Nashar, USDA-ARS, Plant Science and Conservation Laboratory, 1301 N. Western, Stillwater, OK 74075.

Fourteen winter wheat (Triticum aestivum) cultivars were compared for receptivity to leaf colonization by an antibiotic resistant strain (PMM2) of Pseudomonas fluorescens antagonistic to Septoria tritici. The cultivars, in replicated row plots, were inoculated with PMM2, suspended in water, in 1988 and 1989.
Areas under survival curves, calculated from estimates of PFM2 population densities on randomly selected flag and flag-1 leaves were used as indicators of cultivar susceptibility. Popu-
lation densities were determined immediately after inoculation in both years, and thereafter at weekly intervals for a total of five times in 1986 and three times in 1989. Receptivity of cv.
Vona to colonization by PFM2 was significantly (P<0.05) greater than that of any other cultivar in both years. Accord-
ing to Dunn's multiple range test, receptivity among the remain-
ing 13 cultivars did not differ in 1986, but separated into three groups in 1989.

A71

EFFICACY STUDIES OF FIVE FUNGICIDES FOR CONTROLLING RHIZOCTONIA ROOT ROT OF WINTER WHEAT IN TEXAS. J. T. Mathiesen, C. M. Rush, Texas Agricultural Experiment Station, P.O. Drawer 10, Bushland, TX 79012.

Rhizoctonia root rot has been found to be a problem associated with early planted wheat in the Texas Panhandle. Laboratory studies were conducted to determine the minimum fungicide dosages for disease development and to test selected fungicides, Triadimenol, Imazalil, GA196374, UBI1886, and Nusume and Nuzone, for efficacy. A known pathogenic isolate of Rhizoctonia solani, Ag-4, was tested at temperatures from 15-35 C to determine the optimum for disease. Plant emergence in infected soil decreased as the temperature increased, R=0.55. Tests were done both in vitro on amended PDA and in infected soil at 15, 25, and 35 C. Seed treated with Triadimenol had significantly greater emergence and dry matter production at each temperature compared to other treatments. Seed treated with Triadimenol had significantly less infection, both on the seed and coleoptile, for 25 days when compared to non-treated seed.

A72

TRIADIMENOL COMPARSED TO CARBONIC FOR CONTROL OF COMMON BUNT OF WHEAT. E. Williams Jr., Plant Pathology Department, Oklahoma State University, Stillwater, OK 74078-9947.

Comparative efficacy of triadimenol and carbinox against common bunt was evaluated at Perkins (north central) and Duke (southwest) OK using seed and soil inoculated with Tilletia tritici. Fungi were applied on seed at 0.75, 1.5, and 2 X labeled rates for the aboveground mycelium and applied to smutted heads. Smutted heads were inoculated and examined at 21 days with an inoculated Bunt (E. graminis) strain. A significant reduction was observed in smutted heads. Smutted heads were killed on untreated heads at Perkins and Duke, respectively. Triadimenol at 0.15 g a.i./kg reduced bunt to 0.25% at Perkins, and provided total control at Duke. Carbinox at 1.3 g a.i./kg was the lowest rate required for control at Duke, and at Perkins, 3.25% was the lowest level of bunt reduction. Both fungi were highly effective in controlling bunt reduction, but triadimenol provided nearly complete control with less a.i. of chemical.

A73

COMPONENTS OF PARTIAL RESISTANCE TO SEPTORIA NODORUM AMONG BRAZILIAN SPRING WHEATS. A. M. Pires and B. H. Munster, CNTTP/PPA, C. Postal 569, Passo Fundo-RS, Brazil and Department of Plant Pathology, University of Georgia, Griffin, GA 30223.

Thirty-two Brazilian spring wheats were evaluated for partial resistance to Septoria nodorum under greenhouse conditions at the Georgia Experiment Station. There were significant differences among the cultivars tested for all of the components of partial resistance studied. Among the 32 wheats tested the incubation period (IP) was between 5.8 days in the susceptible cultivar BR 4 and longest (14.5 days) in the resistant cultivar CE 14. The latent period (LP) was between 13.7 days in BR 4 and BR 12 and longest (19 days) on CE 14. Disease progress was greatest on CE 14, BR 32, PF 8722, and PF 8723, PF 87584, CE 17, CE 19 and BP A. All the cultivars except IP and LP except BR 8, which was among those with shortest IP and LP. Partial resistance to S. nodorum is available among Brazilian wheats and may be traced to a few cultivars.

A74

COMPONENTS OF PARTIAL RESISTANCE TO SEPTORIA NODORUM IN EIGHT SOUTHEASTERN WINTER WHEAT CULTIVARS. J. A. Yocom and Barry M. Cunfer, Department of Plant Pathology, University of Georgia, Griffin, GA 30223.

Winter wheat cultivars were evaluated for resistance to Septoria nodorum under field conditions. Resistant cultivars Oasis and Saluda had longer incubation (IP) and latent periods (LP), and slower rates of lesion development than cultivars Acala and Gulf. Popu-
lation densities were determined immediately after inoculation in both years, and thereafter at weekly intervals for a total of five times in 1986 and three times in 1989. Receptivity of cv.
Vona to colonization by PFM2 was significantly (P<0.05) greater than that of any other cultivar in both years. Accord-
ing to Dunn's multiple range test, receptivity among the remain-
ing 13 cultivars did not differ in 1986, but separated into three groups in 1989.

A75

COMPONENTS OF PARTIAL RESISTANCE TO SEPTORIA TRITICI IN MOROCCAN WHEAT. A. Farhi and R. M. Hunger, Department of Plant Pathology, Oklahoma State University, Stillwater, OK 74078.

Four spring wheats (Tritium aestivum L.) from the Moroccan Breeding Program (BT4, BT6, BT73, and BT733), that differed in level of resistance to Septoria tritici blight, were quantitatively inoculated under growth chamber conditions to investigate the components of partial resistance to S. tritici. Effects of Desm. The components were measured by infection period, lesion number per gram leaf tissue, and percentage leaf area necrosis on seedlings. The cultivars were evaluated in responses to S. tritici with BT73 having the longest incubation period and lowest number of lesions, and BT74 the shortest incubation period and highest percentage of leaf area necrosis. Results on BT73 were intermediate. Experiments are underway to further characterize these genotypes and develop breeding lines based on one or more components of resistance.

A76


The survival and saprophytic abilities of Septoria tritici and S. nodorum were tested on leaf tissue, filter paper and straw. The number of conidia/pycnidium and percent conidium germination were determined for each species in a field trial conducted from January to May, 1989. The number of conidia produced in pycnidia declined until early Feb. and Apr. for S. nodorum and S. tritici, respectively. Rates of decline in conidium production were not significantly different (P>0.05) between species. Filter paper and straw were inoculated and tested for change in weight at 2, 4 and 6 wk. Substrates inoculated with S. nodorum had greater percentage of weight change (15% on filter paper) than those with S. triteci (6.04% on filter paper). The latter was not significantly different from uninoculated controls. These results indicate that S. nodorum and S. tritici differ in their ability to survive on leaf tissue in the field and to grow saprophytically in culture.

A77

INHERITANCE OF RESISTANCE TO LEAF RUST AND RESPONSE TO CHLORIDE APPLICATION IN SEGREGATING WHEAT POPULATIONS. S.S.A. Bizzri, G.W. Buchanan and F.A. Cholick. Plant Science Department, South Dakota State University, Brookings, SD 57007.

Precision controlled growth chamber experiments were conducted to study genetics of resistance to leaf rust in B73/Fu, F2BC and F2 populations of cultivars SD2980, Shield, Prospect, Pkn1, Pavon, Kohinoor 83 and Sarhad 83. Linkage of genes with recombination values were estimated. In addition, response to low(10 ppm) and high (50 ppm) Chloride ions( CL) in F2BC and F2 plants of SD2980 and Shield studied. Tests of goodness of fit with CL indicated 1-3 genes for resistance to rust depending on cultivar and isolate. With CL, four genes were identified in SD 2980 with linkage in coupling in both cultivars. CL ions increased number of genes for resistance by one compared to plants without CL.
A79
PHOTOTOXINS FROM THE APPLE PATHOGEN BORYEOSPHERA OBUSA
P V Subbalaiah, W S Chilton and T B Sutton, North Carolina State University, Raleigh, NC 27695.
Four isolates of Boryeosophera obusa (Schw.) Shoemaker, causal agent of black rot and frogeye leaf spot of apple were found to produce phototoxins in culture, infected fruit and spore germination fluid (SGF). Mellein was the most abundant toxin isolated from the culture filtrate. Other toxins isolated were tyrosol, 4-hydroxymellein, 5-hydroxymellein and 4-hydroxybenzaldehyde. All toxins except 4-hydroxybenzaldehyde were present in SB obusa-infected fruit, and mellein and 4-hydroxymellein could be detected in SGF. Seven apple cultivars were used in a rapid bioassay to determine phototoxicity of different toxins. Of the apple cultivars, Supergold and Silverspur were highly sensitive to all toxins. Only three apple cultivars (Empire, Stayman and Pirm Gold) showed resistance to mellein and 4-hydroxymellein. There was not a strong correlation between isolate pathogenicity and the amount of toxin production in culture.

A80
A RAPID BIOASSAY TO DETECT MONOGENIC RESISTANCE TO THE NORTHERN LEAF BLIGHT IN SWEET CORN PLANTS.
B Bashan and Y Levy, Department of Life Sciences, Bar-Ilan University, Ramat-Gan 52900, Israel.
Phytotoxic substances were isolated from culture filtrates of Exserohilum turcicum grown in Fries medium, or from susceptible sweet corn plants infected with the fungus. The substances inhibit significantly chlorophyll biosynthesis in susceptible corn seedlings, but not in corn seedlings containing Ht genes which confer resistance to E. turcicum. A linear relationship was found between the phytotoxic extract concentration and the inhibition of Chlorophyll biosynthesis in susceptible plants. The response of ethiobiological seedlings to the phytotoxic extract was tested on 35 sweet corn cultivars with or without Ht genes. The phytotoxic substance was extracted from culture filtrates of 5 different isolates of E. turcicum. A positive correlation was found between the aggressiveness of isolates and the phytotoxic activity.

A81
TEMPERATURE-DEPENDENT RESISTANCE TO DOWNY MILDEW IN MELON PLANTS: STRUCTURAL RESPONSES AND PROTEIN ANALYSIS.
M Balasub R. Cohen and M Bar-Joseph, 1Department of Life Sciences, Bar-Ilan University, Ramat-Gan 52900, Israel and 2ARCO, Volcani Center, Bet Dagan 50250, Israel.
Downy mildew caused by Pseudoperonospora cubensis is one of the most serious diseases of cucurbits in temperate regions. A susceptible reaction type was observed in resistant lines incubated at low temperatures (12 C), namely, enlarged lesions and abundant sporulation. The accumulation of callose, phenolic compounds and lignin-like materials in resistant lines incubated at 12 C were similar to that in susceptible plants incubated at 21 C. The molecular mechanism of the resistance at 21 C is not understood yet. SDS-PAGE analysis of in vitro translated proteins, soluble proteins, intracellular proteins and PR-proteins extracted from resistant and susceptible lines at 48 h after inoculation did not show any detectable differences. However, a 45 KD protein (P45) band was detected in the healthy resistant lines 3110 (near isogenic) and P122411F but not in the susceptible cultivar Hemed. In F1 hybrid (P122411/F X Hemed) the P45 was partially expressed, but in later generations F3, F5 and F7 that are resistant, P45 was expressed as in the resistant lines. P45 was found in cotyledons and leaves but not in roots. Our intention is to use P45 as a genetic marker for resistance and to examine whether P45 is involved in the mechanism of resistance to P. cubensis. Supported by BARD.

A82
LACK OF ASSOCIATION BETWEEN MANSONONE TOLERANCE AND VIRULENCE IN OPHISTOMA ULMI.
R H Proctor and E B Smalley. Department of Plant Pathology, University of Wisconsin, Madison, WI 53706.
Mansones are photoxalins that accumulate in the xylem of elm infected with Ophistem ulmi, the insect of Dutch elm disease. We examined the virulence of mansonone-sensitive mutants of O. ulmi to determine if tolerance to mansonones is necessary for virulence on elm. Mutants were generated by treating yeast-like cells of O. ulmi with the mutagen nitrosourea (NTG) and screening for increased sensitivity to 1,2-naphthoquinone, a mansonone analogue. Strains with increased sensitivity to 1,2-naphthoquinone were also highly sensitive to mansonones E and F. Both mycelial growth and germination of conidia of mutants were highly sensitive to mansonones compared to the wild type. Three mutants were less virulent on American and Siberian elm compared to the wild type strain from which they were derived. However, two mutants were just as virulent as the wild type. Mutants were backcrossed to wild type strains to remove genetic material induced by NTG. After two backcrosses all mansonone sensitive strains were as virulent as the tolerant, wild type strains. These data suggest that the levels of mansonone tolerance exhibited by wild type strains of O. ulmi are not necessary for virulence on elm.

A83
INFECTION CUSHION DEVELOPMENT BY RHIZOCTONIA SOLANI KUNTH ON SOYBEAN LEAVES AND FACTORS AFFECTING IT.
Infection cushion formation by Rhizoctonia solani (AG-1) on soybean leaves was studied with light and scanning electron microscopy. Infection cushion formation started 18 h after inoculation. An inverted "T" shaped foot formed laterally from the extending mycelium. The tips of the cushion spread extended between the grooves formed by the epidermal cells and 28 h after inoculation additional inverted "T" shaped structures were produced which intermixed to form the infection cushion. Miculagenous material binding these structures was also detected, and was greater with the web blight isolate (AG-1LB) compared to the aerial blight isolate (AG-1IA). Isolates of R. solani causing aerial and web blight did not form infection cushions on soybean leaf surface replicas of either resistant or susceptible cultivars. Infection cushions were formed by both aerial and web blight isolates on resistant and susceptible cultivars when inoculated plants were kept in continuous darkness compared to plants kept in continuous light. There was a highly significant correlation between number of infection cushions formed and disease severity.

A84
SPORC ATTACHMENT AND VIRULENCE OF NECTRIA HAEMATOCCOCOA NATING POPULATION I (P. FERMENTI SOLANI F. SP. CIBICICIDAE).
M J Jones and L Epstein. Department of Plant Pathology, University of California, Berkeley, CA 94720.
To study the role of spore attachment in the pathogenesis of the cucurbit rot pathogen Nectria haematoccoca, we isolated mutants with macroconidia that were deficient in attachment. After treatment with 4-hydroxy-3-nitro-N-nitrosoguanidine, we used an assay on polystyrene to enrich for attachment-deficient mutants. Two strains, called Att-, produced macroconidia with a 50% reduction in attachment to polystyrene and to zucchini fruits. The Att- mutants and the wild type were indistinguishable in macroconidial morphology, percentage germination, growth rate, sporotrophy and ability to mate. When macroconidia were inoculated into wounded zucchini fruits, the Att- mutants were as pathogenic as the wild-type strain. However, when macroconidia were inoculated onto the surface of unwounded zucchini, the mutants were less pathogenic. Thus, attachment of N. haematoococa macroconidia to its host surface appears to be a virulence factor and spore attachment may play an important role in the epidemiology of the disease.

A85
GENETIC ANALYSIS OF VIRULENCE IN PHYTOPHthora MEGASPERMA F. SP. GYCINEA.
B G Bhat, A F Schmidtchen, and B A McBain, Dept. of Plant Pathology, Dept. of Agronomy, The Ohio State Univ., Wooster, OH 44691.
Inheritance of virulence against soybean in Phytophthora megasperma f. sp. glycinea (Pmg) was studied by repeated selfing of oospore progenies. Pmg races 1 and 4 were included in the study. Virulence of Pmg strains tested for a hypocotyl inoculation method on a universally susceptible cultivar of soybean and three other differentials with single resistance genes Rp1, Rp3-1 or Rp1-k. Pure breeding single-oospore isolates of races 4 and 1 were obtained after selfing for three and six generations, respectively. In progeny analysis of inbred isolates, race 1 segregated for race 1, avirulent and putative race 4, Race 4 segregated for race 4 and a few (<10%) other race(s). Most of the races at advanced generations took more time to kill soybean seedlings than the field isolates indicating possible expression of inbreeding depression.

A86
GENOMIC RELATIONSHIPS BETWEEN PEA ENATION MOSAIC VIRUS AND THE LUTEOVIRUSES. S A Demler and G A de Zeeën.
Department of Plant Pathology, University of Wisconsin, Madison, WI 53706 and "Department of Botany and Plant Pathology, Michigan State University, East Lansing, MI, 49824.
Sequence comparisons of DNA 1 of pea enation mosaic virus (PEMV) and members of the luteovirus group have uncovered strong organizational and amino acid homologies between these groups. The putative polymerase of
PEMV is encoded by two overlapping out of frame reading frames, consistent with the frameshift hypothesis cited for lentivirus polyprotein expression. The C-terminal portion of the polyprotein homologous to both PLRV and BWVY, and contains all of the helicase and polyprotein motif characteristic of subgroup 2 of the luteoviruses. The coat protein is encoded on an internal open reading frame immediately followed by a second in frame sequence encoding the 20K aphid transmission subunit of PEMV. Both the arrangement and amino acid sequences of these proteins are homologous to the corresponding 3' terminal open reading frames identified in the luteovirus group. This data further strengthens the taxonomic linkage between these two virus groups.

A87
A COMPENSATORY MUTATION OF THE SATELLITE RNA OF TOBACCO RINGSPO T VIRUS RESTORES SPONTANEOUS CIRCULARIZATION AND BIOLOGICAL ACTIVITY. Hans van Tol, Jamal M. Bounayan, and George Bruceing, Department of Plant Pathology, University of California, Davis, CA 95616.

Tobacco ringspot virus supports the replication of a satellite RNA (stToBRV RNA). In vitro, the complementary strand, stToBRV(-)RNA, is a double-stranded molecule and circularization is necessary for RNA synthesis. In some virus strains, a four base pair stem in the stToBRV(-)RNA structure is modified by substituting the central two bases in one strand and, in another construction, in the other strand, such that in a third, doubly-mutated construction the two base pairs should be restored. Neither of the singly-mutated forms of the RNA increased in plants when co-inoculated with ToBRV, and circularisation of the corresponding stToBRV(-)RNAs was very limited. In the doubly-mutated stToBRV RNA, circularization and satellite RNA increase were restored. This result suggests that spontaneous circularisation of stToBRV(-)RNA is necessary for the biological activity of the satellite RNA.

A88
GENETIC MAPPING OF SYMPTOM TIMING AND SEVERITY OF CUCUMBER MOSAIC VIRUS. Marilyn Roossinck and Peter Palukaitis, Department of Plant Pathology, Cornell University, Ithaca, New York, 14853.

Several strains of cucumber mosaic virus (CMV) show phenotypic differences in the time of appearance and severity of symptoms in zucchini squash. Recombinants between two prototype strains, Fny-CMV (a 'fast' strain) and Smy-CMV (a 'slow' strain), have been mapped the symptom differences to RNA 1. The 'fast' strains also display a reduction in the levels of RNA 1, as compared to RNA 2; this alteration maps to both RNAs 1 and 2 of Fny-CMV, implying an interaction between the RNAs 1 and 2 (or their gene products). Making use of full length cDNA clones of Fny-CMV and partial cDNA clones of Smy-CMV, we have constructed a series of recombinant viruses in order to more precisely map the functional domains responsible for these phenotypic differences.

A89
THE 3'-TERMINAL HALF OF GENE VI OF CAILFLOWER MOSAIC VIRUS INFLUENCES SYSTEMIC AND LOCAL RESPONSES IN SOLANACEAE HOSTS. L. M. Wintemustel, E. P. Broglio, J. A. Schoell, Dept. of Plant Pathology, University of Missouri, Columbia, MO 65211, and Dept. of Plant Pathology, University of Kentucky, Lexington, KY 40546.

The 3' portion of gene VI of cauliflower mosaic virus (CaMV) is involved in determining the ability of the virus to systemically infect solanaceous hosts. Reombinants with three strains of CaMV have also demonstrated that sequence differences within the 3' portion of gene VI or the large intergenic region further affect host-viral interactions. Current studies using recombinant viruses have determined that the 3' portion of gene VI of CaMV strain D4 controls the appearance of necrotic local lesions on Nicotiana edwardsonii and contributes to the ability of CaMV recombinant viruses to move systematically in Nicotiana bigelovii. We have sequenced the 3' portion of gene VI of strain D4 and have compared this sequence to CM1841, a strain which fails to produce symptoms on N. bigelovii and N. edwardsonii. Within this region there are three amino acid differences between the two strains which may affect the appearance of necrotic lesions on N. edwardsonii and the development of systemic symptoms on N. bigelovii.

A90
A GENETIC ANALYSIS OF HOST RANGE DETERMINANTS OF CAILFLOWER MOSAIC VIRUS STRAIN W260. R. G. Qiu and J. E. Schoell, Dept. of Plant Pathology, University of Missouri, Columbia, MO 65211.

CaMV strains CM1841 and W260 cause systemic infection in a number of cruciferous hosts such as Brassica campestris. However, strain CM1841 does not infect Nicotiana bigelovii, a systemically infected host for W260. To identify W260 sequences that determine systemic infection of N. bigelovii, we made exchanges between infectious CM1841 and W260 clones and the chimeric viruses were then tested on N. bigelovii. We found that genes 1, 4, and 11 and VI were derived from CM1841 for systemic infection of N. bigelovii. One DNA segment which contained the 3' half of gene V and the 195 promoter and a second DNA segment which contained genes 11 and 13 influenced the concentration of virus in systemically infected leaves. Further constructs are necessary to test the involvement of the 5' half of gene V and the large intergenic region.

A91
A POINT MUTATION IN THE COAT PROTEIN ABOLISHES APHID TRANSMISSIBILITY OF A POTYVIRUS. C. D. Atreya, B. Raccach, and T. P. Piron, Department of Plant Pathology, University of Kentucky, Lexington, KY 40546.

A non-aphid transmissible (NA) variant of tobacco vein motting virus (TVM) was used to test the hypothesis that the viral coat protein (CP) plays a role in aphid transmissibility. Comparison of the nucleotide sequences of an aphid transmissible isolate (TVMA-AT) with that of TVMA-NAT revealed a single nucleotide difference (G→A) at position 848, resulting in a serine to alanine change (S282A) at position 2747. A DNA fragment representing the CP region of TVMA-NAT was substituted into the CP region of a Full-length cDNA clone of TVMA-AT, and transcribed RNA was inoculated to tobacco plants. Aphids were unable to transmit the resultant hybrid virus which had the TVMA-NAT coat protein, although the concentration and infectivity of the hybrid virus in the source plants was as high as that of TVMA-AT. This is the first direct demonstration that a CP mutation affects aphid transmissibility of a potyvirus.

A92
DETECTION OF dsRNA SPECIES AND VIRUS-LIKE PARTICLES IN UNCINULA NECATOR. Osmat J. Aazam, Dennis Gonsalves, Shigetou Namba, David M. Gadoury, and Roger C. Pearson, Cornell University, New York State Agricultural Experiment Station, Geneva, 14456.

In a study of the seasonal distribution of dsRNAs in grapevines infected with Rupesistrum Sterlin Pittin (RSP), we observed several unique dsRNA species by pulsed-field gel electrophoresis that were associated with late-season foliar infection by Uncinula necator, but not with RSP. Homogenates of whole cleistothecia and conidia of U. necator yielded these same dsRNA species. Identity of dsRNA was confirmed by DNAse and RNAse treatments. Molecular weights of the dsRNA species ranged from 9.5 x 10^6 to 6.3 x 10^6 daltons. Spherical virus-like particles were found in negatively stained cell homogenates and thin sections of cleistothecia viewed under transmission electron microscopy. Further work will focus upon the distribution of the virus-like particles in fungal tissues and propagules, the frequency of their occurrence in U. necator, and their possible effects upon virulence, pathogenicity, and survival of the fungus.

A93

Pseudomonas fluorescens 2.79 and P. aureofaciens 30-84 suppress Glomerella cingulata mycelial growth in vitro (GGT), the causal organism of take-all of wheat. Phenazine antibiotics produced by these bacteria protect wheat roots against infection by GGT. Although many soil microorganisms produce antibiotics, the function of such compounds in soil habitats is largely unknown. The objective of this study was to determine the role of phenazine biosynthesis in the survival of fluorescent pseudomonads in soil and in the rhizosphere of wheat. Strains 2.79, 30-84, Tns mutants of these strains defective in phenazine production (Phz^t), and the mutant strains genetically restored for phenazine production (Phz^r) were introduced individually into Thaxton soil loam. Soil was planted to five successive cycles of wheat, and rhizosphere populations of the introduced bacteria were determined 20 days after each planting. Phz^t strains maintained significantly lower populations in the wheat rhizosphere and in soil than did their corresponding parent strains and their respective mutant strains. These data suggest that phenazine production contributes to the survival of 2.79 and 30-84 in soil habitats.

A94
DETECTION OF HERBICOLIN A IN CROWN AND ROOT TISSUES OF WHEAT SEEDLINGS AFTER INOCULATION WITH ERMUNIA HERBICOLA. H. J. Kempp, M. N. Schroc and C. Wolfe, Department of Plant Pathology, University of California, Berkeley, CA 94720.

The herbicolidin was detected in root and crown tissues of wheat seedlings after seeds had been inoculated with E. herbicola. Seeds with approximately 10^8 CFU/s were sown into natural field soil and subsequently kept at 23°C for 4 days. After emergence, the underground plant parts were washed clean from soil and bacteria, homogenized, and extracted with methanol. The concentrated extracts were spotted onto a TLC plate and herbicolidin was detected on the plate by a bioassay with Candida albicans as a test organism. The highest concentration of
A95

THE RELATIONSHIP BETWEEN MICROBIAL ACTIVITY AND SUPPRESSIVENESS OF CANADIAN SPHAGNUM PEAT TO PYTHIUM ROOT ROT OF POINSETTIA. J. A. Boehm and H. A. J. Hotiakin, Dept. of Plant Pathology, The Ohio State University and Ohio Agricultural Res. and Dev. Center, Wooster, OH 44691.

Light (slightly decomposed) and dark ( decomposed) Canadian sphagnum peat varied in suppressiveness to Pythium root rot of poinesettia caused by Pythium ultimum Trow. Root rot and population development of P. ultimum were higher in dark sphagnum peat, in contrast to a lesser decomposed peat and suppressed in a very light, slightly decomposed sphagnum peat. Microbial activity, based on the rate of hydrolysis of fluorescein diacetate, was highest in the suppressive and lowest in the conducive peat potting mixes. When high levels of mineral nitrogen were added, peat mix throughout the initial thirty five days after planting, root rot and Pythium population development were effectively suppressed for the remainder of the three month crop cycle. Using a multiple linear regression there was a significant interaction between suppressiveness of the various peat mixes and the corresponding microbial activity. Suppression was negated by heating the potting mixes at 60 C for 30 min and restored by adding small quantities of nonheated suppressive peat. This suggests that the effect was biological in origin.

A96


During root isolations from field grown soybeans with symptoms of sudden death syndrome (Pseudomonas solani) we observed inhibition of the blue-purple macroconidia of Pseudomonas solani and Macrophomina phaseolina on potato dextrose agar (PDA). We observed that the isolate frequency of M. phaseolina from lateral and apical roots was significantly lower from plants collected in a soybean nontreated field as compared to those from plants collected from a nontreated field. Controlled experiments in vitro, confirmed that P. solani inhibited the growth of M. phaseolina on PDA. P. solani also interfered with the germination of microconidia of M. phaseolina in the growth chamber over time as well as a reduction in average germ tube length. Soybean plants grown in the field on PDA late fall soil reincubated with known concentrations of M. phaseolina alone or M. phaseolina plus F. solani. Using CMR, a selective medium for M. phaseolina, the isolation frequency of M. phaseolina growing in the P. solani plus F. solani soil was one half of that of the isolation frequency of F. solani alone. The results exposed to M. phaseolina alone. We conclude that P. solani inhibits M. phaseolina, although the mechanisms have not been elucidated.

A97

PATHOGENICITY OF AG-2-2 CULTURES OF RHIZOTOCIA SOLANI ISOLATED FROM BEANS AND SUGAR BEET ON BEAN SEEDLINGS. Cheryl A. Engleke and Carol E. Windham, Dept. of Plant Pathology, University of Minnesota, St. Paul, 55108 and Northwest Experiment Station, Crookston, 56716.

Cultures of AG-2-2 of Rhizoctonia solani were isolated from rotated fields of beans and sugar beet. Nine AG-2-2 cultures were evaluated in 32 x 4 in. field for pathogenicity on two cultivars each of fababean, navy bean, and red kidney beans inoculated with sugarbeet rootrot. For each treatment, 30 unifoliate plants were inoculated with one AG-2-2 colonized corn kernel. Basal stems were rated using a 1 to 5 scale (1 = dead; 5 = normal). The highest soil rating was 4.5. Fababean had the highest root rot index of 4.5, followed by soybeans 4.1; fabebeans 4.0, and soybeans 3.5. The two AG-2-2 cultures each fabebeans 4.1 and soybeans 4.0 gave a overall rot index of 4.8; two cultures from fababean 4.3 and soybeans 3.9. Based on these results, rotation of sugar beet with bean crops is not recommended.

A98

MANGANESE SEED CONSENT - AN AMELIORATING FACTOR FOR TAKE-ALL OF CEREALS. T.S. Rosman and D.M. Huber. Purdue University, W. Lafayette, IN 47907.

Manganese is a critical regulator of physiological defense reactions of plants to disease; and the severity of take-all root, crown, and foot rotted of cereals has been correlated with such cultural practices as form of Mn, pH adjustment, and crop rotation which influence the availability of Mn in the soil. This study determined if the Mn content of the root and seed in soils influences the severity of take-all. Five varieties of soft red winter wheat (Triticum aestivum L.) were grown under two widely different ecological conditions to model their Mn seed consent. Four varieties (Carolina, Lincoln, Steele, Twain) differed by 20 to 30 g/Mn kg-1 at the 0-100 cm depth. Varieties (Caldwell) was similar at both locations. All varieties were grown at three field locations in Indiana with natural infestations of Gaeumannomyces graminis var. tritici. Under severe, moderately severe, and no disease pressure Mn content of the root and seed consent were generally more vigorous, had an average of 11% less severe take-all at the head, and yielded an average of 165 kg/ha more grain. No significant differences in vigor, yield, or take-all severity were observed with the variety grown from seed produced under widely different environments but with similar Mn seed consent.

A99

INFLUENCE OF WINTER COVER CROPS ON SOIL POPULATIONS AND ISOLATION FREQUENCIES OF COTTON SEEDLING PATHOGENS. C. E. Rothrock and T. L. Klapatche, Dept. of Plant Pathology, University of Arkansas, Fayetteville, AR 72701 and SWREC, Rt. 3, Box 258, Hope, AR 71810.

Winter legume cover crops reduce soil erosion and provide an environment attractive to beneficial soil organisms. The influence of legume cover crops in crop sequences with cotton on soilborne pathogen populations and colonization of cotton seedlings was examined at two locations. Colonization of seedlings by Rhizoctonia solani increased following hairy vetch cover crop treatments compared to winter fallow near Hope. However, no differences in soil populations of R. solani were detected. Soil populations of Thielaviopsis basicola and seedling cover crops were reduced following hairy vetch cover crop treatments compared to winter fallow near Clarkedale. Soil populations of pathogenic Pythium spp. were greater at both locations following legume cover crops than fallow; however, no differences in isolation frequency of Resistant. The influence of hairy vetch on the seedling disease complex depended on the prevalent pathogens at each location.

A100


Incidence (DI) and severity (DS) of grape powdery mildew (Uncinula necator) on the cultivar Rosette were examined in a 3 -yr study. Treatments were: 3 early, mid-, or late-season sprays of benomyl or triadimenol; 2, 4, or 6 sprays during the season. DI was measured as inclusive leaves/shoots (%) and DS as infected leaf area/shoot (%). Early disease suppression resulted in less fruit infection than mid- or late-season suppression, possibly due to protection from ascospore inoculation in mid-season. The isolation frequency of resistant in existing fruit. DI-DS relationships were affected by fungicide treatments, but were similar within treatments in all 3 years. Late-season sprays permitted DS to exceed 80% and resulted in curvilinear DI-DS relationships. Early- and mid-season sprays, and the 2 - 6 spray treatments reduced DS, and yielded a linear relationship. The 6-spray treatment kept DS at a low and constant level. However, in all treatments, DI in most plots eventually approached 100%. Neither triadimenol nor benomyl eliminated infection. Instead, DS was often reduced to the extent that DI-DS relationships were altered. We suggest that auto-infection (tensio Robinson) is fully operational in U. necator on grape.

A101

COTTONBOLL DISEASE PROGRESS IN A WISCONSIN CRANBERRY FIELD. P. G. Sanderson and S. N. Jeffers. Department of Plant Pathology, University of Wisconsin, Madison 53706.

Spores of Monilia axycocc were collected and disease progress was monitored for 2 years in a commercial cranberry field with a history of cottonboll. Shoots with symptoms were first observed on 7 June 1988 and 10 June 1989. Numbers of symptomatic shoots continued to increase for 1 to 2 weeks, but after 12 weeks, the number of symptoms did not increase. The disease was late blight, in 1988 and 1989, respectively. Conidia were observed on shoots 7 and 6 days after the initial symptoms were seen and were eventually produced on 76% and 85% of all blighted shoots in 1988 and 1989, respectively. All symptoms were found beginning 16 days, 10 days after initial bloom, for 26 days in 1988 and 19 days in 1989, respectively. Bloom, for 33 days in 1989. Conidia peaks began on 30 July. Following the begins the of the ascospores peaks in 1988 and 1989, respectively. Although the onset of symptom development differed between years, the first bloom of spores was caught coincided with full bloom in both years. In 1989, trap plants were placed in the field when symptoms were abundant. No symptoms were observed on expanding shoots; however, an average of 36% of fruit on plants that were in bloom at the time became diseased. The incidence of disease did not increase after bloom in either year. Mycelium of M. axycoccus was detected in approximately 41% of the berries in 1988 and 35% of the berries in 1989.

A102


In 1986, hazelnut orchards with eastern filbert blight (EBF) were found near the northeastern edge of the Willamette Valley in the major production area. From 1986 to 1990, 5,400 ha in 8 counties were surveyed. EBF was detected in the 3 most northern counties within 40 km of the Willamette Valley. EBF was not detected in southern Oregon. EBF was found in 660 ha of commercial orchards, 43 ha of unmanaged orchards, and at 216 residential sites. Fifty one percent of the diseased orchards and symptomatic branches within lightly infected orchards have been destroyed. EBF was very severe in orchards planted with Ennis, Royal, and Davianna, whereas the major cultivar, Barcalenas, appears moderately resistant. Secondary soil have developed around susceptible plantings. Within-orchard spread of EBF is mostly to the northeast, the direction of the prevailing winds.
A103

INFLUENCE OF TEMPERATURE AND LEAF WETNESS DURATION ON INFECTION AND DISEASE DEVELOPMENT BY UROMYCES DIANTHII ON CARNATIONS.
M. Polek and D. M. Ferrin, Department of Plant Pathology, University of California, Riverside, CA 92521

The influence of temperature and leaf wetness duration on disease development by Uromyces diant thi on carnations was examined under constant temperature conditions. Eight-week-old carnation plants (cv. Improved White Sim) were inoculated with a suspension of 10^4uredospores/ml and incubated at 10, 15, 20, 25 and 30 C for leaf wetness durations of 4, 8, 12, 16 and 24 hr. Disease was most severe on plants incubated at 15 or 20 C with a wetness duration of at least 8 hr. Disease did not develop at 10 C and developed poorly at 25 C. The latent period was 22 and 18 days at 15 and 20 C, respectively. In vitro spore germination was examined at 5, 10, 20, 25 and 30 C for incubation periods of 6, 12, 18 and 24 hr. Maximum germination (25%) occurred at 15 and 20 C after 24 hr. Germination was 11 and 9% at 10 and 25 C respectively, whereas it was 3 and 1% at 5 and 30 C respectively.

A107

AN ACTION THRESHOLD FOR MANAGEMENT OF PUMPKIN POWDERY MILDews.
M. T. McGrath, Dept. of Plant Pathology, L. I. Horticultural Research Laboratory, Cornell Univ., Riverhead, N. Y. 11901-1098

A field experiment was conducted in 1989 to evaluate the feasibility of using an IPM approach for managing powdery mildew (PM) caused by Sphaerotheca fuliginea. Observed PM severity was used to time the initial application of triadimefon within a comprehensive fungicide program. Two action thresholds were evaluated: (1) a minimum of 1 out of 50 leaves with PM (AT1) and 2) an average of 1 leaf colony/leaf (AT2). AT1 and AT2 occurred on day 234 and 251, respectively. Other treatments were a control, chlorothalonil, and chlorothalonil plus triadimefon. Treatments were applied 5 times. Average PM severities on both leaf surfaces were similarly low (less than 7% at maturity) for pumpkins treated with either chlorothalonil and chlorothalonil plus triadimefon or triadimefon based on AT1. PM development on lower leaf surfaces was similar to the control (about 15%) in pumpkins treated with only chlorothalonil and it was not controlled adequately with AT2. It appears that PM can be managed using AT1 in an IPM program.

A108

Soil Moisture, Genotype and Vorticillum dahliae Interactions in Potato Early Dying.
M. R. Cappaert, M. L. Rowson, and N. W. Christensen. Dept. of Botany and Plant Pathology, Oregon State University, Corvallis. 97331-2502.

Field microplot studies were initiated in 1989 in the Columbia Basin of Oregon to determine if soil water potential can alter the expression of resistance to potato early dying. Treatments consisted of genotypes, irrigation regimes, and inoculum levels of V. dahliae. Genotypes differed in maturity class and susceptibility to potato early dying. Three irrigation schedules ('dry', 'optimum' or 'wet') were maintained soil moisture within the desired ranges. Plots were irrigated at 1 of 4 day intervals depending upon desired soil water potential and crop water use. Severity of PED was significantly lower (P=0.05) in late compared to early maturing genotypes as well as genotypes that were inoculated compared to susceptible genotypes. Disease severity was significantly greater (P=0.05) in genotypes grown in infested soil which received the "wet" irrigation schedule as compared with genotypes that received the "dry" or "optimum" irrigation schedule. Mean tuber yield was reduced 14% in the high inoculum level compared to the noninfested control across water levels regardless of maturity class or susceptibility. Irrigation regime had no effect on tuber yield.

A109

EFFECT OF TEMPERATURE AND PH ON SPORE ATTACHMENT OF Fusarium Solani F. Sp. Phaseoli On VIGNA RADIATA.
A. G. Schuerger and D. J. Mitchell. The Land, EPOC Center, P.O. Box 10,000, Lake Buena Vista, FL 32830, and Dept. of Plant Pathology, University of Florida, Gainesville, FL 32611.

In vitro and in situ experiments on the growth rate of Fusarium solani f. sp. phaseoli indicate that the optimum temperature and pH for fungal growth are between 25-28 C and 6.0-7.0, respectively. However maximum spore attachment to plant roots by macrocondia occurs at 20 C and a pH of 4.0. Spore attachment is blocked at high and low pH (7.0 and 3.0, respectively) and at high temperature (35 C). Spore attachment to plant roots appears to be positively correlated to the secretion of an amorphous material from the terminal and foot cells of macrocondia. Observations using SEM indicate that the amorphous material is not present on macrocondia in culture nor is it present on macrocondia developing on sporodochia or individual phialides produced in situ.

A106

CONTROL OF GLOBODERA TABACUM SUBSP. SOLANACEARUM WITH INCREASING RATE OF FENAMiphos.
C. S. Johnson, VPI & SU, Southern Piedmont Agricultural Experiment Station, P.O. Box 448, Blackstone, VA 23824.

Effects of fenamiphos rates (0, 1, 7, 3.4, 5.0, and 6.7 kg a.i./ha) on population dynamics of Globodera tabacum subsp. solanacearum (Gts) and agronomic performance of Gts-resistant (NC 557) and susceptible (K 326) flue-cured tobacco cultivars were investigated in on-farm tests conducted in 1987 and 1988. Fewer Gts juveniles were found at higher fenamiphos rates at 2 sampling dates, but numbers of Gts cysts and eggs were not correlated with fenamiphos rate in 1987. Increased rate of fenamiphos reduced Gts cysts and eggs in 3 or 5 sampling dates in 1988. Numbers of Gts juveniles decreased with increasing rate of fenamiphos in each sampling date in 1988. Gts population densities were not significantly (P<0.06) lower on NC 557 than on K 326 until the final, post-harvest sampling date. Yield, gross economic returns, average prices, and flue-cured tobacco grade indices increased with fenamiphos rate in 1987, but similar trends were not statistically (P<0.06) significant in 1988.

A110

AN EVALUATION OF SEED PRIMING TECHNIQUES WITH FIVE SUGAR BEET CULTIVARS.
C. M. Rush, Texas Agricultural Experiment Station, P.O. Drawer 10, Bushland, TX 79012.

A greenhouse study was conducted in which the effects of priming techniques on sugar beet seedling emergence and growth in Pythium, Aphanomyces, or non-infested soil were compared. NaCl and PEG 8000 solutions were compared to a solid matrix priming technique, SPM. Washed and non-treated seed were included as controls. In all soils and cultivars, SPM treated seed had significantly greater emergence after 3 days than all other treatments. After 15 days, stands of SPM treated seed were higher in Pythium infested soil but not in control or Aphanomyces infested soil. All priming techniques resulted in significantly better emergence after 3 days than non-treated seed. Seed primed with PEG often performed no better than washed seed. Primed seed resulted in increased final stands and decreased pre-emergence damping off in Pythium infested soil. No treatment reduced seedling disease in Aphanomyces infested soil. SPM is a superior method of seed priming and the effects of priming are not cultivar specific.
A112

EFFECT OF IAA PRODUCTION ON IN PLANTA SURVIVAL OF PSEUDOMONAS SAVASTANONI, S. E. Silverstone, R. M. Bostock, D. G. Gilchrist, and T. Kosuge. Department of Plant Pathology, University of California, Davis.

Pseudomonas syringae subsp. savastanoi causes tumors on oleander by producing indoleacetic acid (IAA) and cytokinins. Production of IAA by the bacterium is essential for tumor formation and the genes coding for IAA production are located on the plasmid pIAA. The contributions of pIAA and IAA production to the ability of P. savastanoi to grow and survive in oleander leaf tissue were studied. Isogenic bacterial strains which differ only with respect to IAA production were characterized. The strains include one which lacks pIAA (pIAA-), a spontaneous inactively inactivated strain (pIAA+), and the wild-type parental strain (pIAA+). The strains were inoculated separately or in pairs into young leaves. Growth and survival of the bacteria in host tissue were monitored over a three month period by weekly colony counts, colony hybridization, and in situ IAA assays. Growth rates did not differ for the 3 strains, but the wild-type strain survived longer than either mutant. The insertion mutant survived longer than the strain lacking pIAA. Coinoculation with the wild-type strain significantly increased survival of the IAA- mutant. It is suggested that IAA production enhances survival of P. savastanoi in oleander tissue and can be complemented exogenously in planta, and that pIAA encodes additional functions which contribute to ecological fitness.

A113

PSEUDOMONAS SYRINGAE CAN BE DISTINGUISHED AT THE PATHOVAR LEVEL IN RFLP ANALYSIS USING A ROTINASE GENE AS PROBE. M. Henderson, D. C. Hildebrand and W. N. Schroth. Department of Plant Pathology, University of California, Berkeley, CA 94720

All arginine dihydrolase negative strains of Pseudomonas syringae, with the exception of P. s. glycinea are able to hydrolise rutin, a glycoside constituent of many plant species (Caspers and Hildebrand, 1989). The rutinase gene of P. viridiflava F262 was cloned and used to probe EcoRI digested genomic DNA of several different Pseudomonas syringae strains. The probe hybridized to all P. syringae, P. viridiflava and P. cichorii strains tested, as well as certain P. fluorescens and P. putida strains. RFLP patterns for P. syringae strains were identical in most cases for strains isolated from the same host and corresponding to a named pathovar. P. syringae strains from bean and citrus each had RFLP patterns different from P. syringae strains isolated from other hosts. RFLP patterns for each host group of strains appeared to be conserved and unique and may be used for the identification of fluorescent Pseudomonas strains.

A114

DIRECT MAGNETIC IMMUNOISOLATION (DMI) OF XANTHOMONAS CAMPESTIS PV. PELARGONII (XcP). J. B. Jones and J. W. L. van Vuurde. GCREC, Univ. of Florida, Bradenton, FL 34203 and Institute for Plant Protection, P.O. Box 9060, Wageningen, The Netherlands.

In the initial development of magnetic immunosolation (MII), difficulties existed in reducing the saprophyte to pathogen ratio. A modification, DMI, was developed in which the magnet was placed on the sample suspension surface to attract to the surface the paramagnetic iron oxide beads which have the target organism attached. In a test where washings of geranium leaves were spiked with ca. 10^9 survival per ml, bacterial contaminants were reduced to 156.8 and 19.6 on the agar plates, with the conventional procedure (MII) and DMI, respectively, whereas 1250 contaminants were present on the agar plate in the untreated suspension. Recoveries of XcP from the test suspensions were 64.9 and 47.8 percent from MII and DMI, respectively. DMI reduced contaminants significantly compared to MII, but did not significantly reduce recovery of XcP.

A115

MOVEMENT OF GENETICALLY ENGINEERED XANTHOMONAS IN THE ENVIRONMENT. Joe Shae, Fenny Danev, and Joe Kloeper. Department of Botany and Microbiology, Horticulture, and Plant Pathology, Auburn University, Auburn, AL 36849.

The use of genetically engineered microbes (GEMs) has a number of potential agricultural and environmental applications. Effective use of such GEMs will likely depend upon several factors including the ability to monitor the environmental fate of recombinant microbes and their DNA. To address these questions we have genetically modified Xanthomonas campestris pv. campestris (XcC) to express the bioluminescence genes of the transcriptional Tn431 which carries the lux operon of Vibrio fischeri and a tetracycline resistance gene. The movement of this GEM in the environment can be monitored by bioluminescence assays, tetracycline resistance assays and PCR techniques. A limited field release of a bioluminescent strain of XcC is planned for the spring of 1996 under the conditions of a permit issued by the USDA/APHIS. The movement of the bacteria from the inoculation site and their ability to pass Tn431 to other bacteria will be examined.

A116

CHARACTERIZATION OF A GENOMIC REGION ENCODING XANTHOMONADIN PRODUCTION IN XANTHOMONAS CAMPESTIS PV. CAMPESTRIS. A. R. Poplawski, Plant Pathology, PES, University of Idaho, Moscow ID 83843.

Yellow xanthomonadin pigments are thought to be unique to Xanthomonas spp. Their function is unknown. Sequences throughout a 26 kb clone (pG102) from X. c. strain B-24 were needed to restore pigment production to thirteen pigment-negative mutants of B-24. The use of pG102 subclones in a pigment restoration analysis divided the mutants into six restoration groups. Colony blot hybridizations showed that subclones of pG102 were homologous to DNA from 17 pathovars of X. campestris (including pigmentless strains of pv. manihotis) but not to Pseudomonas, Erwinia, or Clavibacter. Over 130 insertions of a promotorless lacZ gene-containing transposon (Tn3-Holho) have been isolated and mapped in pG102. These insertions are being used to further define the pG102 pigment-encoding regions.

A117


Thirteen of 56 non-citrus strains produced reactions on wound-inoculated Swingle citrumelo and Duncan grapefruit leaves similar to those caused by Xc. pv. citricola strains from citrus bacterial spot (CBS) in Florida nurseries. Non-citrus strains of the weakly to moderately aggressive type, Xc. pv. pv. maculifoliolae and three strains from citrus, Strelitzia, elicited necrotic spots on spray-inoculated immature foliage and multiplied in plants as well as a weakly aggressive strain from citrus. Strains of Xc. pv. campesrtis, pv. phaseoli and pv. malvacearum that did not elicicit necrosis failed to multiply in leaves. Most of the weakly to moderately aggressive strains of non-citrus origin could not be separated from the group of weakly aggressive citrus strains by RFLP or fatty acid profile analysis. Other Xc. strains that did not grow in plants or give a necrotic reaction were less related to the groups of citrus and non-citrus strains by these analyses.

A118

FACTORS CONTRIBUTING TO POOR POSTHARVEST DECAY CONTROL OF TABLE GRAPE. J. L. Smilanick, D. J. Henson, D. Luvins, H. Shorey, and J. Knutson. USDA, ARS, PAW, MCHT, 2011 South Peach Avenue, Fresno, CA 93727.

Insufficient box vent size or number, inadequate air movement within pallets of boxes, center-isolated boxes, and tissue-ripe and uniformly mature clusters were found to be the limiting factors associated with poor postharvest decay control of black grapes to control Botrytis cinerea. In some cases, the relative humidity (RH) at 100% was found to be as low as 20-30%. In contrast, only 5% berries decayed when stored at 15°C with 100% RH.
A119

EFFECT OF SINGLE VERSUS MULTIPLE RACE INOCULA ON SCREENING STRAWBERRY PLANTS FOR RESISTANCE TO COLLETOTRICHUM FRAGRANSE. Barbara J. Smith, USDA-ARS, Poplarville, MS 39470

Single and multiple inocula were compared in single inoculations and in a series of 2 or 3 inoculations to develop a technique for screening strawberry plants for resistance to several races of C. fragarum, the causal fungus of anthracnose crown rot. Conidial suspensions of 3 races were applied to 4 plants each of 4 strawberry clones in 20 treatment combinations. Disease severity (DS) was rated on a scale of 0-no symptoms to 6-plant dead. No induced resistance was found. Low DS resulted when conidia of all 3 races were combined and applied in a single inoculation. The DS of plants inoculated with a mixture of 2 or 3 races was usually close to the average DS of plants inoculated with the same races individually. High DS resulted when plants were inoculated with each of the 3 races in sequence, one every 10 days. To identify plants resistant to multiple races, inoculate with each race individually in a series of inoculations.

A120

IDENTIFICATION, OCCURRENCE, AND CRITERIA FOR INFECTION OF APPLE BY ALTERNARIA MALLI. N. M. Filjadic and T. B. Sutton. Department of Plant Pathology, North Carolina State University, Raleigh, NC.

Alternaria mali, cause of Alternaria leaf blotch, was isolated from leaves of cultivars Delicious from several orchards in North Carolina in 1968. Conidial morphology was the same as reported in the literature and similar to that of a culture obtained from ATCC. AM 1 toxin was isolated and similar to a standard obtained from Japan. This is the first report of the disease in the US. In the summer of 1969, 60 orchards in western North Carolina were surveyed for the presence of A. mali. The pathogen was found on Delicious in all orchards. The percent infected leaves ranged from <3 to 65. Criteria for infection of apple anemoids were determined in vitro. Nine different temperatures (range 4-36°C), and eight different wetting durations (range 2-48 hours) were studied. The optimum temperature for infection was 24°C. Infection occurred with as little as 2 hr wetting.

A121

SURVIVAL OF VENTURIA INAEQUALIS CONIDIA AFTER DISCONTINUOUS WETTING PERIODS. C. M. Becker, and T. J. Burr. Department of Plant Pathology, Cornell University, Ithaca, NY 14853.

The viability of V. inaequalis conidia on apple leaves was determined following discontinuous wetting periods. Treatments consisted of combinations of wet-dry-wet periods at 10, 15, 20, and 25°C, with the relative humidities (RH) during dry periods of either 60% or >90%. The first wet period was 0 to 12 hr; the dry periods ranged from 0 to 96 hrs, and the second wet periods were 0, 6, and 24 hrs in length. After the second wet period propagules were stained with fluorescein diacetate and calcicolour, then viewed with epifluorescence microscopy to quantify live propagules. At all temperatures and RHs, nongerminated conidia were unaffected by dry periods up to 96 hrs. The viability of germings, with and without appressoria, was only slightly reduced by dry periods up to 24 hrs. Following dry periods of 96 hrs, the viability of germings without appressoria was reduced up to 45%; viability was most reduced when the RH was >90% at 20 and 25°C. Germs with appressoria were even more affected by drying.

A122

ETIOLOGY AND TRANSMISSION OF STIGMATOMYCISIS DISEASE OF PISTACHIO IN CALIFORNIA. Themis J. Michailides and D. P. Morgan, Dept. of Plant Pathology, Univ. of Calif., Berkeley/ Kearney Ag. Center, 9240 S. Riverbend Ave., Parlier, CA 93648.

Stigmatomycosis of pistachio is characterized by the smelly, rancid, and slimy appearance usually of the entire kernel. A Nematospora sp. was frequently isolated from kernels with stigmatomycosis. Incidence of stigmatomycosis ranged in orchards irrigated by drip 0.7-9.1, by microjets 0.7-13.9, by flooding 0.7-16.0, and by sprinklers 0.7-29.1. Four hemiparasites, stigmas, Thyanta pallidifina, Chlorosporium sp., and Pseudoschaereria sp., were isolated from infected pistachio kernels. All fungi had significantly lower levels of kernel necrosis but not of stigmatomycosis. Fungicide applications did not affect levels of stigmatomycosis. Although insecticide sprays reduced levels of kernel necrosis, their effect on stigmatomycosis was not clear.

A123

RELATIONSHIP BETWEEN XYLEM WOUND RESPONSE IN GRAPEVINES AND SUSCEPTIBILITY TO EUTYPA LATA. G. Munkvold and J. J. Marois, Dept. of Plant Path., Univ. of CA, Davis, 95616.

Grapevines pruned in the fall have been shown to be more susceptible to Eutypa lata than those pruned in the spring. A decline in susceptibility of vines pruned in early March correlated with a rapid increase in the amount of lignin and suberin in the wounded xylem detected by the lignin-thioglycolic acid (LTGA) assay. A majority of wounds inoculated 1 day after pruning became infected; only 25% of wounds inoculated 23 days after pruning became infected. Mean LTGA/mg xylem tissue increased fourfold between 1 and 23 days after pruning. Conversely, vines pruned in late November had a very low rate of lignin accumulation. Significant differences in lignin content among cultivars exist. Differences in levels of lignification among cultivars and pruning dates could account for different levels and durations of susceptibility.

A124

POWDERY MILDEW OF CRANBERRY: A FIRST REPORT. S. N. Jeffers, M. J. Drilis, and P. G. Sanders. Department of Plant Pathology, University of Wisconsin, Madison, WI 53706.

Powdery mildew of cranberry (Vaccinium macrocarpon), although noted once previously (Anon. 1960. USDA Agric. Handbook 165), has never been described. The disease was observed first in mid-summer 1989 on greenhouse-grown, potted plants (cv. Scarsli) that previously had been placed in a commercial cranberry field for several days. Since then, it has been maintained on plants (cv. Stevens) growing indoors and has been transmitted to four additional cultivars -- Ben Lear, Pilgrim, Crowly, and Bain McFarlin -- by shaking infected plants with conidia over healthy plants. Powdery mildew has not been observed on plants in field locations. Mycelia and conidia of the pathogen formed on both upper and lower leaf surfaces and on succulent stem portions. Cleistothecia were produced on the lower leaf surface and stems. Each cleistothecium contained multiple asci and had long appendages that were dichotomously branched at the tips with the ultimate branches recurved. The taxonomic status of this powdery mildew fungus is presently under investigation. It clearly belongs to the genus Microsphaera but has taxonomic characters similar to both M. penicillata and M. vaccinii (= M. penicillata var. vaccinii).
A127

INOCULATION OF STRAWBERRY ROOTS BY COLLETOTRICHUM FRAGRARIAE AND GLOMERELLARIA CINGULATA DOES NOT CAUSE CROWN ROT. C. M. Howard, C. E. Allegretti, and C. K. Chandler, Univ. of Fla., AREC, Dover, FL 33527.

Roots of potted Chandler and Pajaro strawberry plants were inoculated in June by placing 0.5 ml of suspensions containing 3 million spores/ml of C. fragariae or G. cingulata on injured or noninjured roots at 2 points around the turnball of each plant or by placing sections of roots infected by either pathogen in contact with noninjured roots of plants. Observations were made for 5 months. Natural foliar infection by G. cingulata occurred in late July and was controlled by Captan. Four of 20 control plants and 5 of 120 root-inoculated plants died from crown rot, apparently as a result of the foliar infection. G. cingulata was isolated from the crowns of 8 of the wilted plants. These results indicate that crown rot in Florida usually does not result from root infection.

A128

ISOZYME COMPARISONS OF SEPTORIA CITRI FROM AUSTRALIA AND THE UNITED STATES. M. R. Bonde, G. L. Peterson, R. W. Emmett, and J. A. Anderson. USDA-NR, Frederick, MD 21702; Dept. of Agric. and Rural Affairs, Queensland Hort. Res. Inst., Australia; and Univ. of CA, Riverside 92521-0122.

An embargo exists for fresh citrus fruit entering the U.S. from Australia due to reports made decades ago that species of Septoria exist in Australia not present in the U.S. However, there may be only one species, S. citri Pass., with comparable morphology and races of the citrus pathogen. We compared isozymes of 28 isolates of S. citri (18 Australian and 10 U.S.) for 25 enzymes. Except for one Australian isolate (SHC 335), the isolates were essentially identical with an average coefficient of similarity (CS) of 0.97 and no variation for 23 of the 25 enzymes. The average CS comparing SHC 335 to other isolates was 0.98. The data supports the belief that, except for the aberrant isolate SHC 335, these isolates are of the same species.

A129

THE EFFECTS OF POSTHARVEST CALCITON-FUNGICIDE COMBINATIONS ON CONTROL OF APPLE STORAGE DISEASES. H. F. Mollin, USDA-ARS, Horticultural Crops Quality Lab, P.O. Box 167, Beltsville, MD 20705

Preliminary tests demonstrated that Ca and fungicides may interact to protect fruit from postharvest pathogens. This study was conducted to test Ca-fungicide combinations on harvested apples. Fungicides were applied at 10-50% of recommended rates and Ca (CaHPO4) was added. Fruit were wound inoculated, dried, and dipped in fungicide or Ca-fungicide solutions, dried, and stored at 20C. Decay ratings were made 6-12 days after inoculation. Pathogens studied included Botrytis cinerea, Alternaria alternata, and Penicillium expansum. Fungicides tested were Captan, Benomyl, and Imazalil. Ca-fungicide enhanced decay resistance of CaCl2 or fungicide application alone at concentrations as low as 10% of the recommended rate. Benomyl and Imazalil + Ca gave slightly better control than Captan + Ca at all reduced rates. This study demonstrates that postharvest Ca-fungicide treatment can reduce decay and may reduce fungicide residues on fruit.

A130

ROOT ROT OF YOUNG CITRUS CAUSED BY A SPECIES OF THE GANODERMA LUCIDUM COMPLEX. N. Sklar, G. S. Smith, and R. L. Gillettson, Texas A&M Univ. Citrus Center, Weslaco, TX 78596; TMP, 45 Agri-Bldg, Univ. of Missouri, Columbia, 65211: Plant Pathology Dept. Univ. of Arizona, Agri-Bldg # 26, Tucson, AZ 85721.

In 1989, the first author reported on a disease of young citrus in Texas that was associated with a species of the G. lucidum (Gurtt.++) Karst. complex. Since then, we have confirmed the Koch postulates on citrus trees. Thirty-six Citreopara mandarin seedlings grown on germination paper were surface sterilized, planted in 50 ml flasks that contained the Ganoderma isolate 89-385, and grown at 25C. Eight young grapefruit trees, four on sour orange and four on Sweet citrumelo rootstocks, were inoculated with another isolate of Ganoderma from citrus. To date, the Citreopara mandarin and Sweet citrumelo rootstocks have developed root rot and Ganoderma spp. was isolated.

A131


Isolates of Leucocytropha spp. from perennial cankers on stone and pome fruits were characterized into four groups based on colony growth, morphology and temperature optima. Allozyme polymorphisms at eleven loci separated them into five genetic groups using cellulose acetate gel electrophoresis. The genetic groups had cultural characteristics similar to Leucocytropha parasitica. One was found exclusively on peach, whereas the other was widespread on six Prunus spp. Three groups had Leucocytropha cincta sexual states, and of these one was found exclusively on apricot and another exclusively on apple. The third occurred on several Prunus spp. Temperature optima and growth rate of the L. cincta group on apple differed from the other groups.

A132 Withdrawn

A133


The use of sublethal amounts of herbicide, applied as a seed treatment, or as a soil drench as means of protecting tomato plants against the wilt pathogen, F. solani and the bacterial wilt P. syringae, was studied. Seeds soaked for 6 and 24 h in 5 mg/l of dinitrazone (DN), gave rise to plants that were completely free of wilt symptoms after all control plants had died. Minimal impairment of growth performance of plants was observed. DN treatment also reduced symptoms caused by Pseudomonas solanacearum by 60-80%, thereby extending the usefulness of this procedure. The efficacy of chemical treatment for inducing resistance and the activity of the enzyme peroxidase was found to be correlated. This may provide a useful marker for rapid determination of the efficacy of chemicals that induce resistance. Seed treatment offers a more controllable and environmentally safer method of applying chemicals that act on inducing plant disease resistance than those previously tested.

A134

REDUCTION OF FUSARIUM ROOT ROT OF WHEAT BY CALCIUM CHLORIDE IN A GROWTH CHAMBER. G. W. Bucheneu and S. A. Rizvi. Plant Science Department, South Dakota State University, Brookings, SD 57007.

When chloride was varied in nutrient solutions to irrigate spring wheat seedlings in washed sand in a growth chamber, emergence of cv "Butte" but not "Guard" was increased by chloride concentration of 60 mg/l in uninfested sand, but there was no effect of chloride on emergence in sand infested with Fusarium graminearum or on percentage of non-rooted roots in infested sand was reduced by 60 mg/l chloride on "Butte" and a similar trend occurred on "Guard", PC caused reduction in total root length of 80% on "Butte" and 48% on "Guard".

A135


Two rows of non-treated pinto cultivar U.I. 114 plants were inoculated with a mixture of Uromyces appendiculatus var. appendiculatus (subspecies at Spence) at the Colorado State University Bay Farm Research Facility in 1988 and 1989. Uromyces was isolated from leaf blight lesions on bean of the same age in Colorado. Two-row plots, 1.85 m long, of pinto bean cultivar U.I. 114 or Olathe were treated with 0, 5, 10, 25, 50, or 100 g a.i./A of the systemic fungicide Tilt (propiconazole) 42, 58, and 70 days after planting in 1988 and 65 and 80 days after planting in 1989. Average pustule size, pustules per cm² of leaflet, and yield were recorded. Data indicate that sub-lethal fungicide application and the genetic resistance of Olathe reduced infection efficiency. Fungicide treatment rates were correlated with yield of U.I. 114 (susceptible) but had no effect on pustule size on either cultivar.
A136

REDUCTION OF THREE DISEASES IN PEANUT CAUSED BY SCLEROTIUM ROLFSII, RHIZOCTONIA SOLANI, AND CYLCINDROCLADUM CORTALIAEAE WITH DMINICINOLE. T. A. Kucherek and F. M. Shokes, University of Florida, Gainesville 32611 and Quincy, 32351.

Southern stem blight (SSB), caused by S. rolfsii, limb rot (LR), caused by R. solani, and cylindrocladium black rot (CBR), caused by C. cortaleaeae, were reduced significantly (P=0.05) in peanut (Arachis hypogaea) by foliar spray of diniconazole and triadimefon. CBR and LR occurred with three mid-season sprays with 0.28 kg a.i./ha/application (broadcast-equivalent rate). Higher rates or more frequent applications increased control of SSB up to 100%, but vines were stunted and yields were reduced. With 0.28 kg a.i./ha in each of three mid-season sprays, CBR was reduced by 45% (P=0.05) in the cultivated treatment and by 33% in florunner. With 0.56 kg a.i. in 4, 5, 6, or 7 applications, LR was reduced by at least 89% (P=0.05) without excessive plant growth regulator effects. Radial growth of isolates of S. rolfsii, R. solani, and C. cortaleaeae was reduced, respectively, by 100, 69, and 47% on acidified PGA amended with 5 ppm of diniconazole.

A137


Rice sprouts were treated with a mixture of benomyl fungicide and Dazyl detector dye. A-15-8 at 1.1 kg a.e./ha each. Sprays were made at the panicle 2.5 cm, heading, and heading growth stages and when the canopy was wet from dew or dry. Plants were allowed to dry and were evaluated in the laboratory under a UV light for dye distribution. The upper, middle, and lower third of the plant were rated for color. Sprays of 0-10 rating scale where 0 indicated no dye and 10 indicated heavy dye coverage. More fungicide/dye was deposited in the lower canopy at the panicle 2.5 cm stage than at the boot or heading stages. Less wetness reduced lower canopy deposition and caused the panicle 2.5 cm stage but increased deposition at heading. Water droplets running off of the plant were the probable cause of fungicide loss at the early growth stage. Less wetness apparently increased redistribution to the lower foliage in the more closed canopy at heading.

A138

SENSITIVITY OF GRAPE POWDER WILDFER DETERMINED BY ISOLATES FROM CALIFORNIA TOWARD TRENAMON, MYCLOBUTANIL AND TRIADIMIFENOM. D. G. Umett and W. D. Gubler. University of California, Davis 95616.

Using an excited leaf disc assay, the sensitivity of grape powdery mildew (Uncinula necator Schw. Burr.) isolates to the DM fungicides Trenamono, myclobutanil and triadimefon was determined. Mildew isolates from vineyards where little or no DM fungicides were used rarely had EC50 values towards Trenamono and myclobutanil exceeding 1 mg/L or 5 mg/L for triadimefon. In contrast, isolates from vineyards where there is a loss of efficacy towards triadimefon had occurred or where DM fungicides were frequently applied showed reduced sensitivity towards both triadimefon and myclobutanil, and to a lesser degree, towards fenpropimorph. The results indicate heavy mildew pressure where DM fungicides are frequently used, a shift in the powdery mildew population towards reduced sensitivity can occur and that there is a potential for cross-resistance among the different DM fungicides.

A139

ALGA BLIGHT OF POUTERIA SAPOTA CAUSED BY CEPHALSPORESIUM VIRESCENS. R. T. McMillan, Jr., University of Florida, IFAS, Tropical Research and Education Center, Homestead, FL 33031.

Alga blight caused by Cephalosporium virescens Kunze was observed on Pouteria sapota (Jack.) Moore & Stearn in south Florida in 1988. Lesions on twigs and branches appear circular and light brown in color. Lesions are covered with a thin gray-green velvety layer during most of the year. The leaves expand in the rainy season between April and May due to the presence of fruiting bodies. Affected bark becomes cracked and scaly and the infected branches are stunted with sparse foliage. Cephalosporium virescens is easily controlled by good cultural practices, water fertilization, pruning, and 2 sprays of copper at 1 pound per 100 gallons.

A140

DISEASE ENHANCEMENT AND TURFGRASS QUALITY AS INFLUENCED BY FUNGICIDES. P. H. Berndeno and M. C. McIntosh, Dept. of Agronomy, University of Maryland, College Park, MD 20742.

The main objectives of this field study were to identify turf quality and non-target disease enhancement effects of five fungicides applied to four Kentucky bluegrass, KB (Poa pratensis) and two perennial ryegrass, PRG (Lolium perenne) cultivars. Fungicides were applied monthly from Apr through Sept between 1986 and 1988. Fungicide quality of PRG by keeping low levels of Rhizoctonia solani in abundance and they did not enhance any disease in PRG. Quality of KBG was improved significantly. Most notably, triadimefon improved quality by controlling stripe smut (Fusarium lateritium) in 'Merion' and summer patch (Magnaporthe poae) in Merion and 'Sydsport'. Chlorothalonil and thiram were associated with a significant increase in smut in Merion, and chlorothalonil increased summer patch in Merion, Sydsport, and Vantage. Triadimefon tests produced improved leaf spot (Drechslera poae) in 'South Dakota' KBG. Pyridone did not exacerbate any disease, provided long residual leaf spot control, and elicited a color enhancement.

A141

REMISSION OF DERMATITIS SYMPTOMS IN SOUTHERN HARDWOOD AS TREATED WITH BENZIMIDAZOLE FUNGICIDES. P. P. Van Arsdale, Professional Tree Service, Inc., 2112 Cavitt, Bryan, TX 77801.

Benzoin, Topin-m, or thiabendazole were applied to 7 species of oaks, 4 elm, persimmon, sweetgum, 4 ashes, and 18 other native hardwood species in treatments of declining ornamental hardwood trees. Isolation tests have shown their xylems to be inhabited with Cephalosporium dioiscoi [=Phyllostomum obscurum]. Positive isolations were obtained from 33 of 106 species. Post treatment tests showed a 95% reduction in the number of cultures obtained from xylem chips of treated trees. Treated trees developed larger, greener leaves, longer inner bark, longer inner bark, and Vantage. Isolation tests produced dormant leaf spot (Drechslera poae) in 'South Dakota' KBG. Pyridone did not exacerbate any disease, provided long residual leaf spot control, and elicited a color enhancement.

A142

Control of Necrotic Ring Spot on Kentucky Bluegrass in Colorado. D. C. Volkz, W. Brown, Jr., and E. Milus.

Isolations of fungi associated with patch diseases in Colorado since 1987 have shown that Necrotic Ring Spot, Leptosporangia kikuchii, is the predominant patch disease present in Kentucky Bluegrass. Evaluations of selected fungicides and an organic top dressing were made in conjunction with an enhanced cultural disease control program on a naturally infected necrotic ringspot site of predominantly Kentucky Bluegrass turf. Each plot (3 x 5 m) except for the undisturbed control, received an enhanced cultural practice program which included weed control and core aerating. All fungicides followed aerating (Spotless) provided a significant reduction in number of rings and percentage of diseased turf. No other treatment significantly affected disease incidence or intensity.

A143

INFLUENCE OF FUNGICIDES ON POPULATION DYNAMICS OF PHYТОPHAGA PARASITICA CAUSING ROOT ROT AND WILT OF VINCA ROSEA. D. M. Ferrin and R. G. Rohde, Department of Plant Pathology, University of California, Riverside, CA 92521.

Soil populations of Phytophthora parasitica were monitored in container-grown Vinca rosea either not treated or treated with labeled rates and frequencies of metalaxyl (Subdue) or fosetyl aluminum (Alitide) in separate experiments. Metalaxyl was most effective in reducing populations to 0.2 oz./100 gal. every 4 wk. When applied at 0.5 or 2 oz./100 gal. every 4 or 8 wk, respectively, populations were generally less than for nontreated plants, but were not reduced significantly. Fresh root weights were not significantly different for treatments applied every 4 wk, but they were significantly greater than with no treatment or treatment every 8 wk. Fosetyl-Al was more effective in reducing populations when applied as a soil drench at 8 oz./100 gal. to a 6 inch band. Soil drenching nontreated populations equal to or greater than nontreated plants. Fresh root weights were not significantly different for any of the fosetyl-Al treatments.

A144

ASSESSMENT OF HUMAN HEALTH RISK FROM PESTICIDE RESIDUES. J. L. Tomerlin, TAS Inc., 1000 Potomac St. NW, Washington, DC 20007.

The Environmental Protection Agency (EPA) regulates the magnitude of pesticide residues remaining in or on crops.
As part of the regulatory decision making procedure, estimates of exposure are calculated as the product of mean food consumption estimates and estimates of the pesticide residues. The exposure estimate is then compared to a reference dose, a measure of toxicological significance determined from animal studies. In the absence of guidance estimates of residues in food as eaten, particularly for processed or cooked foods, EPA calculates worst case exposure estimates, thereby overestimating exposure and consequent human health risk. Such exposure assessments are part of EPA's normal registration, special review, and reregistration procedures. Pesticide residue data from processing studies, cooking studies, storage degradation studies, and commodity surveys in storage may yield exposure estimates which more closely approximate actual exposure.

A145
PROGRAM FOR ANALYZING ECONOMIC IMPACT OF PESTICIDE CANCELLATION. J.E. Bailey and H.D. Tilmon. North Carolina State University, Raleigh, NC 27695-7616 and University of Delaware, Newark, DE 19717-1303.

Public concerns over pesticide safety have increased the rate at which reevaluation and removal of pesticides have occurred. University agriculturalists are increasingly called upon to supply information on pesticides such as types and amounts used, alternative materials, and economic consequences resulting from the loss of these materials. A computer model was developed to analyze the economic impact of sequential deletion of all pesticides used for a particular crop/pest combination. The model was designed to utilize data furnished by agricultural experts who work with the identified pests in their respective fields. Issues addressed are: 1) likely deletion order of alternatives, 2) quality and yield changes resulting from the use of these compounds, and 3) the likely proportion in which the alternative chemicals (or cultural practices) would be substituted or not used at all. This program can be used to standardize the approach used by different individuals involved in a risk-benefit study, and exposes the logic used in the analysis for future criticism and improvement.

A146
EFFECTS OF WATER STRESS ON CHESTNUT BLIGHT CAUSED BY CRYPHYCNESTRIA PARASITICA. S.J. Guo and L. Shade. Dept. of Plant Pathology, University of Kentucky, Lexington, KY 40546-0091.

Mycelial growth and conidial germination of virulent (V) and cytoplasmic hypovirulent (CH) strains of C. parasitica were monitored on corn meal agar media osmotically adjusted with NaCl, KCl, sucrose and a salt mixture of NaCl/KCl/NaNO3/3K2. Inhibition of mycelial growth generally was not found on a sucrose or KCl induced osmotic potential above -2.0 MPa. The sucrose containing CH agent HVt were more sensitive to sodium than V strains or those strains containing CH agent H2. Conidia were less sensitive than mycelium to an osmotic potential of -0.6 MPa induced by sucrose or KCl. Conidia from CH strains with agents HVt and H2 were more sensitive to sodium than conidia from their isogenic V strains. The osmotic potential of American chestnut bark measured monthly during the year was between -0.8 and -2.1MPa. Excised stem segments of American chestnut were inoculated with mycelium and agar after water stress was induced by incubation in a water bath at 25°C in 10% polyethylene glycol with reduced relative humidity. Canker expansion was significantly greater on those stems with increased water stress. These results indicate that the impact of water stress is greater on the host than on the pathogen.

A147
INVESTIGATION OF GENETIC RELATEDNESS AMONG dsRNAs ASSOCIATED WITH CRYPHYCNESTRIA PARASITICA ISOLES FROM WEST VIRGINIA. S.A. Emelk^1, B.I. Hillman^2, W.L. MacDonald^2 and P.J. Bedker^1. ^1West Virginia University, Division of Plant & Soil Sciences, Morgantown, WV 26506 and ^2Rutgers University, Cook College, Department of Plant Pathology, New Brunswick, NJ 08903.

Worldwide, double-stranded (ds) RNAs responsible for hypovirulence in the chestnut blight fungus Cryphyenestria parasitica (syn. Endothia parasitica), are intercalated to varying degrees. To study the interrelationships of dsRNA found in West Virginia, recombinant cDNA libraries were constructed in the plasmid pUC8 using dsRNAs from two different isolates. The first isolate, designated D^2, has two bands of approximately 1.5 and 1.1 kb. The second isolate, designated C^2, contains 11 bands ranging in size from 1 to 2 kb. Recombinant pUC8 plasmids extracted from ampicillin resistant colonies that were white on X-gal plates contained inserts of up to 3 kb. Two plasmids from each library were S^32P-labeled and used to probe dsRNA preparations from these and other dsRNAs of European and North American origin. In each case, the recombinant plasmid used as a probe hybridized only to its own template dsRNA, indicating that D^2 and C^2 RNAs neither have close affinities to one another, or to the other dsRNAs tested.

A148
THE USE OF PROPICONAZOLE FOR CONTROL OF OAK WILT IN LIVE OAK. D. N. Appel. Department of Plant Pathology and Microbiology, Texas Agricultural Experiment Station, College Station, TX 77843.

The sterilizing fungicide propiconazole was evaluated in live oak for control of oak wilt caused by Ceratocystis fagacearum. Specimen growth of the fungus in vitro was inhibited by 0.1 g/mL A1 using a paper disc plate method. Wilt development was suppressed 100% in 2 to 3-year-old containerized live oak inoculated with 10-50 ml of 250 mg/mL A1 followed by inoculation with pathogen conidia. Since June, 1987, mature live oaks at high risk to natural infection distributed throughout Central Texas were injected with propiconazole. In the first year injected, eight of ten treated trees, 0% to 58% crown loss with typical oak wilt symptoms for over 24 months. However, eight trees treated preventatively at rates of 100-500 μg/ml and 1 L/in d/bhr progressed from 0% to 3% crown loss; no symptom of oak wilt developed. Similar results were observed in more recent plots, involving a total of 99 trees. More modest levels of disease suppression were observed in trees injected therapeutically.

A149
PLANTING SINGLE OR MULTILINE FAMILIES OF LOLLIBOLLY OR SLASH PINES ON SITES WITH A HISTORY OF DUSIFORM RUST. C. H. Wallshuw. USDA, Forest Service, Southern Forest Experiment Station, Rt. 1 Box 124-A, Asheve, NC 28806.

A number of commercial forest managers in the South have chosen to plant single families of lollibolly (Pinus taeda L.) or slash (P. elliottii Engelm. var. elliottii) pine in blocks of 20 to 50 acres. These families generally have superior growth and better than average resistance to fusiform rust. But planting single families might encourage pathogen changes on sites with abundant oaks and climate favorable to the fungus. However, most resistant pine families appear to cope with alterations in the pathogen population. These families maintain their resistance on different field sites and when challenged with a variety of fungal isolates in the greenhouse. Planting multiline (many families) appears best when single families show significant genotype × environment or genotype × fungus isolate interactions.

A150
AGROBACTERIUM MEDIATED TRANSFORMATION OF POPULUS X EURAMERICANA "OGY" USING THE PROTEINASE INHIBITOR II GENE (PIN II) TO INCREASE PEST RESISTANCE. S. A. Heuchel, H. S. McNabb, Jr., N. B. Klopfenstein, and R. W. Thornburg. Dept. of Plant Pathology and Forestry, Iowa State University, Ames, IA 50011.

Attempts to increase pest resistance of Populus x euramericana "Ogy" by transformation with the Proteinase Inhibitor II (PIN II) gene (pin II) are in progress. PIN II is expressed in sin and chymotrypsin. Transforms were obtained using an Agrobacterium binary vector system with a disarmed Ti plasmid and plasmid pH104 containing pin II regulated by a J5S promoter, and a selectable gene encoding neomycin phosphotransferase II (NPT II). Putative transformed shoots from co-cultured leaves were selected using 3 kanamycin selections. To date, 10 plantlets from 1-ml mix show putative PIN II expression. An additional 13 plants are in the third regeneration. DNA and immunoassays (Southern and Western blots, and ELISA) to test pin II integration and expression are in progress. Preliminary ELISA results suggest pin II expression is at least one transformant. Bioassays for pest resistance will be conducted after verification of pin II.

A151

Effects of pathogenic Armillaria spp. or Phellinus weirii on stand growth and development are represented by the Western Root Disease Model. This model can be used to predict effects of root disease and associated bark beetle activity on the forest over the next several decades and evaluate effects of a wide assortment of silvicultural practices, including stump removal for disease control. The model operates through a keyword system that allows users to choose among various trees and conditions and conduct a local analysis. A critical assumption in the model is that a tree can only become an inoculum source if its root system was at least partially colonized by pathogenic Armillaria spp. or P. weirii before to disease is detected. The model includes a series of workshops designed to collect information about the disease process from many experts on the biology of the root diseases and their hosts and from forest land managers knowledgeable about the resource values being affected. The model is being adapted to simulate effects of annosus root disease.

A152
EPIDEMIOLOGY AND CONTROL OF CYCLANUS MINUS NEEDLECAST OF SCOTCH PINE IN MICHIGAN. G.C. Adams. Dept. of Botany and Plant Pathology, Michigan State University, East Lansing, MI 48824.
Cumulative infection of Scotch pine by *G. minus* following three years of fungicide treatment on ten different application schedules was compared with the number of high-quality trees harvested per plot. Infection was reduced significantly in all three complements of needles when 4 or 5 annual applications of a fungicide formulation of chlorothalonil were used at 8-week intervals. However, disease control did not increase the quantity of high-quality trees at harvest nor decrease the amount of needles cast in fall. A dispersable granular formulation of chlorothalonil was not effective. As expected, rainfall discharge varied yearly. Discharge was correlated with occurrence of daily precipitation, but not with quantity of daily rainfall, nor with cumulative monthly rainfall. No significant differences in infection were evident among 70 seed sources of *Pinus sylvestris* when current-year needles were evaluated, but differences appeared in infection incidence of one- and two-year-old needles.

**A153**

**SUDDEN DEATH OF EUCALYPTUS GLOBULUS.** A. H. McCain and L. R. Costello. Department of Plant Pathology, University of California, Berkeley, CA 94720.

Sudden death is descriptive of a disease of *Eucalyptus globulus* that occurs in the greater San Francisco Bay area of California. The disease is "spotty" in that not every tree in a group is affected. Large trees (dbh 0.5-2 m) that appear normal in the spring are dead by the end of summer. The bark appears normal from the outside. The xylem just beneath the bark of affected trees is dead in cross-section. No necrotic tissue extends into the phloem. In trunk cross sections, the discoloration is barely visible in the xylem and does not extend inward into older tissue. An unidentified fungus is readily isolated in pure culture from the discolorated tissue. There is no recovery as occurs with some canker diseases. As far as can be determined from the literature, this is a new disease of *E. globulus*.

**A154**

**DOGWOOD ANTHRACNOSIS FUNGUS, DISCULA SP., ISOLATED FROM NECROTIC AND SYMPTOMATIC DOGWOOD FRUITS AND SEEDS.** Jerry O. Britton, U.S.D.A. Forest Service, SEFES, Green St., Athens, GA 30602.

Dogwood fruits were collected in Sept. from trees with symptoms of anthracnose in 11 locations in SW and NW North Carolina, and were sub-divided into four symptom types. The fleshy pulp was removed from 25 seeds in each group. All fruits and seeds were surface-sterilized in 10% bleach:10% ethanol, placed on acidified potato dextrose agar, and incubated 2 wks. Discula sp. was isolated from 2% of symptomless fruit, 2% of shriveled red fruit, 8% of the fruit with necrotic lesions, and 12% of the entire fruit set. Necrotic tissue was obtained from infected fruits. The isolations from seed samples in the first three symptom categories. However, Discula sp. was isolated from 49% of the seeds in the entire necrotic category, compared with only 12% of similar, uninfected fruit. Thus, isolations from whole fruits may underestimate the incidence of Discula infection, and infected seeds may provide a dispensal mechanism for dogwood anthracnose.

**A155**

**NEW DISEASES OF FOREST TREES IN ISRAEL.** R. J. Buck, Z. Solod, and I. S. Ben-Ze’ev, Department of Plant Pathology, North Carolina State University, Box 7616, Raleigh, NC 27695 and ARO - Volcani Center, P.O. Box 6, Bet Dagan, Israel.

Thirty-seven new fungal diseases were found on 9 different tree species in Israel including Aleppo Pine, Italian Cypress, 3 species of Juniper, 2 species of oak, Carob and Cedar of Lebanon. All pathogen isolates were isolated from symptomatic tissue, pure cultures were established, and trees growing under greenhouse conditions inoculated. The tenets of Koch’s postulates were accomplished on all suspected pathogens. A partial list of new diseases include: Cupressus sempervirens: Diplodia pinea Isp. cupressi, Serioidium cardinale, Botryodiplodia theobromae, Fusarium oxysporum, Pinus halepensis: Alternaria alternata, Diplodia pinea, Fusarium oxysporum, Botryodiplodia theobromae, Phomopsis sp., Phomopsis juniperina, Fusarium solani, Pestalotiopsis juniperina, Sclerotinia juniperina, Quercus ilex, & Quercus calliprinos: Chalara sp., Cladosporium sp., and Alternaria alternata.

**A156**

**STEM CANKER OF PLANTATION BLACK WALNUT IN FIVE CENTRAL STATES.** J. F. Cummings-Carlson, M. E. Misle, and J. G. O’Brien, Wisconsin Department of Natural Resources, 3911 Fish Hatchery Road, Madison, WI 53711, and USDA Forest Service, State and Private Forestry, 1992 Foulkwell Ave., St. Paul, MN, 55108.

A survey was conducted in 1989 to determine the incidence of stem canker of plantation grown black walnut, its association with *T. sitophila*, and its relationship to site and silvicultural practices in Illinois, Iowa, Minnesota, Missouri, and Wisconsin. Diseased tissue was cultured to determine the *Fusarium* spp., associated with canker sites. Site characteristics and silvicultural practices were noted. Fifty percent of cankers were present in 8% of the 183 plantings surveyed. Regionwide, 10% of trees surveyed had walnut canker. Within plantations, disease incidence ranged from 0% to 74%. *Fusarium solani* was present in 15% of the 183 plantings. Regionwide, 6.8% of the trees surveyed on upland sites had canker versus 13.3% on bottomland sites. Thirty percent of the cankers were associated with a wound and pruning wounds accounted for 48% of the wound associated cankers.

**A157**

**RESISTANCE OF MONTEREY PINE (PINUS Radiata) TO PITCH CANKER CAUSED BY FUSARIUM SUBGUTTULATUM.** M. E. Schultz, T. R. Gordon, and A. H. McCain. Department of Plant Pathology, Berkeley, CA 94720.

Five populations and 4 interpolation crosses of Monterey pine were tested for susceptibility to pitch canker. Each population (and population cross) was represented by a minimum of 11 different clones. Each clone was represented by 25 conidia of *F. subguttulatum* placed originally separated in 1981. One set of ramets was potted and maintained in juvenile condition; the second set was allowed to mature by outplanting. Plants were inoculated with a spore suspension containing 50% conidia. Lesions from crosses between resistant and susceptible populations were intermediate in susceptibility. More lesions formed on potted than on outplanted ramets. These data suggest that the populations of both planted and native Monterey pine vary considerably in their susceptibility to pitch canker.

**A158**

**RELATIONSHIP BETWEEN IN VITRO ASCOSPORE AND TOXIC METABOLITE BIOASSAYS OF POPULUS TREMULOIDES TISSUE CULTURE PLANTLETS.** B. M. Kruiger and P. D. Manton, SUNY College of Environmental Science and Forestry. 13210.

Two in vitro bioassays, utilizing ascospores and toxic metabolites, have been used to screen *P. tremuloides* for resistance to *Hypoxylon magnum*. Twelve clones in tissue culture were used to test the hypothesis that sensitivity to toxic metabolites is correlated with resistance to ascospore inoculation. Ascospores germinated equally well on plantlets from all clones but subsequent hyphal growth and infection was influenced by the presence of resistant clones. The same clones were tested with toxic metabolites from three *H. magnum* isolates. Significant differences were detected in clonal response to both bioassays however, sensitivity to toxic metabolites was not correlated with sensitivity to the other. This suggests that susceptibility of *P. tremuloides* to ascospore infection is not dependent on sensitivity to pathogen toxins.

**A159**

**PURIFICATION OF IRON-CHELATING COMPOUNDS FROM GLEOPHYLLUM TRAEBUM.** J. Jellison, V. Chandoke and R. Bushway. 202 Deering Hall, University of Maine, Orono, ME 04469.

Iron plays an important role in the metabolic functions of fungi that cause wood deterioration. *Gleophyllum trabeum* is able to produce low molecular weight iron-chelators. Production of these chelators (siderophores) can be induced by iron starvation. Purification of biological chelators from fungal cultures or from wood infected by *G. trabeum* has been achieved by freeze drying, ultrafiltration, and HPLC analysis. Developing a better understanding of the action of siderophores, their role in scavenging metals for fungal metabolism, and the possible function of siderophores in lignocellulose degradation, will help us to better understand how wood deterioration occurs.
Freshly milled pine bark amended with ammonia nitrogen (0.6 kg/m³) was composted 10–12 wk in windrows at a moisture content of 60–73% (wet weight) using process temperatures <68 C. Plug mixes (particles <0.65 cm diam) and potting mixes (particles <1.5 cm diam) were prepared by blending compost with various amounts of sphagnum peat, perlite, vermiculite and mineral additives. Highest levels of natural suppression to Pythium damping-off and root rots were induced and plant dry weight provided was highest in media amended with 15% (v/v) compost if media contained 40–50% moisture (w/w) and were incubated >4 days before planting. Benomyl did not reduce suppressiveness in media containing >40% moisture (w/w). The suppressive effect lasted through 4 mo in pot crops such as poinsettia and begonia. Batches of media naturally suppressive to Pythium over a 2-yr period varied in suppressiveness to Rhizoctonia.


The parasitic fitness of sensitive isolates of Botrytis cinerea (BC), E. elliptica (EB) and B. tulipae (BT) was compared to single and multiple fungicide resistant ones. Lesion development on tulip (BC & BT) and lily (BE), and sporulation for BC and BT were assessed by inoculation on detached leaves. For BC, lesion size averaged 1.5 cm² after 7 days, and there were no differences (P>0.05) between fungicide groupings. Sporulation of BC isolates resistant to both benimidazole and dicarboximide (15%) of the 15% isolates were half that of sensitive isolates. For sensitive isolates of BT, lesion size (2.5 cm²) and sporulation (180 spores/cm²) were greater (P<0.05) than benimidazole resistant isolates (0.5 cm² and 800 spores/cm²). For BE, lesion size was greater (P<0.05) for sensitive isolates (9.4 mm), than benimidazole (8.5 mm), dicarboximide (6.0 mm), or multiple resistant ones (6.6 mm).

A165 A NEW AZALEA FLOWER BLIGHT CAUSED BY A PYCNIDIAL COELOMYCETE. G. R. Holcombe, Dept. of Plant Pathology & Crop Physiology, Louisiana Agricultural Experiment Station, Louisiana State Univ. Agricultural Center, Baton Rouge 70803.

A new flower blight disease of azaleas was observed on the fall-flowering varieties Pink Camellia and Fashion at the LSU Burden and Hammond Research Stations in October 1989. Symptoms of the new disease were similar to those caused by Oomycetes, and canflower discoloration and death, except that infected tissue was firm instead of mushy and pycnidia formed on blighted flowers. A fungus belonging to the pycnidium-forming Coelomycetes was consistently isolated from infected flowers. Pathogenicity tests were positive and the same fungus was later reisolated from inoculated flowers. The fungus appears to be a species of Macrophoma and efforts are continuing toward a positive identification. Fungicidal tests on detached flowers indicate that Bayleton, Dithane N-45 and Chiptco 26019 show promise as effective controls.

A166 THE SUSCEPTIBILITY OF FINE FESSES TO ISOLATES OF MAGNAPORTHE POGAE AND GAUMANNOMYCETES INCURSAN. M. L. Kemp, B. B. Clarke, and C. R. Funk. Rutgers University, New Brunswick, NJ 08903.

M. paga and G. incrustans are root-infecting fungi recently associated with patch diseases of some turfgrasses. The susceptibility of fine fescue to isolates of these pathogens is under investigation. M. paga and G. incrustans were isolated from hard fescue (E. lanata) turf. Summer symptoms were observed near the edge of New Jersey. A fine fescue field trial inoculated with two isolates of M. paga in June 1989 developed summer symptoms seven weeks later. Strong creeping red fescues (E. rubra rubra) showed significantly better resistance than isolates than hard fescues, slender creeping red fescues (E. rubra littoralis), and Chewings fescues (E. rubra). Preliminary pathogenicity studies indicated that G. incurans may also be pathogenic to species of fine fescue. Results of laboratory and field inoculations of the 1989 Natural Fine fescue test with isolates of M. paga and G. incrustans will be reported.

A167 PATHOGENICITY STUDIES OF FUNGI ASSOCIATED WITH BERMUDAGRASS DECLINE. M. D. Elliott, University of Florida - IFAS, Fort Lauderdale Research and Ed. Center, Fort Lauderdale, FL 33314.

Pathogenicity studies were conducted in a plant growth chamber with Five Gaumannymycetes graminis var. graminis (Eg) isolates, six G. incrustans (GI) isolates and seven Phaeophila sp. (EH) isolates. 'Tifgreen' bermudagrass and 'Florida' ryegrass were used as hosts. The growth medium was either homogenous grade vermiculite or a top soil mix of 80% sand/20% top soil. After 30 days of incubation, the Eg isolates were pathogenic on ryegrass in both media; none demonstrated pathogenicity on bermudagrass. Three Eg isolates were pathogenic on ryegrass and bermudagrass in both growth media. However, there were fewer symptomatic roots found on bermudagrass than ryegrass. None of the Ph isolates appeared to be pathogenic to either grass. All of the Eg and GI isolates were recovered from infected plant tissue to fulfill Koch's postulates. Most of the Ph isolates were also recovered from root tissue indicating they were capable of colonizing the roots.


Growth and (asexual) reproduction rates of ten Discula isolates were determined in constant light (cLT) and constant dark (cDK). A complete defined medium (CM) was used in all studies. Growth increases (determined by colony dry weight) as great as 37% (range = 0-37%) in cultures grown in cLT as compared to cDK were observed. Isolates differ in their response to light with respect to growth rates, and 3 have been identified for which light does not appear to be a strong regulator of growth in vitro. Growth enhancement in cLT is observed in both solid and liquid CM. Cultures grown in cLT (1.8% agar CM) sporulate profusely. To date, sporulation has not been observed following the incubation of cultures in cDK.
A168

Fifteen species of Pyrithum were identified from 65 isolates from bentgrass, bermsod grass, centipedegrass, and tall fescue with root rot and blight during 1989 in North Carolina. Homothallic species included P. vanterpoolii (13 isolates), P. graminicola (10), P. comosum (6), P. stenotaphri (4), P. myriotyltum (3), P. aphanofermentum (2), P. volutum (1), and isolate each of P. aristotilorum, P. oilgandrum, P. paroecandrum, and P. tardicrescens. Heterothallic isolates included P. ceterispermum (6), P. intermedia (5) and P. carolinisn. Of the 7 unidentified species, 5 were homothallic and 2 were heterothallic. P. intermedia was isolated twice in association with P. volutum and once with P. rostratum. Because differences in sensitivity to fungicides have been observed among these Pyrithum species in related research, a pictorial key and procedures for rapid identification was developed. Determination of pathogenicity and fungicide sensitivity of these species is in progress.

A169
INFLUENCE OF RELATIVE HUMIDITY ON STEM BLIGHT OF GERANIUM. M. K. Hausbeck and S. P. Pennypacker, Dept. of Plant Pathology, The Penn. State University, University Park, PA 16802.

All stems of geranium (Pelargonium x hortorum) stock plants inoculated with Botrytis cinerea and incubated in a dew chamber within 12 hr of excising cuttings (stem wounding) became blighted. Disease incidence decreased to 38 and 28% when plants were inoculated 24 hr and 3 days, respectively, following stem wounding and placement in an environment of >60% relative humidity (RH) prior to incubation in a dew chamber. The area under the incidence curve for the disease, and stem blight disease progression was least when cuttings were inoculated in an environment of >60% RH for 24 hr following inoculation, prior to incubation in a dew chamber. When plants were inoculated and subjected to <60% RH for 3 days prior to inoculation in a dew chamber, an average of 69, 85, and 50% of the stems, respectively, became blighted. According to AUDPC data, a minimum of 24 hr in an environment of >60% RH following inoculation significantly reduced stem blight incidence.

A170
RESIDUAL ACTIVITY OF FUNGICIDES APPLIED TO GERANIUMS IN THE GREENHOUSE. G. W. Moorman and R. J. Lease, The Pennsylvania State University, Department of Plant Pathology, 211 Buckhorn Laboratory, University Park, PA 16802.

To use mixtures of fungicides for the management of fungicide resistant pear borer populations, the chemicals must have residual activity similar to the chemical which is "at risk" to the development of resistance and maintain effective control of the target organism. Fungicides were applied singly at the rate for seed geraniums (Pelargonium x hortorum) at 100 ml per 100 stems in a greenhouse. The potted plants were placed in a dew chamber for 3 weeks; 1 cm diameter disks were excised from leaves. The disks were inoculated with Botrytis cinerea spores suspended in 0.1M dextrose and were then inoculated for 10 da at 20°C in 16 hr light/8 hr dark. The number of diseased disks was recorded. Control, as compared to diseased on inoculated fungicide-free tissue, was calculated. Control provided by the fungicides was as follows: vinclozolin (the "at risk" chemical) provided 100% control initially, and 60% by wk 3; chlorothalonil, 83% initially, 75% at wk 3; maneb, 53% to 40% for all 3 wks; cupric hydroxide, 38% initially, 15% at wk 3; zineb, 43% initially, 15% by wk 3; and dichloran, 10% initially, 3% by wk 3.

A171
IDENTIFICATION OF LEPTOSPIERA KORRAE WITH CLONED DNA PROBES. N. Tisserat, S. Hlubet, and A. Nus. Department of Plant Pathology, Kansas State University, Manhattan, KS 66506.

Differential of Leptospira korrae from other enteric fungi associated with path-type diseases of turfgrasses can be difficult because of similarities in colony morphology and the inability to induce ascocarp formation in some isolates. A more reliable means of identification was developed using two different cloned DNA fragments from L. korrae. The probes, 0.8 and 1.2 Kbp in size, were specific to L. korrae, and did not hybridize to genomic DNA of other path-disease causing fungi, including L. parvum, Gaeumannomyces rufus, L. graminis var. graminis, and Uromyces phaseoli. Phylogenetic analysis of the DNA probe hybridization patterns from EcoRI digests of genomic L. korrae DNA implied that they belonged to the same repetitive element family. No polymorphisms were detected between L. korrae isolates with either probe.

A172
SERIOLOGICAL AND ULTRASTRUCTURAL ANALYSIS OF A ROD-SHAPED VIRUS IN A ST. AUGUSTINEGRASS LINE IMPORTED INTO THE UNITED STATES FROM URBANIA. C. Edward McClellan, R. W. Toler, and D. R. Huff. Department of Plant Pathology and Microbiology, Texas A&M University, College Station, TX 77843, *B-Four Corporation Research Center, Box 321, West Columbia, TX 79486.

In 1963, Stenotaphrum secundatum (Wall.) P. 291594 was introduced into the United States from Zimbabwe and has been maintained in Georgia and more recently in Texas. P. 291594 was found to be infected with a rod-shaped virus with a length of 712 nm and a width of 12 nm. The maximum length found was 781 nm, and 70% of the particles were between 678 and 753 nm. Immunoblot electron microscopy (IEM) using sugar cane mosaic virus (STMV) antisera from the American Type Culture Center showed trace reactions to infected P. 291594 and strong cross-reactivity to maize dwarf mosaic virus (BMV) infected sorghum. IEM using STMV and BMV antisera gave negative reactions with infected P. 291594. This section of P. 291594 contained morphous viral inclusions in epidermal cells.

A173

Rosa dillecta cultivars were bud grafted-inoculated with leaf pieces infected with apple mosaic virus (APMV). After two years each plot of twelve plants contained between one and six ELISA-tested positive plants with foliar symptoms. Flowers from symptomatic and nonsymptomatic plants were cut and graded for bloom height, bloom width, number, height and weight during a 25 week period. Compared to nonsymptomatic ELISA-tested negative plants in the same plots, symptomatic plants had a mean reduction overall of 32% in rose cuts: Bridal Pink 25%, Gold Rush 43%, Lavande 49%, Royalty 31%, Samantha 7%; Sonia 38%. The low percentage of cuts in Samantha suggests a variadic difference in tolerance to APMV. Although no dramatic decrease in height or weight occurred, the severe reduction in flower cut due to virus infection indicates the need for APmV-clam commercial operations.

A174

Chromosomes of the aflatoxin producing fungi Aspergillus flavus and A. parasiticus versicolor grouped in complex homogeneous electrically charged (CHEP) gel electrophoresis. Linkage group studies have indicated A. flavus has eight chromosomes (Papa, Can. J. Microbiol. 50:68-73, 1984). CHEP electrophoresis showed that all eight chromosomes are grouped in two size ranges: one group of 4 chromosomes of approximately 3-5 megabases and one group of 1-2 chromosomes larger than the S. pombe 7.0 megabase chromosome. Although similar, the chromosome of A. parasiticus and A. versicolor are not identical in size. Karyotype profiles of A. flavus isolates suggest chromosome transfer mechanisms have occurred in some strains. Chromosome separation conditions and chromosome profile comparisons among and between related Aspergillus spp. are discussed.

A175

Uredospores of the bean rust fungus, Uromyces appendiculatus, differentiate to form infection structures in response to the topography of stomatal guard cells or to 0.5 μm ridges on a plastic surface. Twenty differentiation-specific clones have been isolated by cascade-hybridization. They were divided into six classes based on cross hybridization. One of the clones, 13k, 1.2 kbp in size, encoded for a 230 amino acid product including a predominate one of 1.0kb. The gene specifying the 1.0kb transcript (INF56) was subcloned on a 2.5kb fragment, sequenced, and the open-reading frame was determined. It contains a 63bp intron and encodes for a 230 amino acid polypeptide. The amino acid sequence is unique and cross-hybridize, but hybridization patterns from EcoRI digests of genomic L. korrae DNA implied that they belonged to the same repetitive element family. No polymorphisms were detected between L. korrae isolates with either probe.
A176
CLONING AND PRELIMINARY CHARACTERIZATION OF AN ENDO-
POLYGALACTURONASE GENE FROM COCHLIOBOLUS CARBONUM. J. S.
Scott-Craig and J. D. Walton, MSU-DOE Plant Research
Laboratory, Michigan State University, East Lansing,
Michigan, 48824.

Purified endo-polygalacturonase (PG) from Cochliobolus carbonum
was digested with trypsin and both intact enzyme and purified
fragments were sequenced by Edman degradation. Degenerate
oligonucleotide primers were synthesized based on the aligned
sequence data and were used to prime DNA synthesis from genomic
DNA using the polymerase chain reaction. An 800bp PCR product
was used as a probe to identify genomic and cDNA clones of the
PG gene. Northern analysis indicates that the gene encodes a 1.3
kilobase mRNA which is present at high levels when the
fungus is grown on pectin and at very low levels when the fungus
is grown on sucrose. The DNA sequence and intron structure of
the gene have been determined and deletion derivatives have been
constructed and re-introduced into C. carbonum by transformation.

A177
CHROMOSOME-LENGTH POLYMORPHISMS AMONG FOURTEEN RACES OF USTILAGO
HORDEI: CHARACTERIZATION AND MEIOTIC SEgregation. K. McCluskey
and D. Mills, Department of Botany and Plant Pathology, Oregon
State University, Corvallis, Oregon, 97331-2902.

Chromosome-length polymorphisms were identified among seventeen
races of Ustilago hordei, the causal agent of covered smut of
barley. Fourteen of the strains represent different races; the
three additional strains complete a meiotic tetrad of one race. A
chromosome-length polymorphism segregated 2:2 among the
members of this tetrad. Several other tetrads have been
examined, and all have conserved karyotypes. Among the strains
representing the fourteen races, chromosomes varied in size from
170 to 3,500 kilobases, and in number from 16 to 21. Homologous
chromosomes which vary in size by up to one hundred kb among
different strains were identified by Southern
hybridization. Random homologous DNA probes have allowed
distinction between two chromosomes that are nearly identical in size.
Conserved genes from other fungi are being used to
identify linkage groups. Novel techniques for chromosome sample
preparation without the need for protoplast formation have been
developed.

A178
TRANSFORMATION OF AN ECTOMYCORRZHAZI FUNGUS. V. Barrett, J. Shaw,
P.A. Lemke, Department of Botany and Microbiology, Auburn
University, Auburn, Alabama 36849.

Transformation of the ectomycorrhizal basidiozyme, Laccaria
laccata, has been based on selection for resistance to hygro-
mycin B using a plasmid with the Aspergillus nidulans ugp
promoter and the Escherichia coli hpt structural gene (Barrett
et al. 1990 Appl. Environ. Microbiol. 56:1844). The obser-
vation that promoters and structural genes function in heterol-
gous fungi implies considerable similarity for mechanisms of
gen expression. We have begun experiments to assess promoter
efficiency in L. laccata. Using protoplasts, we have transfor-
mation protocol for L. laccata with sequences coding for several heterologous
promoters and are currently exploring the use of these promoters
in conjuction with the reporter genes B-galactosidase and luciferase.
Quantification of gene expression from known pro-
moters will provide the basis for cloning homologous L. laccata
sequences with promoter activity. Such sequences will allow
efficient expression of introduceed genes in the transformed fungus.

A179
CHARACTERIZATION OF CIRCULAR PLASMIDS FOUND IN THREE SPECIES OF PYTHIUM. Frank N. Martin, Plant Pathology Department,
University of Florida, Gainesville, Fl 32611.

Circular plasmids have been identified in two isolates of P. aphanidermatum
and one isolate each of P. tatorum and an unidentified echinulate isolate.
Plasmids were multimeric, with unit lengths ranging from 3.36 to 4.94 kb
and were unique for each isolate based on restriction maps of cloned
plasmids and Southern hybridization analysis. Hybridization studies
demonstrated no sequence similarity between the plasmids and the nuclear
or mitochondrial genome of the isolates from which they were recovered. For
the two isolates, Southern transfers of total DNA and
hybridization with cloned plasmids provided plasmid DNA fragments which
did not correspond to the sizes expected from the restriction map or from
partial digests, indicating that rearrangements of the plasmid had occurred.
Investigations to determine if the plasmids are of mitochondrial origin are
in progress.

A180
DETECTION OF SEQUENCE HOMOLOGY BETWEEN DOUBLE-STRANDED RNA SPECIES ISOLATED FROM PHYTOPHORA INFESTANS. J. R. Newhouse,
F. W. Tooley, and O. P. Smith. USDA-ARS, Frederick, MD 21701.

Northern-blot hybridization analysis was employed to evaluate
the sequence homology between species of double-stranded (ds)
RNA from Mexican, Dutch, and potato isolates of Phytophthora
infestans. Double-stranded RNA species extracted from two
Mexican isolates (without bands in common) were prepared for use as two separate 32P-labeled DNA probes. Hybrid-
ization was observed only between conglutinate dsRNA species of the
isolates from Mexico, the Netherlands, and Peru. No
homology was detected between dsRNA species of the two Mexican
isolates, and neither probe showed homology with a high
molecular weight dsRNA band from the Dutch isolate. These
results indicate that at least three non-homologous groups of
dsRNA exist in P. infestans, and support the hypothesis that
at least one type isolate of the fungus recently found in
Europe may have originated in Mexico.

A181
RFLP BASED PHYLOGENY OF FUNGALIS OXYSPORUM-F. SP. LUCIDICERCUS (FOL) REVEALS NO ASSOCIATION BETWEEN RACE AND GENEALLY ISOLATED POPULATIONS. K. S. Elias, T. Katan, and D. Zmir, Volcani Ctr., Bet Dagan, Hebrew Univ Jerusalem-
Fed. Agric., and Otto Warburg Ctr., Rehovot, ISRAEL.

RFLPs were employed to estimate genetic diversity in FOL. We utilized 110 isolates of
FOL previously characterized for form species, race, geographic origin, vegetative
compatibility group (VCG), and isozyme electrophoretic phenotype. Fifty DNA clones
from a random genomic library from one isolate of FOL, were used as probes for
Southern hybridization to total genomic DNA cut with 4 restriction enzymes.
Polymerophisms were recorded, coefficients of similarity were calculated, and cluster
analyses was performed. Few RFLPs were observed among isolates within a VCG
whereas the majority of RFLPs occurred among isolates between VCGs. This suggests
that VCGs are genetically isolated populations with differing ancestral progenitors.
In addition, races could not be distinguished by RFLPs. It appears races have arisen
several times, i.e. at least once within each VCG of FOL.

A182

Length polymorphisms in restriction fragments containing moderately
repetitive DNA sequences have been identified in the fungus Fusarium
oxysporum. Arbitrarily chosen genomic DNA clones pEY1, pEY7 and pEY10,
containing, respectively, 1.1, 2.3 and 1.2 kb of fungal DNA, were used to
identify the repetitive sequences. When used as a probe for hybridization to
restriction endonuclease digested DNA from various strains, distinctive
banding patterns were observed for each strain of the four formae
specialies of F. oxysporum studied. These probes have utility for phylogenetic
analysis and for DNA fingerprinting individual strains. Identification of
generic markers specific for strains will aid in epidemiological studies of the
fungus.

A183
ANALYSIS OF THE dsRNA ASSOCIATED WITH NB88-58, A HYPERVERULENT STRAIN OF THE CHESTNUT BLIGHT FUNGUS FROM NEW JERSEY. B.L. Hillman and Y. Tian, Dept. of Plant
Pathology, Rutgers University, New Brunswick, NJ 08903.

NB88-58 is a hypervirulent strain of the chestnut blight fungus characterized by a single dsRNA species of
approximately 13 kb. We have made cDNA libraries
representing the genome of this dsRNA beginning with oligo d(T) and random primers. 95 cDNA clones were
mapped relative to one another by Southern blotting, and 45 of these were mapped in greater detail by restriction
and nucleotide sequence analysis such that a contiguous map of overlapping cDNA clones was
obtained. The sequences of the 5’ terminal 3 kb and 3’ terminal 2 kb (relative to the plus strand), and a
several internal regions were determined and compared to that of the French-derived strain EF1173. The overall
similarity between these two strains was approximately 50%, with some regions of notable conservation, and the
two appear to have similar genetic organizations.

The hypovirulent strain EP747 of the chestnut blight fungus, derived from dsRNA of Italian strain EP420, is characterized by two dsRNA species, the larger of which is approximately 3.3 kb in length. One dsRNA molecule is present in high abundance, representing the genome of this dsRNA. We have used those cDNA clones to examine relationships between EP427 and other chestnut blight-associated dsRNAs, and between this dsRNA and the genomic DNAs of several Cylindrocladium parasiticum strains. The dsRNA of EP747 was more closely related to that of EP113 than to any other dsRNA tested, as determined by slot hybridization analysis. The cDNA clones tested showed no significant sequence similarity with any C. parasiticum genomic DNA, including that of Platypus fragaria derived from EP747 and their ascospore progeny, as determined by Southern blots capable of detecting single copy genes.

A185

INCREASE IN IMPORTANCE OF CUCUMBER MOSAIC VIRUS INFECTION IN GREENHOUSE-GROWN BANANAS IN MOROCCO. M. Bouhadou and E. J. Lockhart, Complexo Hortícola, B.P. 438, Agadir, Morocco/Department of Plant Pathology, University of Minnesota, St. Paul, MN 55108.

Until recently, cucumber mosaic virus (CMV) infection was of trivial importance in Morocco and other areas throughout the world, where there has been rapid expansion of the crop under plastic greenhouses. The CMV strain occurring in banana produced mild mosaic symptoms and had little effect on plant growth. Within the last 2 years, outbreaks of CMV in banana have occurred, involving a strain of CMV not previously recorded in Morocco. Symptoms include severe yellow mosaic and systemic necrosis similar to hepatitis. The disease has been widely distributed and is now present in more than 100% of some greenhouses. The severe CMV strain is readily aphid-transmitted to and from banana, and has spread to weeds and vegetable crops. Necrosis of melon and watermelon has been observed. Control measures for CMV in infected banana plantings are being developed. The disease is speculated that the severe strain of CMV was introduced recently into Morocco in infected banana plantings. A187

GENETIC MULTIPLE VIRUS RESISTANT BELL PEPPERS. B. Villalobos, Texas Agricultural Experiment Station, 2415 E. Hwy 83, Weslaco, Texas 78596.

Bell peppers, one of 20 different domesticated Capsicum annuum L. types, has for many years been the most important fresh market and home garden pepper. Increased demand for this high Vitamin C, low calorie vegetable salad item has stimulated production in Texas and throughout the world. The most recognized commercial bell pepper cultivars are susceptible to virus diseases. The Texas Agricultural Experiment Station at Weslaco has released two new bell pepper varieties and has also developed hundreds of advanced inbred lines with resistance to tobacco mosaic virus, pepper mottle virus, potato virus Y, tobacco mosaic virus, tobacco ringspot virus, and cucumber mosaic virus. Improved inbred lines of large, four lobed bell type peppers are being proposed for release to the seed industry for development of hybrid or open-pollinated varieties.

A188

SELECTIVE AND DIFFERENTIAL ISOLATION OF PHYTOPHORA SPP. FROM THEBROMA CACAO L. AND CITRUS SPP. M. L. Oliveira and J. A. Menge, Department of Plant Pathology, University of California, Riverside, CA 92521.

Phytophthora which cause black pod of cacao in Brazil, P. palmivora, P. capsici and P. citrophthora, were tested for the production of pectate lyase, polygalacturonase, lipase, protease, deoxyribonuclease, phosphatase, urease, cellulase and amylase. These studies led to the development of a differential and selective medium which was used for macroscopic identification of several phytophthora species from the three species of Phytophthora. It contained agar 15 g, peptone 5 g, beef extract 3 g, soluble starch 2 g, pimaricin 0.01 g, ampicillin 0.125 g, pentaclorontrobenzene 0.153 g, mycinfen 0.075 g and rifampicin 0.01 g plus l-H2O and permitted reliable quantitative differential isolation of Phytophthora from infected tissues and naturally or artifically infected soil. It proved useful for studies involving survival and competition of the three Phytophthora species and for selective isolation from other hosts such as citrus infected with more than one Phytophthora pathogen.

A189

DISTRIBUTION OF CITRUS TRISTEZA VIRUS IN A SWEET ORANGE GROVE CONTAINING YOUNG AND OLD TREES. J. G. Lee and J. A. Dodd, Department of Plant Pathology, University of California, Riverside, CA 92521.

dsRNA analysis and ELISA was used to determine the incidence of citrus tristeza virus (CTV) in a grove containing navel sweet orange trees. 26 yr old Frost Rauccelar alternatingly planted with 4 yr old Washington. The rootstock for both was Troyer citrange. An initial survey revealed that 100% of the old trees and 26% (13/50) of the young trees were infected with CTV. Eleven of the young trees that tested positive for CTV were near the eastern edge of the grove suggesting aphid transmission into the grove from this direction and not at random from old trees. Titer of CTV was higher in old compared to young trees. Expression of a specific dsRNA (0.5 X 106) was more frequent in old trees than young trees. No other strain specific dsRNAs have been detected in samples from trees in this grove.

A190


Over 100 populations of root-knot nematodes (SER) were collected from bean (Phaseolus vulgaris) roots and soils from the major production areas of Colombia and Peru. Species were identified using morphological and molecular criteria. The most frequent species, as it occurred in the states included in the survey in both countries and was detected in 65% of the samples. H. javanica was detected in 14% of the collections and occurred in both hot and cool regions. H. kapra was detected only in cool-temperature regions and was present in 7% of the collections. H. arenaria occurred in association with other SER species only in 40 samples. Seven collections consisted of a mixture of two or more of the above species.

A191

PECTINOLYTIC ACTIVITY IN XANTHOMONAS CAMPESTRIS PV. VESICATORIA. C. Bouillieau, G. V. Minavage and R. E. Stahl. Plant Pathology Department, Universty of Florida, Gainesville, FL 32611.

Although pectinolyis is not a feature commonly associated with X. c. pv. vesicatoria (Xcv), several pectinolytic strains that cause bacterial spot of tomato pepper were found occurring in commercial strains from South America. Studies were undertaken to characterize the pectinolytic activity in strain Xv 56. Most of the pectinolytic activity was due to the secretion of an unique pectate lyase. The pl of this enzyme was estimated to be 8.8. Neither pectin lyase nor polygalacturonase activity was detected. From a genomic library of DNA from Xv 56, a cosmid conferring the ability to degradative pectate was isolated. This cosmid was identified. A 1.4 kb fragment of this cosmid hybridized to genomic DNA of other pectinolytic xanthomonads but not to genomic DNA of Erwinia chrysanthemi.
A192
We evaluated restriction fragment length polymorphisms among 53 strains of Pseudomonas syringae pv. syringae. Twenty-nine strains were isolated from brown spot lesions on bean, nine strains were isolated from lima bean, and the rest from 10 other hosts. Southern DNA digested with EcoRI or Hind III were hybridized to two random probes from a cosmid library of P. syringae, and a hsp locus (pHR11) cloned from P. syringae. The size of hybridizing fragments was determined and a similarity matrix was computed on a computer with strains on a pairwise basis for the presence or absence of fragments. Data were converted into a phenogram using an unweighted pair-group method with arithmetic mean algorithm. This study confirms Pseudomonas syringae pv. syringae to be composed of two pathogenic races. Both of race 1 and race 2 isolates included in this study were identified as P. syringae isolated from bean were more closely related to each other than to strains from other hosts. However, the bean strains formed two sub-groups that are only 60% related, and few strains had identical restriction profiles. These data are consistent with the hypothesis that P. syringae pathogenic on bean arose from 1 or 2 founding events rather than from multiple foundings. Construction of restriction maps of the hsp region in P. syringae isolated from bean is in progress.

A193
TRANSCRIPTIONAL ACTIVITY OF FLUORESCENT SIDEROPHORE GENES FROM PSEUDOMONAS SYRINGAE IN SITU ON LEAF AND ROOT SURFACES. S.E. Lindow and J.E. Loper, Dept. Plant Pathology, UC, Berkeley, CA 94720, and USDA, ARS, HCRL, Corvallis, OR 97333
Plasmid pEJI701 was constructed by cloning a 4.8 kb DNA fragment, which is essential for fluorescent siderophore production (flu) of P. syringae, upstream of a promoterless ice nucleation gene (inaZ) in the stable plasmid, pVS961. P. syringae psb* and Pseudomonas fluorescens strains harboring pEJI701 expressed ca. 1 ice nuc/cell/when grown in an iron-deplete minimal medium and only ca. 10^5 nuc/cell/when the medium was supplemented with 10^-5 M FeCl3. In contrast, cells harboring pVS961, containing incSB from P. syringae, constitutively expressed inaZ, expressed ca. 10^5 nuc/cell when grown in both iron-replete and iron-deplete media. The effects of iron on the E. coli/in A. rhizogenes and hsp genes were also studied. The results of the expression of the inaZ gene in fluorescent P. syringae isolates indicate that iron availability may be an important factor in the expression of the inaZ gene in fluorescent P. syringae.

A194
PHYSICAL AND GENETIC CHARACTERIZATION OF THE CLOSED COR REGION FROM PLASMID pAC002 PSEUDOMONAS SYRINGAE pv. TOMATO DCT601 S.W. Ma, V.L. Morris, and D.A. Cuppels, Agriculture Canada, Res. Centre and Dept. of Microbiol. & Immunol., Univ. of Western Ontario, London, ON, N6G 2V4, Canada
Pseudomonas syringae pv. tomato, causal agent for bacterial speck of tomato, produces the chlorosis-inducing phytotoxin coronatine. In P. syringae pv. tomato DCT601, coronatine production (cor region) is chromosomally-located; in strain DCT601, they are present on pAC002, a 106-kb plasmid. Southern blot analysis demonstrated that the cor region was plasmid-born. Deletion analysis of the cor region in 9 of 9 transformed tomato with a transposon Tn5 isolated from the tomato. Additionally, the transposon Tn5 had been used to insert an ampicillin resistance cassette into the cor region in strain DCT601. The coronatine production was abolished in the cor region and the strain DCT601.

A195
CHARACTERIZATION OF A PUTATIVE REGULATORY LOCUS REQUIRED FOR ENDOPOLYAGLUTANURONASE PRODUCTION IN PSEUDOMONAS SOLANACEARUM. Caitlin Allen, Merellee M. Atkinson, and Luis Sequeira. Department of Plant Pathology, University of Wisconsin-Madison, Madison WI 53706.
An extracellular polygalacturanase (PG) produced by P. solanacearum is believed to play a role in bacterial virulence. We have cloned the structural gene for this enzyme, pchA, and have found that it is physically linked to the ystC2 locus of hsp locus, which is a putative transposable element. At first, a second, unlinked locus, pchR, was required for expression of the pchA gene. When a pch::Tn5 insertion mutant, which does not produce this PG, was complemented in trans by the wild-type region, it produced 80% of the wild-type level of the enzyme. This suggests that pchR encodes a positive trans-acting regulatory gene. This work is derived from the A. tumefaciens virA locus hybridized to pch indicating that pchR may be related to a family of bacterial two-component regulatory systems. To test the hypothesis, DNA sequencing of pchA and phosphorylation studies with pchR-encoded proteins are being completed.

A196
A CLOSED REGION OF PLASMID PTT23A IS REQUIRED FOR CORONATINE SYNTHESIS IN PSEUDOMONAS SYRINGAE pv. TOMATO PTT232. S. A. Young and C. L. Bender. Dept. of Plant Pathology, Oklahoma State University, Stillwater, OK 74078-9947
Plasmid pPTT23A is involved in coronatine biosynthesis in P. syringae pv. tomato PTT232. Previously, coronatine-defective (cor) mutants of PTT23.2 contained either Tn5 insertions or deletions in plasmid pPTT23A or were missing pPTT23A entirely. In the present study, library of PTT232 was constructed in pLAFR3. A 52 kb cosmid clone, designated pPS51, was transformed by electroporation into three classes of cor mutants and two nonproducing of the transposon Tn12 and P. syringae P551. Organic acids were extracted from these transformants and analyzed by reverse-phase high performance liquid chromatography and a potato disc bioassay. Cosmid pPS51 restored coronatine production to five mutants containing either Tn5 insertions or deletions in pPTT23A. However, the acquisition of pPS51 did not confer coronatine production to a mutant lacking pPTT23A or to the two nonproducers of coronatine.

A197
MOLECULAR CLONING OF CELL WALL DEGRADING ENZYMES FROM AGROBACTERIUM tumefaciens BIOTOX 3. K.M. Ophel, B.A. Jones and A. Kerr. Department of Plant Pathology, Waite Agricultural Research Institute, Glen Osmond, South Australia, 5064.
Both tumorigenic and nontumorigenic strains of the grapevine pathogen, Agrobacterium tumefaciens biotox 3 (AT3), produce a root-specific decay of grapevine (Burr et al., 1987). All AT3 strains tested in our study have both endopolygalacturonase and cellulase activities. A genome library of a wild type AT3 strain was constructed and used to screen a library of both enzymes were isolated. The genes are expressed both in E. coli and in Agrobacterium biotox 3. Production of either enzyme alone does not cause root decay of grapevine. Cloning and sequencing of the genes from AT3 strains. The enzymes of AT3 mutants have been obtained. The cloned mutants have been used to construct marker exchange mutants in AT3. The AT3 mutants will be screened for activity on grape roots in order to investigate the role(s) of the enzymes in pathogenicity.

A198
USE OF PUTATIVE TRANSPORTABLE ELEMENTS AS PROBES FOR POPULATION STUDIES OF THE BACTERIAL BLIGHT PATHOGEN OF RICE. M.R. Bamdad, R. Nelson, J.E. Leach, T.W. Mow, and H. Leung. International Rice Research Institute, P.O. Box 933, Manila, Philippines and Kansas State Univ., Manhattan, KS 66506
The transposon trapping vector pJ39ac (Kennedy and Stankovic, 1990. J. Bacteriol. 172:143) was used to isolate putative transportable elements from Xanthomonas campestris pv. oryzae (Xco), the causal agent of bacterial blight of rice. Restriction analysis of the 153 clones isolated from the Xco DNA demonstrated that 153 clones isolated from the Xco DNA demonstrated that the isolated DNA was mapped using restriction enzymes BglII, EcoRI, and XhoI. The genetic and transcriptional organization of the plasmid-encoded cor genes was determined by plasmid-encoded cor genes was determined by insertional mutagenesis using Tn3::SpL and then compared to that of the chromosomally-located cor region of strain DC3000.

A199
MOLECULAR CLONING OF AN ERWINIA AMYLOVORA rcsB GENE REQUIRED IN POLYSACCHARIDE SYNTHESIS. A. Mendez and A.K. Chatterjee, Department of Plant Pathology, University of Missouri, Columbia, Missouri 65211.
The production of extracellular polysaccharides (EPS) by E. amylovora (Er) is required in its pathogenicity on apples and pears. Our previous work with the cloned rcsa gene (rcs-regulation of capsule -EPS) synthesis) suggested that it, in conjunction with another gene product (i.e. RcsB), activates polysaccharide synthesis in Er. The presence of an rcsb homolog in Er was indicated by the restoration of EPS production in Er strain Ee by an E. coli rcsb but not by the Er rcsA gene. By screening an Er cosmid library in E. coli rcsB strain (S1086) we obtained colonies that were mucoid at 20-30°C in a medium supplemented with 0.2% glucose. The mucoid phenotype was not expressed at 37°C or in the absence of glucose or metabolizable sugar. The cosmid complemented both rcsA and rcsB mutations in E. coli. From one of the cosmids, we have obtained subclones that complemented either the rcsB mutation or the rcsB mutation. Thus, in E. coli at least two genes are required for the activation of EPS synthesis.
A200
A NEW SET OF ERWINIA CHRYSANTHEMI FECITIC ENZYMES PRODUCED DURING GROWTH ON PLANT MATERIAL. S. Kalem and A. Collmer. Department of Plant Pathology, Cornell University, Ithaca, NY 14853.

Erwinia chrysanthemi E16 is known to produce a number of pectolytic enzymes which can contribute to its maceration ability. A mutant containing site-directed mutations in the genes pelh, pelk, pelA, pelB, pelC, respectively coding for exopolygalacturonase, D-polygalacturonase, endopolygalacturonase, and the four known isozymes of pectate lyase retained significant maceration activity. Activity-stained isoelectric-focusing gel analysis of culture supernatants revealed that the mutant produced several new pectic enzymes when grown on plant tissue extracts or chrysanthemum cell walls, but not when grown on pectate or a variety of other carbon sources. Filter-sterilized, pectolytic, culture supernatant and pectate lyase activity in nuclease and macerated tissue tissues. Analysis and manipulation of DNA fragments cloned from the mutant to produce pectolytic Escherichia coli transformants will clarify the role of these enzymes in pathogenesis.

A201

Transgenic tobacco plants expressing the papaya ringspot virus (PRV) coat protein gene were obtained by transformation with Agrobacterium tumefaciens. Strongly infected reaction was obtained when leaf extracts from transgenic plants were tested with anti-PRV monoclonal antibody in ELISA. The coat protein comprised 0.2% of the total leaf soluble proteins. A protein band with similar molecular weight to that of PRV coat protein was detected in leaf extracts of ELISA-positive transgenic plants by Western blot analysis with the anti-PRV antibody. Transgenic plants with high levels of PRV coat protein expression showed significant delay in symptom expression and severity after inoculation with other potyviruses, including tobacco etch, pepper mottle, and potato virus Y.

A202

Bean golden mosaic virus (BGMV) causes a serious disease of Phaseolus vulgaris in Latin America and is transmitted by Bemisia tabaci. Isolates from Brazil are not mechanically transmitted by the vector. Two BGMV isolates from the Caribbean and Central America are mechanically transmitted. Sequence analysis of full-length clones of BGMV isolates from Guatemala (BGMV-GA) and the Dominican Republic (BGMV-DR) showed that each isolate was composed of two DNA components, designated A and B, which have four and two RNAs, respectively. Sequence analysis indicated that BGMV-DR and BGMV-GA were closely related to a BGMV isolate from Puerto Rico but not to one from Brazil, which is more similar to tomato golden mosaic bean virus. Beaus inoculated with cloned DNAs of BGMV-GA/Component A and BGMV-DR/Component B or with BGMV-DR/Component A and BGMV-GA/Component B developed typical and attenuated symptoms, respectively, implying component asymmetry.

A203

Geminiviruses are small plant viruses that possess a single-stranded DNA genome. Bipartite, whitefly-transmitted geminiviruses are a major constraint on bean production in the tropics. Four isolates of bean-infecting geminiviruses—bean golden mosaic virus from Brazil (BGMV-BZ), Guatemala, and the Dominican Republic and bean dwarf mosaic virus—were cloned. Full-length linear double-stranded DNA components A and B were not infectious when mechanically co-inkoculated onto bean primary leaves by surface abrasion but were infectious when inoculated into raddices of beans by electric discharge particle acceleration. Infection of beans by cloned DNAs of BGMV-BZ, which has never been mechanically transmitted as virions or cloned DNAs, was achieved using particle acceleration and indicated that this method circumvents plant barriers to mechanical transmission. Particle acceleration will facilitate genetic analysis of these geminiviruses and may allow for efficient introduction of viral nucleic acids or virions of other viruses into hosts that are refractory to mechanical transmission.

A204
POLYMERASE CHAIN REACTION: MOLECULAR TECHNOLOGY TO ENHANCE DETECTION AND DIAGNOSIS OF POME FRUIT VIRIIDS.

A205
COAT GENES OF CONEA CHLOROTIC MOTTLE VIRUS AND BROME MOSAIC VIRUS CAN BE EXCHANGED WITHOUT AFFECTING HOST SPECIFICITY OR SYSTEMIC MOVEMENT. R.F. Allison, M. Janda, C. Thompson, P. Aliquist, Institute for Molecular Virology and Dept. of Plant Pathology, Univ. of Wisconsin, Madison, WI 53706.

Although the tripartite genomes of conea chlorotic mottle virus (CCMV) and brome mosaic virus (BMV) share common organization and sequence similarity, these viruses have distinct host ranges. We have shown that BMV coat proteins can be efficiently expressed in CCMV vector, while CCMV infects only dicotyledonous plants. In vitro transcripts from full length cDNA clones of BMV or CCMV RNAs are infectious when inoculated into their natural hosts. In protoplasts RNAs 162 of either virus replicate in the absence of RNA3; no replication, however, is detectable with heterologous combinations of RNAs 162. Combinations of RNAs 162 from one virus plus the heterologous RNA3 replicate and are encapsidated in protoplasts, but similar coinoculations do not initiate systemic infections. Therefore, the 1A or coat proteins, encoded by dicistronic RNA3, must either multiply or cooperatively influence host range. The role of the two RNA3 genes in host specificity has been assessed by exchanging individual genes between the viruses. Whole plant inoculations indicated that BMV and CCMV coat protein genes can be exchanged freely without affecting host specificity. Involvement of the 1A protein in host specificity is confirmed, since exchange of these genes abolishes systemic infections in both hosts.

A206
BOTH HELPER VIRUS AND SATELLITE RNA AFFECT SYMPTOMS OF TURNIP CRINKLE VIRUS INFECTION. C.W. Collmer and S.H. Howell, Byoe Thompson Institute, Cornell University, Tower Road, Ithaca, NY 14853.

Previous studies on the three satellite RNAs associated with the isolate of turnip crinkle virus now designated TCV-J1 (John Innis isolate) have identified satellite RNAs as a virus. Addition of this satellite to helper virus (TCV-J1) inoculum containing the smallest satellite RNA D results in an intensification of the normally mild symptoms in turnip. In an attempt to correctly assess the effects of the satellite RNAs and helper virus, we tested each satellite with TCV RNA derived from a cDNA clone of TCV-D (Berkeley isolate). The much milder symptoms caused by the satellites with this virus isolate indicate a role for the helper virus in symptom intensification. Ongoing sequence analysis of the TCV-J1 isolate has thus far revealed relatively few nucleotide differences from TCV-B, thus offering the opportunity to localize determinants for symptom expression in the viral genome.

A207

Sequence homology of gene I of some caulimoviruses with the 30K transport protein of TMV as well as localization studies by immuno-electronmicroscopy of cauliflower mosaic virus (CaMV) gene I protein suggest that gene I of the caulimovirus might be involved in viral movement. In order to understand the relationship between the gene I protein and intercellular transport of virus, D. inoxia plants were transformed with gene I of peanut chlorotic streak virus (PCS); a member of the gourd group that infects Nicotiana and other non-hosts. Southern blot analysis of transgenic plants indicated the genomic integration of gene I. Expression of gene I protein was confirmed by western blot analysis using an affinity purified antibody. A 204 amino acid synthetic peptide corresponding to the carboxy-terminus of the protein.

A208
CLONING OF GENES ENCODING EXTRACELLULAR METALLOPROTEASES FROM ERWINIA CHRYSANTHEMI E16. G.S. Dahler, F. Barras and T. J. Keen. Department of Plant Pathology, University of California, Riverside, CA 92521.
A 14-kb BamHI-EcoRI DNA fragment cloned from strain EC16 contained a gene encoding a protease inhibitor as well as three tandem prt genes encoding metalloproteases. The prt genes were separated from the inhibitor gene by a ca. 4-kb region that was necessary for extracellular secretion of the proteases. When individually subcloned from its downstream vector promoters, the prt genes each led to substantial extracellular secretion of the proteases in E. coli, provided that the ‘required region’ was supplied in cis or trans. The prtC gene was sequenced and had a homology of 0.7% to 2.2% with proteases phylogenetically distant from Serratia spp. and E. chrysanthemi strain B374. Mutations in strain EC16 that reduced protease production did not detectably affect virulence in chrysanthemum stems.

A209
CHARACTERIZATION OF A GENE REQUIRED IN TRANSCRIPTIONAL ACTIVATION OF THE PECTIN LYASE STRUCTURAL GENE, pnlA, OF ERWINIA CAROTOVORA SUBSP. CAROTOVORA (ECRC-3069). J. L. McCann1, A. Chatterjee2 and A. K. Chatterjee, University of Missouri, Columbia, Missouri 65211.

Pectin lyase (Pnl) production occurs in many strains of soft-rotting Erwinia species in response to DNA-damaging agents. This induction in ECRC strain 71 requires RecA function. Complementation of regulatory mutations in EC71 and reconstitution of an inducible Pnl system in a RecA- E. coli led to the detection of a cosmid carrying the activator gene, pnnR. By subcloning, pnnR was localized on a 6.7-kb EcoRI DNA fragment. pnnR was inactivated using the mini-Nu-λ element Mu dill734. Insertion of the element in one orientation yielded a β-galactosidase-inducible phenotype in response to the DNA-damaging agent acridine orange (Lasch C.) and RecA and RecA LexA- E. coli strains. These findings suggest that the stimulation of Pnl production by DNA-damaging agents results from an increased pool of the pnnR product that is required for transcription of pnlA.

A210
EXPRESSON OF THE ERWINIA CAROTOVORA SUBSP. CAROTOVORA (ECRC-3069) AEP GENES IS REQUIRED IN THE PRODUCTION OF EXTRACELLULAR PROTEINS. H. Murata1 and A. K. Chatterjee2, Department of Plant Pathology, University of Missouri, Columbia, Missouri 65211.

In Ec71, we identified an aep gene that is necessary for production of extracellular enzymes including pectate lyases (Pels). We subsequently constructed an aep- lacZ transcriptional fusion, and placed the DNA on the chromosome of a LacZ- strain, AC506E. The resulting Aep- strain (AC5026) failed to produce extracellular enzymes but was inducible for β-galactosidase production; induction ratios were ca. 3 and 5 when grown in presence of citrus pectin and celery extract (CE), respectively. Under these growth conditions Pel production in an Aep+ strain was also stimulated. The kinetics of the induction of β-galactosidase and extracellular enzymes in AC5024 carrying an aep+ plasmid revealed that a high rate of pel, protease and cellulase production commenced after the initiation of β-galactosidase synthesis in a CE medium. Thus, aep gene expression is necessary for the synthesis of the extracellular enzymes in Ec71.

A211

The pexX gene encoding extracellular exo-poly-O-D-glucuronoxylomaltotetraose (exoPG) was isolated from a genomic library of the pectate lyase-deficient E. chrysanthemi mutant DM1005 (a NalR, KanR, Laplace derivative of EC16). The cloned pexX gene was sequenced and expressed in its original N-terminal, and most of the enzyme was localized in the periplasm. The nucleotide sequence of pexX revealed the presence of an aminoterminal signal peptide and an open reading frame encoding a preprotein of 66,493 bases. A signal peptide was truncated when 10% of the C-terminus of exoPG was removed by subcloning. Analysis of the constructed mutants, CUCPB5008 (Pel+, Peh) and CUCPB5009 (Pel-, Peh), indicated that exoPG can contribute significantly to bacterial utilization of polygalacturonate, the pitting phenotype on pectate semi-solid agar and the induction of pectate lyase, but not to the maceration capability of the bacterium.

A212
DIFFERENTIATION OF Pseudomonas syringae pv. morrisonorum FROM P. S._pv. syringae USING A DNA PROBE FROM P. S. pv. tomato. J. M. Paterson and A. L. Jones. Department of Botany and Plant Pathology, Michigan State University, East Lansing, MI 48824.

A DNA hybridization probe developed in Georgia for detection of Pseudomonas syringae pv. tomato (Pst) was evaluated for its ability to differentiate isolates of P. s. pv. morrisonorum (Psm) from P. s. pv. syringae (Pss) collected from cherry, prune and plum in Michigan and other geographical locations. The DNA probe was specific for isolates of E. coli and Pst digested genomic DNA revealed that the DNA probe hybridized to multiple restriction fragments. Eight and twelve isolates of Psm contained a common 3.5 kilobase (kb) EcoR I and PstI fragment, respectively. No restriction fragments of Pss were detected with the Pst probe. The ability of the Pst probe to detect plant pathogens in situ will be discussed.

A213
MOLECULAR ANALYSIS OF A LOCUS FROM Pseudomonas syringae pv. syringae REQUIRED FOR LESION FORMATION. E. M. Hrabak & D. K. Willis, Dept. of Plant Pathology and USDA/ARS, University of Wisconsin, Madison 53706.

The wild-type lemA locus of P. syringae pv. syringae B728a is required for development of typical brown spot lesions on bean (Phaseolus vulgaris). Mutations in this locus affect lesion development, as well as production of an extracellular protein that is toxic to bean seedlings. Tn3-HoHo1 was used to mutagenize a 9.7 kilobase (kb) cosmid subclone carrying the intact lemA locus. The direction of transcription and length (<4.3 kb) of the lemA locus has been determined. Sequencing of this region is in progress. Because of the pleiotropic phenotype of lemA mutants, it is hypothesized that lemA may be a regulatory locus.

A214
TPHOS A TAGGING OF Pseudomonas syringae pv. syringae HRP GENES ENCODY POTENTIALLY EXPORTED PROTEINS. H. C. Huang1, S. W. Hutcherson2 and A. Collmer1. 1Department of Plant Pathology, Cornell University, Ithaca, NY 14853. 2Department of Botany, University of Maryland, College Park, MD 20742.

Cosmid phr111 contains a cluster of hrp genes from Pseudomonas syringae pv. syringae (Pss) that enables P. fluorescens to elicit the hypersensitive response (HR) in tobacco leaves. phr111 was mutagenized with Tnpho1 in Escherichia coli G118, then mobilized by triparental mating into P. fluorescens and screened for loss of HR elicitation. Hrp mutations were marker-exchanged into the genome of Pss and determined to define 11 complementation groups based on analysis of merodiploids. Two of the Pss mutants produced blue colonies on media containing 5-bromo-4-chloro-3-indolyl-phosphate, indicating that Tnpho1-generated hybrids expressed alkaline phosphatase activity. Western blot analysis revealed that the hybrid proteins had molecular weights of 58 K and 61 K and were produced only in minimal medium. The results indicate that some Hrp proteins have either periplasmic domains or are exported out of the cytoplasm.

A215
FURTHER CHARACTERIZATION OF A GENE CLUSTER REQUIRED FOR EPS PRODUCTION AND VIRULENCE IN Pseudomonas solanacearum. Douglas Cook and Luis Sequeira, University of Wisconsin-Madison, Department of Plant Pathology, Madison, WI 53706.

A 28 kb cosmid clone that complements two EPS-avr1-1 avr2-2 Tn2 mutants of P. solanacearum was characterized by saturation mutagenesis. A gene cluster of 6.5 kb contains five complementation units required for virulence, four of which are essential for maximal EPS production. Tn2-mediated gene fusions were used to quantify gene expression in each complementation units by measuring B-glucuronidase activity both in planta and in broth cultures. Two classes of EPS-affected mutants could be distinguished visually based on colony morphology and chemically according to level of N-acetylglucosamine (NAG) in purified EPS. All but one of the mutants grew as well as wildtype in broth and rapid growth in planta growth of the mutants was substantially reduced. One group of mutants produced no visible EPS, grew poorly or not at all in the plant and were weakly virulent. Another group produced an intermediate level of EPS, grew slower than wildtype in the plant and retained moderate virulence.

A216
BACTERIAL UTILIZATION OF TRANSGENEIC PLANT SYNTHETIZED AND SECRETED MANNITOL OPINES. M. A. Sadd1 and S. K. Farrand, Department of Plant Pathology, University of Illinois at Urbana-Champaign, IL 61801.
An Agrobacterium-mediated binary transformation system was constructed to introduce manninyl opine anabolic genes from an octopine-type Ti-plasmid into plants. Transgenic tobacco plants were generated which expressed resistance to kanamycin and synthesized the manninyl opines. One regenerant examined in detail exhibited a selected feature: the plant was kanamycin-resistant and the manninyl opine biosynthesis cosegregated with kanamycin resistance at a frequency of 1:0. Analysis of R3 progeny from R2 selections showed segregation patterns consistent with the T-DNA insert being located on a single chromosome genome. Genomic Southern analysis confirmed the presence of T-DNA in DNA from the transgenic plant. Transgenic plants grown autotrophically in a mineral salts solution secreted the opines from their roots into the medium. Agrobacterium and Agrobacterium strains containing genes conferring catabolism of the manninyl opines could grow in mineral salts solution at the expense of the plant synthesized and secreted opines. The genes 0.1 and 0.2 of pl95595 T-region are sufficient for the biosynthesis and secretion of the manninyl opines, that these opines are synthesized and secreted from the roots of transgenic plants grown under autotrophic conditions, and that the secreted opines can be utilized by plant-beneficial bacteria.

A217

COPPER-BINDING OUTER MEMBRANE AND PERIPLASMIC PROTEINS FROM THE COPPER RESISTANCE OPERON OF PSEUDOMONA SYRINGEAE PV. TOMATO, J.-S. Cha and D. A. Cooksey. Department of Plant Pathology, University of California, Riverside, CA 92521.

P. syringae strains carrying the plasmid-borne copper resistance operon (cop) accumulate copper when grown in the presence of high levels of cupric sulfate. Proteins produced from the cop operon were purified by cellular fractionation methods, ion exchange chromatography, and gel filtration chromatography. Purified CopA and CopC proteins bound copper, as measured by atomic absorption spectrophotometry. CopA was associated with the outer membrane; CopC was periplasmic. CopB was tightly associated with the outer membrane fraction that bound copper, but after purification from the membrane, CopB did not contain copper. Accumulation of copper in the outer membrane and periplasmic cop gene products, and probably other outer membrane components, may prevent the entry of toxic levels of copper ions into the cell and therefore confer resistance.

A218

CHARACTERIZATION OF ERWINIA STEWARTII MUTANTS UNABLE TO CAUSE WATERSOAKING SYMPTOMS ON CORN. D. L. Coplin, D. R. Majerczak, L. D. Tuttle, R. D. Frederick, D. K. Estes, and J. Costa. Dept. of Plant Pathology, The Ohio State University, Columbus OH 43210.

Cosmid pES1044 from Erwinia stewartii contains two large clusters of genes (uaA and uaB) that are needed for this bacterium to cause watersoaked lesions on corn seedlings. Chromosomal deletion mutants of the es region, DM3001 and DM3020, were isolated. Ths, ThsIc, and ThsIIc, mutations of pES1044 and complementation analysis confirmed that uA and uB are separate transcription units; a third complementation group, which was represented by a single mutation, was also identified. Initial growth of uA and uB mutants in seedlings was not impaired. DM3020, which is deleted for uA and uB, produced extracellular polysaccharide (EPS) that was similar to wild-type EPS in sugar composition and proton and carbon NMR spectra. uA gene function was restored in plants, and in a minimal salts-glucose-casamin acid medium during mid-log phase, but not in rich media or during other phases of growth.

A219

COMPLEMENTATION OF HRP MUTANTS OF ERWINIA AMYLOVORA WITH DNA OF ERWINIA STEWARTII. S. V. BEER, R. J. Laby, and D. L. Coplin. Departments of Plant Pathology, Cornell University, Ithaca, NY 14853 and The Ohio State University, Columbus, OH 43210.

Transposon-induced Hr of E. amylovora mutants are not pathogenic to immature pear fruit and fail to elicit the hypersensitive response in tobacco. The cloned hrc gene cluster of E. amylovora restores the Hrp phenotype. A cosmid, pES1044, contains the wts (water-soaking) region of E. stewartii that is required for symptoms of Stewart’s wilt. The cosmid hybridized with certain subclones of the Hrp cluster of E. amylovora. Hrp function was restored fully by pES1044 to some of those mutants with insertions in the regions of hybridization. The cloned hrc gene cluster of E. amylovora is in progress. The observation of interspecific complementation for pathogenicity indicates conservation of genetic and functional homology between two species of Erwinia.

A220

THE CULTIVAR-SPECIFIC ELICITOR ASSOCIATED WITH EXPRESSION OF AVIRULENCE GENE D FUNCTIONS IN SOYBEAN CELL CULTURE. Stanley J. Tamaki, A. Diane Lawrence, Noel Keen, and Mark Staynor. Department of Molecular Biology, University of Wyoming, Laramie, WY 82071; CerealGen, Inc., University of California-Richmond Field Station, Richmond, CA 94804-4698; Department of Plant Pathology, University of California at Riverside, Riverside, CA 92521-0122.

Expression of the pathogen avirulence gene D in Pseudomonas syringae pathovars as well as in Erwinia carotovora, results in the biosynthesis of a protein, which is induced in vivo and is common to all pathovars (the cultivar specific elicitor or SE). The SE induces a strong necrotic reaction on the leaves of soybean cultivars which express the dominant disease resistance gene, Rpp4. Cultivars which carry the recessive allele at this locus show no visible signs of necrosis upon inoculation. In leaf disks from soybean lines which express Rpp4, the SE inhibits the incorporation of [55]methionine into protein. Thus, we have initiated studies to determine the minimum level of tissue organization required to maintain cultivar-specific sensitivity to the SE compound. Soybean callus cultures derived from hypocotyl tissue were digested with cell wall macerating enzymes yielding mainly single cells with a small proportion of protoplasts. A 20-min preincubation of these cells with low concentrations of SE, inhibited [55]methionine incorporation by 70% in Rpp4 cells but only 15% in Rpp4 cells. At ten-fold higher SE concentrations, [55]methionine incorporation is completely inhibited in both genotypes. Thus, at appropriate SE levels, soybean cell cultures show a cultivar-specific response to the SE.

A221


Xcp pv. translucens strain Xi-216 causes leaf streak on barley, wheat, oats, rye, and triticale. Three thousand Tn5 Gus insertional derivatives of strain Xi-216 were inoculated on the host plants. Proteogens from each of the inserts was identified in virulence on one of the hosts but not affected on the rest (host specific virulence, Hsv) were identified at frequencies of 0.078 (Barley), 0.04% (Wheat), 0.07% (Oats), 0.10% (Rye) and 0.07% (Triticale). Proteogens associated with virulence on homologous hosts (Vir), and failing to give a hypersensitive response on tobacco and cotton were obtained at a frequency of 0.42%. The cloned mutated region from one Vir derivative hybridized to the cloned hrc gene (gvi2) from Pseudomonas solenomcarus (C.A. Bouchon et al., 1986, J. Bacteriol. 169:5062-32). Complementation of several Vir and Hsv mutants with DNA fragments from Xi-216 was achieved. Based on Tn5 insertional analysis, two loci may be required for virulence of Xi-216 on barley. Growth in plants, and analysis of DNA fragments which complement host specific virulence mutants will be discussed.

A222

IDENTIFICATION AND PARTIAL CHARACTERIZATION OF PLASMIDS PRESENT IN THE BEET LEAFHOPPER TRANSMITTED VIRESCENCE AGENT. M. E. Shaw, B. C. Kirkpatrick, and D. A. Golino. Department of Plant Pathology, University of California, and *USDA/ARS, Davis, CA 95616.

Southern blot analyses, using cesium chloride purified supercoiled DNA as probe, identified at least two plasmids in pest species Circulifer tenellus leafhoppers infected with a type strain (PC-85-13) of the best leafhopper transmitted virescence agent (BLTVA-MLO). No plasmids were detected in healthy hosts. The plasmids were approximately 18 and 3 kbp in size. Southern blot analyses, using native and cloned BLTVA plasmid fragments as probes, showed the BLTVA plasmids shared no homology with the plasmids that are present in strains of the western aster yellows MLO. The 18 kbp BLTVA plasmid clone will be used to facilitate cloning full-length copies of this plasmid in lambda EMBL-3.
**A224**

**SEROLOGICAL DIFFERENTIATION OF MAIZE DWARF MOSAIC VIRUS (MDMV) STRAINS A, B, D, E, F AND O. S. L. Lenardon1, D. T. Gordon1, and R. E. Gengery2. 1Dept. of Plant Pathology, The Ohio State Univ. 2USDA-ARS, Wooster, OH 44691.**

Polyconal antisera (Poa) s to MDMV strains A, B, D, E, F and O, collected 1 wk after a single injection with intact virions (As-1wk), reacted with homologous capsid protein (Hmp), but not with homologous capsid core protein (HmpCCP) in western blots. CCPs were obtained from intact virions by lysyl endopeptidase proteolysis followed by SDS-PAGE. CCPs were similarly obtained, but were not enzyme treated. As-1wk distinguished A, D, E and F from B and O, but not from each other. PCAs collected after several wk (As-swk) reacted with both HmpCP and HmpCCP. As-swk to A, D, E and F, cross-absorbed (xab) with heterologous CCPs (HmpCP), reacted with HmpCPs and HCPs not used for xab, but not with the HTPCP used for xab. Apparently, As-1wk to each strain contained antibodies (Ab) only to N-termius epitopes, whereas As-swk contained Ab to N-termius and CCP epitopes. As-1wk and As-swk to A, D, E and F contained Abs specific to the N-termius of the immunizing strain.

**A228**


A closterovirus has been mechanically transmitted from a grapevine leafroll-infected grapevine to Nicotiana occidentalis, where it induced necrotic local lesions on inoculated leaves; and curling, yellowing and mosaic symptoms on young leaves. The virus also induced very mild chlorosis symptom on young leaves of N. benthamiana, but was not transmitted to N. tabacum, P. argentatum, Datura stramonium, Gomphrena globosa, Cuprea maxima, and Cucumis sativus. The modal length of the virus is 800 nm, and in SDS-PAGE analysis, a 24 Kd coat protein band was identified. Several dsRNA bands ranging in molecular weight from about 3.5 x 10^6 to 5 x 10^6 Mr were isolated. Polyconal antibodies were produced to the closterovirus in rabbits, used in double diffusion. ELISA, ISEM, and Western blot assays. The antibodies reacted with the closterovirus, and also with grapevine A and apple stem pitting virus. The relatedness of the virus with other similar closteroviruses, the distribution of the virus in grapevines, and the association of the virus with grapevine leafroll disease are under investigation.

**A229**

**BIOLOGICAL FACTORS AFFECTING LEAFHOPPER TRANSMISSION OF PURIFIED MAIZE CHLOROPHYLL DISEASE VIRUS (MCDV). R. Casame, L. Ralph, and R. E. Gengery. USDA-ARS, Dept. of Plant Pathology and Entomology, The Ohio State University, OARDC, Wooster, OH 44691.**

Purified MCDV (WS strain), when acquired through membrane feeding, was transmitted by *Grainneilla inquisitor* if the leafhoppers were allowed an initial acquisition on MCDV (Ms strain)-infected corn. Conversely, MCDV-WS-infected corn could transmit purified MCDV-Ms. After removal from MCDV-Ms-infected plants, G. nirifrons feeding MCDV-Ms for up to 24 hours failed to transmit the virus. The feeding on healthy corn; the ability of the leafhopper to acquire and transmit purified MCDV-WS was retained for 36 hours. In transmissions where G. nirifrons fed initially on MCDV-infected plants and then on purified MCDV-Ms, purified MCDV could be transmitted without the helper virus, G. m. nicotianae, suggesting that a factor from MCDV-infected plants other than virus assists in transmission of MCDV. Increasing the time for acquisition feeding of G. nirifrons on MCDV-Ms-infected corn or on purified MCDV-WS did not significantly change the transmission frequency of MCDV-WS. Amblysuper gus, an experimental vector of MCDV, also transmitted purified MCDV-WS after an initial acquisition feeding on MCDV-Ms-infected corn.

**A230**


The ability of naturally occurring mild strains of citrus tristeza virus (CTV) to suppress the spread of severe strains of CTV into citrus propagated on susceptible rootstock was tested. Sour orange rootstock budded with Valencia sweet orange either infected with one of four mild strains of CTV or uninfected were planted at Ft. Pierce, FL where severe strains of CTV are prevalent. Decline was first observed 2.5 years after planting in both trees protected with mild isolates and in unprotected trees. After 6 years, the percentage of decline was 37.5, 39.8, 38.9, and 42.0 for the 4 CTV-infected trees containing the 4 mild strains of CTV, respectively, compared to 47.4 for the unprotected trees. After 8 years the percentages were 75.0, 75.7, 74.4, and 72.7, respectively, compared to 86.0 for the unprotected trees. These percentages were not significantly different. The breakdown of cross-protection was documented serologically using monoclonal antibodies which are specific for severe CTV strains occurring at Ft. Pierce, FL.

**A231**

**PRELIMINARY IDENTIFICATION OF VIRUSES IN CERTAIN ORNAMENTAL SPECIES IN CALIFORNIA. J. F. Peterson, Department of Plant Science, Macdonald College of McGill University, 2111 Lakeshore Rd., Ste-Anne-de-Bellevue, Quebec, Canada, H9X 1C0**

Many of the cut flowers sold during the winter are imported. Striping of leaves and spathes shows on most iris, presumably Dutch bulbous iris, *I. x hollandica*, from The Netherlands. Results of host range tests and immunosorbent electron microscopy studies indicated that these flowers were infected by a combination of iris mild mosaic (IMMV) and narcissus latent virus (NLV). A carlavirus, possibly lily symptomless virus, was revealed by electron microscopy of crude mixtures of preparations of symptomatic leaves from European cut lilies, as well as some Asiatic hybrid lilies grown from imported bulbs. One cultivar of peony (*Paeonia officinalis*), imported from local multiplication shippers, showed the symptoms apparently associated with a strain of tobacco rattle virus (TRV). TRV has been recorded only once previously in Canada, and could pose an added threat to potato production.

Double-stranded (ds)RNA was isolated from leaves of ‘Wayne’ soybean infected with the dwarfing (D) or yellowing (Y) strain of soybean dwarf virus (SDV). Each strain produced two virus-specific dsRNAs. SDV-D dsRNAs were estimated to have molecular weights of 3.4 and 1.9 x 10^6 dal; corresponding dsRNA for SDV-Y were 3.6 and 2.2 x 10^6 dal. Northern-blot hybridization analyses showed the strains share sequence homology and that the two dsRNAs correspond to viral genomic-length and 3' subgenomic-length species. Because SDV-D and SDV-Y are closely related ecologically, immunological methods for in vitro strain differentiation are not practical. The ability to differentiate SDV-D from SDV-Y based on dsRNA profiles should facilitate investigations into the etiology of SDV-like viruses reported to occur in U.S. forage legumes.

A233 PARTIAL CHARACTERIZATION OF cDNA LIBRARIES TO AN ISOLATE OF ZUCCHINI YELLOW MOSAIC VIRUS (ZYMV) FROM FLORIDA. G. C. Wiler, E. Hiebert, and D. E. Puricelli. Department of Plant Pathology, University of Florida. Gainesville, FL 32611.

Complementary DNAs prepared to an aphid transmissible isolate of ZYMV from Florida were cloned in the expression vector λgt11. Clones representing the coding regions for the capsid protein (CP), cylindrical inclusion protein (CIP) and the yellowish-green phase, were identified by serological detection of fusion proteins. Clones ranged in size from 0.6-4.5kbp and together represented ca. 80% of the genome. Sequences obtained showed 98% similarity of the CP with the 3’ end of a Connecticut ZYMV isolate (Grumet & Fang, Phytopathology 79:1194). A primer was developed from the ZYMV CIP sequence and was used to clone specifically for the 5’ end of ZYMV using the A-ZapII vector. Clones from this study will be used to analyze the products encoded by the 5’ end of the ZYMV genome.

A234 HEAVY METALS BIND TO RNA INSIDE MAIZE CHLOROTIC MOTTELE VIRUS PARTICLES AND REDUCE THE VIRUS SURFACE CHARGE. R. W. Skopp, L. C. Lane, Dept. of Plant Pathology, Univ. of Nebraska, Lincoln, NE, 68583-0722.

Maize chlorotic mottle virus, a carmovirus, is more stable in acid than at pH 7. At pH 6 it has two anionic electrophoretic forms. Carboxypeptidase A converts the slow form to the fast form. Briefly heating virions at 50 C with heavy metals such as calcium, zinc, or iron produces discrete forms with reduced mobilities. Heating metal ion treated virus with excess EDTA restores the “normal” electrophoretic mobility. Neither cytoines nor histidines appear to be involved in heavy metal binding. Heating MCMV in the presence of zinc ions hydrolyzes virion RNA. Model studies with hollyhock mosaic tymovirus show that zinc ions reduce the electrophoretic mobility of virion bottom component, but does not affect the mobility of top component. These studies suggest that heavy metal ions penetrate virion capsids, bind to RNA and reduce virion surface charge.


Chloroplasts from potato virus Y (PVY) infected, healthy or mock-inoculated tobacco (Nicotiana tabacum) were obtained by differential centrifugation and used with tobacco to separate the coat protein (CP) adhering to their outer membranes. Using western blotting techniques, chloroplasts from infected tobacco yielded significant amounts of coat protein, whereas healthy and mock-inoculated controls had none. Using similar amounts of protein for SDS-PAGE, chloroplasts from infected plants had slightly less CP than total sap protein extracts. Reconstruction experiments and treatments with and without thermolysin confirmed that CP was specifically located within the chloroplast envelope. Additional experiments revealed the presence of helper component in chloroplasts. The function of these potyvirus gene products in chloroplasts is unknown; work is underway to create transgenic plants engineered to express these proteins in their chloroplasts.

A236 CELL-FREE TRANSLATION OF THE BEET WESTERN YELLOWS VIRUS STV ISOLATE VIRION RNAs AND IN VITRO TRANSCRIPTS OF CAPSID PROTEIN cDNA CLONES OF ORF-DNA CLONES. S.S. Chin, C. A. Blish, and B. W. Falk. Department of Plant Pathology, University of California, Davis, CA 95616.

Three cDNA clones representing the beet western yellow virus (BWYV) cap protein ORF, readthrough region, and cap protein ORF plus readthrough region were generated by Eco III deletion from a 3.2 kb 3' proximal clone and subcloned into plasmid pBR322. In vitro transcripts were generated from these clones compared with the in vitro translation products from BWYV STv virion RNA. Three major protein products of ca. 66 Kd, 30 Kd, and 27 Kd were seen in the virion RNA preparations. A ca. 21 Kd protein specifically immunoprecipitated with BWYV antiserum and demonstrates that the cap protein was made only from transcripts and not from the virion genomic RNA. Northern blot analysis using cap protein cDNAs revealed the presence of a ca. 2.9 kb RNA in total RNA extracts from BWYV STv-infected plants. These data suggest that the BWYV cap protein is generated via a subgenomic mRNA strategy.


Verticillium dahliae microsclerotia and fertilizer were applied to soil in microplots at three 15 cm depth intervals. Nutrient placement affected host root length density, root diameter, and root infection. Fertilizer significantly increased the proportion of host root length density that occurred in the top 7.5 cm method in the bottom (30-65 cm) interval. The percentage and total number of roots that were infected were reduced by the fertilization treatment.Depth affected the pachagon propagule dynamics. The top (0-15 cm) contained the greatest in-season increase of microsclerotia, 5 to 13.2 mgs/g. Using stepwise multiple regression, inoculum density and root radius explained 75% of variation in root infections. A log-log transformation of percent infection and inoculum density with a slope of 0.61, and explained 82% of the variation. A probability model explained 81% of the variation in root infections. Fertilizer reduced the propagule competency value and increased the rhizosphere width.

A238 SURVIVAL OF PEPHOTYPHUS CINNAMOMI IN RIVER AND FARM POND WATER USED FOR IRRIGATION IN SOUTH AFRICA. Sharon L. von Broembaek. Department of Plant Pathology, Stillwater, OK 74078-9947.

P. cinnamomi is prevalent in river systems of the southwestern Cape Province of South Africa, where river water is used for irrigating crops directly or after holding in farm ponds. Natural populations of Phytophthora spp. declined to be not detectable levels within 3 days with high pH after a period filled with highly infected (>1000 colony forming units/L) river water and were reduced >99% after 7-10 days storage in the laboratory. Axenically produced P. cinnamomi zoospores and chlamydospores stored filtered river water (FWM) survived longest at 8 and 12 C. For zoospores, P. cinnamomi could be recovered after 42 days in FW at 8 C, but extinction was 99% after 10 days in all types of filtered river water. Zoospores suspended in 50 m nylon mesh containers survived up to 11 days in a river and up to 17 days in a farm pond. Storage of infected river water in farm ponds for several weeks could greatly reduce inoculum in irrigation water taken from the surface of these ponds.

A239 CHARACTERIZATION OF GROWTH AND SPORULATION IN VITRO AMONG ISOLATES OF MYCOSPHAERELLA FJENIUS. Luis Jacome and Wolfgang Schuh, Department of Plant Pathology, The Pennsylvania State University, University Park, PA 16802.

Benomyl-sensitive and -resistant isolates of Mycosphaerella fijiensis were obtained from infected banana leaf tissue from Honduras, C. A. Fungal growth and conidia production on mycological agar were monitored over time at 20 C in the dark. After 12 days at 25 C, conidia production on 7 days at 25 C. Higher rates of growth were observed as T increased. No fungal growth was observed at 35C. Growth, expressed as the area under the fungal growth curve (AUCF) responded linearly (R^2=0.84,98) at all T. However, AUCF at 20C was better explained by a quadratic function (R^2=0.71,99) and AUCF (R^2=0.78,99) at all T. Spore production decreased as T increased. Significant differences among isolates were observed.
Disease incidence, severity and latency period were studied at three locations with annual mean temperatures of 23°C, 21°C and 19°C, associated with specific altitudes. Incidence and severity were highly correlated (r=0.8). Incidence and severity increased with increasing temperatures, while LP became shorter. Yield, foliage and disease incidence are closely associated. Therefore, leaf rust follows and intensifies the biennial crop pattern in the typical region. The disease progress curve may differ among years and localities. However, in a typical crop year the disease increases starting in June and reaches its highest values from November to January. During February, March and April, the disease incidence falls, mainly due to active foliage growth. In May and part of June disease incidence is at its lowest level. These results are being used for epidemic forecasting and for timing of fungicide sprays.

### A245

**EFFECTS OF GENOTYPE FREQUENCY ON STRIPE RUST SEVERITY AND EFFECTS OF GENOTYPE FREQUENCY AND STRIPE RUST ON PLANT-PLANT INTERACTIONS IN WHEAT CULTIVAR MIXTURES.** M. R. Finck and C. C. Mundt. Dept. of Botany and Plant Pathology, Oregon State University, Corvallis, OR 97331-2902.

Mixtures and pure stands of five wheat cultivars possessing different race-specific genes for resistance to *Puccinia striiformis* were grown in varying mixture ratios in the field at two locations. Yield components and disease severity were measured on individual plants on a per-cultivar basis. The effects of frequency and density of a genotype on disease severity were dependent on the genotypes in the mixtures and on the disease pressure, suggesting that factors other than frequency and diversity might affect disease severity. Plant-plant interactions between genotypes differed among different mixture ratios, between diseased and non-diseased mixtures, between years, and between locations. This suggests that biotic and abiotic environmental factors greatly affect plant-plant interactions in the field.

### A246


Acceleration or retardation of a stochastic epidemic, attributable to any factor, can be measured by ratios of mean times to epidemic completion for different levels of that factor. When disease is spread by a vector population, the effects of varying levels of vector preference for diseased and healthy plants, or of attractiveness and repulsion of vectors by diseased and healthy plants, measured in this manner, depend on the proportions of plants that are susceptible to the disease.

### A247

**EPIDEMIOLOGY OF PEANUT WEB BLOTCH IN EASTERN NEW MEXICO.** C. M. Liddell, Department of Entomology, Plant Pathology and Weed Science, New Mexico State University, Las Cruces, NM 88003.

Web blotch is a serious foliar disease of Valencia peanuts in eastern New Mexico. The disease is currently controlled by scheduled fungicide applications. A prediction model is being designed to improve efficacy and reduce the frequency of fungicide applications. The causal agent in *Phoma arachidicola* and both teleomorph and anamorph stages are present in the field. The disease is favored by temperatures below 29°C and diurnal cycles of relative humidity above 85% with periods over 95%. Conidia were detected on lesions from field plants at midseason and pseudonidia developed on necrotic tissue after incubation in the laboratory. In 1989, disease incidence was 100% in irrigated field plots and disease severity remained near 10-15% throughout the season. Overhead irrigation increased disease incidence to 100% within 5 days although disease severity remained low due to below normal rainfall.

### A248

**Use of stability analysis to predict the effects of soil moisture on yield of tobacco infected with *Meloidogyne incognita*.** T. A. Wheeler, N. M. Schneider, K. R. Barker.

Department of Plant Pathology, North Carolina State University, Raleigh, NC 27695-7616. USDA-ARS, Oxford, NC.
Flue-cured tobacco (cv. NC27-NF) was transplanted into microplots infested with initial levels (Pi) of Meloidogyne incognita ranging from 0 to 20000 eggs/500 cm³ soil. Numbers of nematodes/plot and leaf weight were determined by sampling destructively 10 times during 1988 and 1989. Stability analysis of 2-linked, differential equations representing the host-parasite system, showed that with moderate irrigation (0 to 41 kPa) a stable, nonoscillatory system was present. With low levels of irrigation (0 to 510 kPa) or Pi >20000, a stable, oscillatory system was common, with no water added, or plant-limiting nutrient deficiencies present, unstable systems developed at Pi lower than 10000. Highest yields were associated with stable, nonoscillatory systems.

Vigorous American chestnut sprouts were inoculated on opposite sides of the stems at 46 and 76 cm from the ground with a virulent isolate of *G. parasitica* (SCS-19) from New Jersey. After 4 wks, the lower cankers were challenged with the isogenic, dsRNA containing hypovirulent isolate (MB88-58) on the margin perpendicular to the original inoculation. This challenge had a significant (P < 0.01) effect on canker expansion using regression analysis. Stems were removed over time, isolations were made from cankers, and conversion was determined by culturable pathogen. An analysis of 3P-labeled recombinant plasmid dsRNA. The first converted isolate from challenged cankers was obtained at day 6. At days 12 and 32 all isolates recovered were converted. In total for 13 harvest dates, 68 of 147 (46.3%) and 2 of 168 (1.2%) of the isolates from the lower and upper cankers were converted, respectively.

A250

ESTIMATION OF WHEAT LEAF RUST ON LEAVES AT DIFFERENT POSITIONS FROM WHOLE PLANT DISEASE DATA. K. V. Sabba Rao, X. B. Yang, and J.P. Snow. Department of Plant Pathology and Crop Physiology, Louisiana Agricultural Experiment Station, Louisiana State University Agricultural Center, Baton Rouge, LA 70803.

In modeling crop-pest interactions in crops, disease data on different leaves on plants is needed. Crop models simulate plant growth by leaf development at different positions on plants. In conventional crop disease surveys, overall disease on a crop is recorded. A method to estimate the disease on leaves at different positions from the whole plant disease severity values and disease progress curves is needed. Leaf rust data collected during the 1986-87 and 1987-88 wheat growing seasons were used to study the relationship between rust on leaves at different positions and mean disease per plant. Theoretically, environmental effects are the same for different leaves and the leaf infection rate (d) was assumed to be identical for all leaves. Development of leaf rust on the ith leaf was expressed as $d_i = d_0 \cdot e^{-k \cdot X_i}$ or in an integrated form $x_i = x_0 \cdot e^{-k \cdot T}$, where $x_i$ is the rust on the ith leaf at time $t$, $x_0$ is the number of leaves per plant, $X_i$ is the mean disease on the plant at time $t$, and $t$ is the time of leaf initiation. The equation fitted the field data and gave satisfactory prediction.

A251

COST/BENEFIT COMPARISON OF A WEATHER-BASED FUNGICIDE SCHEDULING PROGRAM VERSUS A CALENDAR SPRAY PROGRAM TO CONTROL LATE LEAF RUST OF PEANUT. E. West, W. Nutter, Jr., and F. D. Mills. Dept. Plant Pathology, Univ. of Georgia, Athens, GA 30602 and Dept. of Agriculture, Ablene Christian University, Ablene, TX 79699.

Field experiments were conducted at Athens, Plains, and Tifton, GA, in 1986 and 1987 to compare a weather-based forecasting system and the currently used calendar-based fungicide spray program. In 1988 eight calendar based sprays were applied at each location whereas only three sprays were applied according to the forecast in Athens and Plains, and four sprays were used at Tifton. Seven calendar-based sprays were applied at each location in 1989. The models were applied to the forecasting model. Although disease levels at harvest were lowest when the calendar-based schedule was used, pod yields were equal to or slightly higher when the forecasting model was followed. Risk-rated yields were higher for the forecasting model (14 to 100 $ net return per ha higher than the calendar-based model). While the accuracy for profit remained unchanged (99% for both systems).

A252

CONVERSION OF CRYPTOCOCCUS PARASITICA INDUCED CANCERS ON AMERICAN CHESTNUT STEM. R. J. Bednek and R. L. Kovacev. Department of Plant Pathology, Rutgers University, New Brunswick, NJ 08903.

A253

OCCURRENCE OF BASAL DECAY IN WESTERN HEMLOCK AS A FUNCTION OF TREE HEIGHT AND DIAMETER. D. C. Shaw and R.L. Edmonds, College of Forest Resources, AR-10, University of Washington, Seattle, WA, 98195.

Heterobasidion annosum and Armillaria species cause basal decay in coastal western hemlock. We randomly sampled 35 year-old trees in Chinese chestnut forest and unthinned forest stands 20 years after thinning on the Olympic Peninsula, Washington. Armillaria sp. was consistently present on small dead trees on an area of decay infections. H. annosum was present on trees covering the range of heights and diameters. This supports the presence of Armillaria as an opportunistic and atack the litter and vine trees in western Washington. This organism, however, can successfully attack trees of any height and diameter. Analysis of the distribution of basal decay by height and diameter of host trees aids in understanding the biology of these decay organisms.

A254

EVIDENCE FOR HERITABLE VARIATION IN RESISTANCE TO BLIGHT IN CHINESE CHESTNUT. Frederick K. Hoiberg, American Chestnut Foundation, Rt 1, Box 77, Meadowview, VA 24361.

Arthur Graves produced a number of first hybrids of Chinese and American chestnut between 1937 and 1939. Twenty-five of these were planted at the White Memorial Foundation in Litchfield, CT, in the late 1940s. Sixteen were surviving in 1989. Four of the surviving trees are progeny of one hybrid, tree R177, and five are from another hybrid, tree R177. The American chestnut male parent may have differed depending on the Chinese chestnut female parent. Four of five of the original trees with Chinese parent R177 were living in 1989, whereas all four of the original trees with Chinese parent R177 were killed by naturally occurring chestnut blight; the trees survive due to sprouts which originated after the original stem was killed. These data suggest that there are heritable differences in blight resistance among Chinese chestnut trees.

A255


Pitch canker of Monterey pine recently appeared in Santa Cruz County, where it is associated with the feeding activity of scolytid and ambelid beetles. *Pseudomonas subtillis* was isolated from 15-29% of both twig and cone beetles (*Pityogastus carrelli, Ips mexicanus, I. paracensfrontus and Armillius punctulatus*). The fungus was isolated from less than 10% of woodcote beetles. It was isolated from the twigs, branches and cones these beetles infested. Cone whors accounts for 30-60% of the infection courts, depending on the site. We hypothesize that cone and twig beetles spread the fungus, while Ips spp. kill diseased trees. Monterey pine has continuous cone production, possibly because of its shorts life span relative to other pines.

A256

Regions of water depletion surrounding roots of lobel pine seedlings were observed with magnetic resonance imaging (MRI). The relationship between signal intensity, water binding, water content, and instrument settings is complex. However, a linear relationship was found between water content of wet sand (5-25% water) and signal intensity. This presents the possibility of determining water content from signal intensity normalized against a reference material. A 5mm diameter NMR sample tube filled with a reference solution was placed in the field of view of the wet sand. For a pulse sequence repetition time of 800-3200 ms at 2T, a linear relationship was observed between water content and signal intensity normalized against 25% CuSO4 (0.07M/75% D2O solution. A curvilinear relationship fitting a polynomial function was found for water content and signal intensity normalized against a solution of 25% CuSO4 (0.05M/75% D2O). These relationships are being studied further, with the objective of quantitatively describing water uptake dynamics of plant roots.

A257
SPECIFIC DIAGNOSIS OF ASH YELLOWS BY MEANS OF BIOTINYLATED CLONED DNA PROBE. R. E. Davis, W. A. Sinclair, J. M. Lee, and E. L. Dally. Microbiology and Plant Pathology Laboratory, Agricultural Research Service, USDA, Beltsville, MD 20705, and Department of Plant Pathology, Cornell University, Ithaca, NY 14853.

Cloned biotin-labeled fragments of DNA from Ciceras租as infected with an Ashy MLO were employed as probes in dot hybridizations. Four probes all hybridized with nucleic acid from C.租as containing the Ashy MLO but not with nucleic acid from healthy C.租as. These probes were shown to contain chromosomal DNA of the MLO. Under stringent conditions of hybridization (52 C), none of the probes hybridized with nucleic acid from C.租as carrying the MLOs associated with 10 other plant diseases. Two probes were tested for diagnosis of Ashy in naturally infected white as (Fraxinus Americana). These probes hybridized with nucleic acid from diseased trees but not with that from healthy trees. These probes will facilitate searches for insect vector(s) and alternative plant hosts of Ashy MLO.

A258

Isolates of T. symbiotsite (Ts) and other fungi were obtained from beetles emerging from a provenance plantation of white Fir trees undergoing attack by the fir engraver. Additional isolates of Ts were obtained from infested stands in selected geographic areas corresponding to seed sources of the provenance plantation. Growth rate of Ts on 1.25% malt extract medium amended with 800 mg/ml cycloheximide was about 80% of non-amended medium. Cultures of Ts isolates from different geographic areas differed in rates of pigment development and growth on both media. Also, morphological features of conidiphores and conidia of this fungus do not conform to descriptions of other Trichosporum species. In the provenance study plantation, the insect parasite Benavaria bassiana was isolated in high frequency from emergent beetles and may relate to population density of the bark beetles.

A260

Black spruce seedlings lifted from compartments known to have Cylindrocladium root rot at two Ontario forest nurseries were outplanted and monitored for survival and growth through two growing seasons. Significant levels of mortality caused by Cylindrocladium floridatum Sob. & C. P. Seym. occurred in seedlings with symptomatic shoots or with main root lesions and non-symptomatic shoots, compared to seedlings with only lateral root lesions or non-symptomatic roots and shoots. More than 30% of the outplanted seedlings died by the end of the second growing season; >76% of this mortality occurred in the first 6 months. Differences in annual growth increment among the four categories of surviving seedlings were not significant at the end of the second season.

A261
TOMATO MOSAIC TOBAMOVIRUS TRANSMITTED FROM WATERS IN THE ADIRONDACK MTS. Y. Jacobii and J. D. Castello. SUNY College of Environmental Science and Forestry, Syracuse, NY 13210.

Tobamoviruses, consistently recovered from waters draining forest stands in the Adirondack high peaks, were identified as tomato mosaic virus (ToMV) based upon serological and host range tests. Three water isolates were compared with the L-strain and dogwood and lillac isolates identified by host range, agar gel double diffusion (AGDD) and indirect ELISA. In reciprocal AGDD, tests all six isolates were identical. In indirect ELISA, however, the L-strain and the lillac isolate were identical to only one of the water isolates and could be distinguished from the other two isolates by different reciprocal dilution endpoints against twelve tobamovirus antisera. The three water isolates also were distinguishable from the lillac isolate by symptom production in indicator plants. ToMW was consistently detected by ELISA in the roots but not the needles of inoculated balsam fir (Abies balsamea (L.) Mill.) seedlings five months post inoculation.

A262
TOMATO MOSAIC TOBAMOVIRUS TRANSMITTED FROM LILAC. J. D. Castello and C. R. Hiben. SUNY College of Environmental Science and Forestry, Syracuse, NY 13210 and Brooklyn Botanic Garden Research Center, 712 Kitchawan Rd., Ossining, NY 10562.

A virus was mechanically transmitted to Xanthi tobacco from symptomatic foliage of lilac, Syringa x nancea McHale with 'Rutilant' growing at the Arnold Arboretum, Jamaica Plain, MA. Field symptoms consisted of foliar chlorotic mosaic and mottle. Dieback symptoms and non-symptomatic shoots or mottle was also infected with MLO, as determined by DAPI. It was not possible to attribute particular symptoms to virus infection alone. Based upon host range, symptomatology, and serological tests the virus was identified as tomato mosaic virus. The virus was back transmitted to healthy seedlings of S. x henryi cv 'Lutea' and white ash (Fraxinus Americana L.). No symptoms developed in lillac but a systemic chlorotic mottle and mosaic developed in white ash. The virus was subsequently recovered from young foliage of both lilac and white ash.

A263
DNA RESTRICTION FRAGMENT ANALYSIS OF NECTRIA COCCINEA VAR. FAGINATA AND N. GALLIGENA ASSOCIATED WITH BEECH BARK DISEASE. E. M. Mahoney. USDA Forest Service, Hamden, CT 06514, and Cornell University, Department of Plant Pathology, Ithaca, NY 14853.

Both Nectria coccinea var. faginata (Ncf) and N. galligena (Ng) kill American beech (Fagus grandifolia) infected by the beech scale (Cryptococcus fagiisporus) in North America. Ng is a globally distributed pathogen on many arboresal species, but Ncf is known only on American beech and only in northeastern North America. The phylogenetic relationship of these fungi was explored through restriction fragment analysis of total genomic DNA from single ascospore isolates obtained from American beech. Southern blots representing four different restriction enzymes were probed with randomly selected low-copy number, nuclear DNA clones derived from isolate EM-252 of Ncf. Analyses indicated that Ng is the more variable species and that Ncf has not evolved recently from Ng.
RESPONSE OF FOUR TREE SPECIES TO VARYING DOSES OF OZONE IN NORTHCENTRAL PENNSYLVANIA IN 1988 AND 1989.

M. Simini, J. M. Skelly, and D. D. Davis, Department of Plant Pathology, 211 Buckhout Lab, The Pennsylvania State University, University Park, PA 16802.

Four 2-year-old seedlings of each of Prunus serotina Ehrh., Liriodendron tulipifera L., Quercus rubra L. Wangen., and Acer rubrum L. were planted in each of 16 plots in a randomized complete block design at 3 sites (lat. 41° 19', long. 79° 02'; lat. 41° 07', long. 78° 31'; lat. 41° 20', long. 77° 20') in northcentral PA. Seedlings were exposed to ambient air or to charcoal-filtered air in open-top field exposure chambers containing 95%, 60% or 40% of the ozone (O_3) in ambient air. The total 03 dose was greater at the western sites than at the eastern-most site and 03 was much greater in 1988 than in 1989. Foliar stipple injury of E. pteronia and L. tulipifera was correlated positively with 03 dose at all sites in 1988 and 1989, and premature coloration and abscission of foliage was correlated positively with 03 dose at the high 03 site during both growing seasons. Seedlings of Q. rubra and A. rubrum were unaffected by elevated levels of 03 in this study.

A265

Relationships among forest litter burning, seedling density, and black cherry leaf spot severity in northern Pennsylvania.

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Lithium, caused by Blumeriella jaapii (Kohm.) v. Arx, causes defoliation and death of black cherry (Prunus serotina Ehrh.) seedlings. Litter in ten 5-m radius plots in a maturing stand was burned prior to seedling emergence; ten similar plots were not burned. Survival, number of leaves, and disease severity (foliage estimated visually as ≤10%, 11–50%, or ≥51% symptomatic) were recorded on four dates for the same 25 current-year seedlings/plot. Black cherry seedlings were counted in midsummer in the square foot surrounding each sample seedling. Numbers of living sample seedlings, sample seedlings with leaves, leaves/sample seedling, and sample seedlings in the lowest severity class, were greater in burned than unburned plots on all dates. These variables tended to be correlated positively with seedling density in burned plots (mean=2.5) and negatively with seedling density in unburned plots (mean=8.1). Practices that reduce pathogen survival (in leaf litter) and seedling density may lessen leaf spot's impact on regeneration.

A269

GENETIC CONTAINMENT SYSTEMS FOR BIOCONTROL AGENTS. D. C. Sands and R. V. Miller. Dept. of Plant Pathology, Montana State University, Bozeman 59717, and Mycogen Corporation, Ruston, LA 71270.

Biocontrol agents, whether natural or genetically manipulated, cannot be released until perhaps the are considered environmentally safe. Yet, it is possible to adequately assess risks with our present technology due to limited conclusions possible with microcosm studies and the inability to detect minute numbers of a given microbe. Combined with the lack of prior data bases, it is difficult, if not impossible, to predict the impact of releasing most biocontrol agents. We propose that microbes be genetically constrained, at least during preliminary small-scale releases aimed at ascertaining safety and efficacy. Such constrained microbes could include auxospores in a mutant phage that is not commonly free in the environment, temperature-sensitive isolates, non-sporulating strains, isolates incapable of surviving drying, conditional lethal mutants, and/or use of suicide plasmid systems.

A270

CONDIAITION OF COLLETOTRICHUM TRUNCATUM IN SUBMERGED CULTURE: THE NUTRITIONAL ENVIRONMENT INFLUENCES CONIDIAL GERMINATION AND DISEASE DEVELOPMENT ON SESEBIA EXALTATA. D.A. Schisler, M.A. Jackson, and R.J. Bothast, USDA-ARS, NERRC, Peoria, IL 61604.

Colletotrichum truncatum has considerable potential for development as a mycoherbicide for controlling the noxious weed, hemp sesbania (Sesbania exaltata). Conidia of C. truncatum (NRRL 13737) were produced in semi-defined liquid media with C/N ratios of "40:1", "15:1", and "5:1". Conidia produced in "5:1" medium were longer and thinner than "15:1" and "40:1" conidia and a higher proportion contained 2, rather than 1, nuclei per conidium. After either 6 or 12 h on cellulose membranes, a greater proportion of "5:1" conidia germinated than "15:1" and "40:1" conidia, but treatments did not differ when germination was assessed on attached S. exaltata leaves. Results from equality of variance tests implied that the leaf environment had the greatest influence on "15:1" conidial germination. All conidial treatments reduced plant growth, though "5:1" and "15:1" conidia induced greater reductions in shoot height and shoot dry weight than did "40:1" conidia.

A271

SUBMERGED-CULTURE SPOREULATION IN ALTERNARIA SPP. AND IDENTIFICATION OF NEW CONIDIA BY FLUORESCENCE MICROSCOPY. K.M. Howard, M.G. Smart, and R.J. Bothast, USDA-ARS, NERRC, Peoria, IL 61604.

Alternaria crassa and Alternaria cassiniae are important fungal pathogens of Jimsonweed (Datura stramonium) and Sicklepod (Cassia obtusifolia, respectively). Both show potential for use as mycoherbicides. Failure to produce conidia in submerged culture has limited commercialization of these weed pathogens. Studies were conducted in column bioreactors containing minimal medium (MM) or Richards V8 (RV-8). Conidial inocula were stained with Nile red fluorescent dye and added to the column bioreactors yielding final concentrations of 1.2 x 10^5 and 8.3 x 10^5 conidia/ml for A. crassa and A. cassiniae, respectively. Between 1.5 x 10^6 and 3.3 x 10^6 conidia/ml were recovered for each fungus after 96 h in MM and approximately 30% of the total spores recovered were identified as new
conditiva. New conidiat were distinguished from inocula by their inability to fluoresce. This is the first report of either fungus sporulating in liquid culture.

A272


Super sweet corn, especially hybrids containing the SU-2 gene, is prone to stand establishment problems. We initiated a program to select emergence-promoting rhizobacteria to improve establishment. Greenhouse trials indicated that several Streptomyces and Bacillus strains were able to elicit significant effects on emergence, final stand and vegetative biomass, although some variation in performance could be attributed to culture. In initial field trials in Florida (1988), several strains, including S. proteomycans strain 1-102 and P. putida strains GR12-2 and 61-9A elicited significant effects on emergence. Ontario field trials in 1988 demonstrated a significant increase in final stand by P. fluorescens 31-12 and S. liquefaciens 2-68. Both strains as well as 1-102 also had a significant effect on yield. Subsequent studies were focused on compatibility with the fungicide Captan, and in 1989 Ontario field trials, strain 1-102 elicited a significant increase in stand relative to the Captan control when applied to treated seed.

A273

DUAL-STAIN FLUORESCENCE MICROSCOPY TO OBSERVE THE INTERACTION OF PYRENOMORA WITH A BIOCONTROL FUNGUS IN WHEAT STRAW. W.F. Pfender, J. Rabie, and H. King. Dept. of Plant Pathology, Kansas State University, Manhattan, KS 66506-5502.

Pyrenophora tritici-repentis, causal agent of wheat tan spot, was visualized by means of indirect fluoresce antibody staining, using antibodies produced in chicken and anti-chicken IgG conjugated to fluorescein as FITC. Ligninomycetes, a basidiomycete that can reduce acetyl acetoacetate to pyruvate. Pyrenophora in straw, was stained with thymidine (as TRITC) conjugated to wheat germ agglutinin. The interaction was observed in sterilized wheat straw inoculated with the two fungi. To prevent autofluorescence of plant tissue from masking the TRITC staining, the tissue was counterstained with toluidine blue-O. With this method, we observed that the fungi have different colonization patterns. Also, Ligninomycete conidia grew in close association with Pyrenophora, and could occasionally be seen growing inside the hyphae of the pathogen as early as 24 hr after their mycelia had grown into close proximity.

A274


Biomass of organisms to be used in biocontrol must be inexpensive to produce in liquid fermentation, capable of withstanding drying, and insensitive to environmental fluctuations, and have a long shelf life. Different minimal media were compared and minimal media such as Czapek Box or Richard's media were found to produce high proportions of conidia, but overall yields were low. Addition of V8 juice to these media increased yields by 8 to 10-fold, yielded only 1% of the conidia resistant to vacuum drying. However, addition of osmoticants, i.e. polyethylene glycol (PEG), MgCl2, or mannitol to Richard's medium with V8 juice (RMB), provided a high level of conidial production, and these conidia were resistant to vacuum drying. Further, conidia produced in RMB+PEG were insensitive to storage relative to humidity, more effective in biocontrol net treatment of cucumber against infection of Pythium than those produced in RMB, and survived longer on treated seeds.

A275

BIOLOGICAL AND CHEMICAL CONTROL OF RHIZOCTONIA SOLANI AG-4 IN SNAP BEAN. D. R. Sumner, J. A. Lewis and R. D. Gitaitis.

1Department of Plant Pathology, University of Georgia, Coastal Plain Experiment Station, Tifton, GA 31793 and USDA/ARS, Beltsville, MD 20705.

Snap bean cv. Strike was grown in field plots of Tifton loamy sand infested with Rhizoctonia solani anastomosis group (AG), as a double-crop following corn. For three successive years, Fluitolani, PCNB, metalaxyl, metalaxyl + flutolanil, Cladiocins vires (GI-21), Trichoderma hamatum (TRI-3), the binucleate Rhizoctonia-like fungus CAG-2, and Pseudomonas capicola were applied as treatments for two or more years. Fluitolani consistently increased plant stand, reduced populations of R. solani in soil and increased yield of green pods compared with the control. Fluitolani was significantly more effective than CAG-2 and TRI-4 in reducing R. solani yields under different conditions. Biologicals were variable, but each treatment increased plant numbers in 1 of 3 yrs, and GI-21, CAG-2, and P. capicola increased yield in 1 of 3 yrs.

A276

DAMPING-OFF OF MARIGOLD IN RELATION TO MICROBIAL RECOLONIZATION OF STEAMED AND FUMIGATED SOILS. T. Isaketz, A. R. Weinhold, J. G. Hancock, and M. N. Schrot, Department of Plant Pathology, University of California, Berkeley, CA 94720.

The incidence of damping-off of marigold following the addition of Rhizoctonia solani to two soils which had been steamed or fumigated with methyl bromide/chloropicrin was compared with the microbial recolonization of these soils. Disease incidence was highest in freshly steamed and fumigated soils, which coincided with a marked reduction in bacterial, fungal and actinomycete populations. The addition of 1 t non-treated soil (w/w) to freshly steamed or fumigated soil resulted in an increase in the population of bacteria and a decrease in disease incidence. The population of fungi or actinomycetes did not increase. A steamed soil which was recolonized nine weeks by survivors was more suppressive to damping-off than the non-treated soil. In this soil, microbial populations were at the same level as non-treated soil, but species diversity was lower. These results suggest that microflora can be manipulated in treated soils to make them suppressive to soilborne pathogens.

A277


Alternative methods for control of the soil-borne plant pathogens Fusarium solani, Rhizoctonia solani and Pythium ultimum are being sought since adequate chemical control has not been achieved, and there is heightened concern over toxic accumulations of agri-chemicals. More than 100 plant growth-promoting rhizobacteria (PGPR) were screened for their ability to protect white bean seedling roots from these three pathogens. Results indicate that mode of application has a major impact on the efficacy of PGPR. There is a positive correlation of the ability of strains to inhibit fungal growth on solid media and their ability to suppress pathogen activity on white bean seedlings. Similarly, there is a correlation between antibiosis in liquid broth and the application a PGPR to pathogen-infested soil. In most cases, PGPR were not as effective as soil treatments were not as effective when applied as a seed treatment.

A278

STUDIES ON THE MECHANISM OF HIGH VARIATION IN PYRICULARIA ORYZAE (MAGNAPORTHE GRISEA). B.C. Wu and C. W. Magill, Department of Plant Pathology and Microbiology, Texas A&M University, College Station, TX 77843.

Many mechanisms (i.e. mutation, heterokaryosis, aneuploidy, sexual and assexual recombination) have been proposed, but none fully explains, the unusually high pathotype variation observed in Pyricularia oryzae. Careful examination of monochonal cultures of unstable isolates over 4 generations revealed an apparent mutation rate of one out of sixty. Variability is not random in the population, but is exhibited only in progeny from some individuals. Frequent reversal to the parental type is not observed, and chromosome rearrangements are associated with pathogenically changes. We propose that a transposable element is present in the genome of P. oryzae and that it is active in unstable strains. Insertion into an avirulence allele can inactivate it, causing a change in pathotype. To test this hypothesis, we are examining both stable and unstable isolates from the same monochonal culture for genomic differences at the molecular level.

A279


Colletotrichum gloeosporioides isolates causing post bloom fruit drop of citrus show variability in colony characteristics (secreting and non-secreting) and virulence. Isolates from diseased plants from Florida and different geographic locations of the world have been tested for polymorphism in 26S ribosomal DNA (rDNA), p-nitrophenyl butyrate esterase (PB activity) and chromosome—
sized DNA molecules separated by CHEF gel–electrophoresis. Preliminary observations based on DNA restriction fragment length polymorphism using a Neurospora crassa 28S rDNA probe suggest that two distinct forms of rDNA are present in C. tropicalis and that from single-celled isolations of sectored cultures may contain only one of the two forms of rDNA, and isolates may show greater than 50% reduction in PBE activity compared to wild type. Experiments in progress seek to understand the vegetative rearrangement of rDNA and PBE activity by examining the molecular karyotype of wild type and derived strains.

A280
ANALYSIS OF CHROMOSOME-SIZED DNA MOLECULES IN STRAINS OF TWO FORMAE SPECIALES OF PUSARIUM OXYSPORUM AND PROTOPLAST FUSION PRODUCTS E.A. Momoh, F.N. Martin, J.W. Kimbrough, and H.C. Kistler, Department of Plant Pathology, University of Florida, Gainesville, FL 32611.

To determine whether genetic recombination could occur, protoplasts of Pusarium oxysporum f.sp. conglutinans strain ATCC 9990 (a cabbagew pathogen), and of P. oxysporum f.sp. raphani strain ATCC 16601 (a radish pathogen) were fused in the presence of Prot-Fusion was confirmed by restriction fragment length polymorphism (RFLP) analysis of nuclear DNA. Chromosomes of parental strains and single-spored derived cultures from fused strains were separated using a contour clamped homogenous electrical field (CHEF) gel electrophoresis. Chromosome-sized molecules differed greatly in mobility between parental strains, with a minimum of 11 and 8 bands detected for ATCC 16601 and ATCC 9990, respectively. Chromosomal banding patterns of fusion products were identical to parental strains.

A281
RFLP GROUPS AND PHYSICAL MAP OF MITOCHONDRIAL DNA FROM PUSARIUM OXYSPORUM F.S. NIVEUM. D. H. Kim, R. D. Marlyn, and C. W. Magill, Department of Plant Pathology and Microbiology, Texas A&M University, College Station 77843.

A restriction map of mitochondrial DNA (mtDNA) from Pusarium oxysporum f.sp. niveum, causal agent of Pusarium wilt of watermelon, was constructed to facilitate understanding of the relationship among races and to identify variable regions in mtDNA among RFLP groups. Mitochondrial DNA was isolated from a race 1 isolate and digested with four restriction enzymes: EcoRI, HindIII, HpaI, and PstI, either singly or in selected pairs. The digestions yielded 4, 9, 21, and 10 fragments with the respective enzymes. Restriction patterns of mtDNA were examined on agarose gels and by hybridization with cloned PstI fragments from FON18 and RFLP hybridization with each PstI fragment probe was used to determine the physical order of restriction sites based on overlapping regions. Among the cloned PstI fragments, fragment FON3 (1.5kb) had homology with HpaI and the 9.1kb and 2.0kb fragments and seems to be an important region in discriminating among the five RFLP groups detected within the 44 isolates examined. The RFLPs among the isolates appeared to be primarily a result of base substitutions within the mtDNA; however, one insertion and one deletion event was detected in two of the isolates.

A282
ISOLATION AND CLONING OF A PROTEIN KINASE HOMOLOG FROM COCCILIOBULUS HETEROSTROPHUS. S. C. Deacon, T. Tewell, and W. E. Timmerlade. 1st Dept. of Plant Pathology, Clemson Univ., SC. 2nd Dept. Plant Pathology and Genetics, Univ. of Georgia, GA.

Increasing concern about food safety necessitates the need for new, safe disease control strategies. One approach is to target genes controlling fungal propagation and dissemination. Several important genes regulating sporulation have been isolated from A. nidulans and have been used to identify corresponding genes from closely related pathogenic fungi. Other sporulation specific genes have been cloned, but nothing is known about their function. One such gene, CAN4, is expressed to high levels during conidiation in A. nidulans. Sequence analysis reveals two open reading frames. The first potentially encodes a hydrophobic protein of 15.6 kDa, pl 4.5 and the other encodes a highly basic protein of 11.1 kDa, pl 12.1. Both reading frames contain the same two small introns, with splice junctions typical for A. nidulans. Forced expression of CAN4 in vegetative hyphae results in reduced hyphal extension, but does not initiate asexual reproduction. When CAN4 is deleted, conidial appres appear darker and encased in a droplet of liquid. The conidia themselves seems to be less hydrophobic, suggesting a wild type. Expression from the conidial cell wall lacks the hydrophobic rodlet layer. We propose that CAN4 is required for a structural component of the conidial cell wall and may contribute to the survival of the conidia.

A285
COMPARISON OF PROMOTER STRENGTH IN COCCILIOBULUS HETEROSTROPHUS. Sally Van Weert and O. Yoder, Department of Plant Pathology, Cornell University, Ithaca, NY 14853.

Translational fusions were made between lacZ and the promoter regions of 3 C. heterostrophus genes: GPD1, TRP1, and PRO1. Each fusion fragment was inserted as a single copy at the same chromosomal site in C. heterostrophus and assayed for expression by growth on plates that repressed endogenous δ-galactosidase activity. The GPD1::lacZ fusion expressed 6 times more δ-galactosidase activity and had a more abundant transcript than either the TRP1::lacZ or PRO1::lacZ fusion. These promoters will be useful to study the effects of altered virulence gene expression on host-parasite relationships of C. heterostrophus.

A286
MOLECULAR CHARACTERIZATION OF MITOCHONDRIAL GENOME IN CRYPTOCOCCUS PARASITICA. N. Mahanti and D.W. Fulbright, Department of Botany and Plant Pathology, Michigan State University, East Lansing, MI 48824.

One hypovirulent strain of Cryptococcus parasitica (CL25) from Michigan has all the characteristics of dsRNA associated hypovirulent strains but harbors no detectable amounts of dsRNA. Genetic studies with CL25 show that a cytoplasmic agent other than dsRNA might be involved in the CL25 type of hypovirulence. Restriction digest analysis of the mitochondrial genome of both hypervirulent and hypovirulent strains using the restriction enzyme Sau3A showed that a band of about 2.5 kb is present in the CL25 strain but absent in other strains screened so far including CL25-16, a virulent strain from the same collection of the CL25 strain has been cloned and the relationship of this RFLP fragment with the CL25 type of hypovirulence is under investigation.

A287

Although the presence of dsRNA molecules is closely correlated with hypovirulence in C. parasitica, little is known about the
role dsRNA plays in the reduction of fungal virulence. To elucidate this role, dsRNA cloned into genomic vectors in G.
parasiticus strain GH2 were made. These dsRNA clones were then
used to probe Southern blots of total fungal DNA. Homology was
detected between a dsRNA clone of GH2 dsRNA and several G.
parasiticus strains, both virulent and hypovirulent. Using the
polymerase chain reaction (PCR) technique, a product which shares
homology with a GH2 dsRNA clone was produced from the DNA of
several strains. This PCR product has been cloned, and will
be used as a probe to clone this entire GH2 region of homology
from the fungus genome.

A288
MOLECULAR CHARACTERIZATION OF THE BETA-TUBULIN GENE FROM
BENOMYL-SENSITIVE AND BENOMYL-RESISTANT FIELD STRAINS OF
VENTURIA INAEQUALIS. H. Koernraadt, S.C. Somerville and A.L.
Jones. Dept. of Botany and Plant Pathology and Pesticide
Research Center, Michigan State University, East Lansing,
MI 48824.

Benomyl-resistance in Venturia inaequalis has been attributed to
mutations in the beta-tubulin gene. Field strains with
 differing levels of resistance provide a resource for
characterizing the molecular changes responsible for the
 varying degrees of benomyl resistance. The beta-tubulin gene
from a sensitive and a highly resistant isolate of V.
inaequaaliis were cloned from genomic libraries using a
Phytophthora parasitica beta-tubulin probe. Sequence analysis showed that the V.
inaequalis beta-tubulin gene has six introns and encodes a protein of 446 amino-acids.
A comparison of the sequences of the beta-tubulin clones from the
benomyl-sensitive and benomyl-resistant field strains will be
presented.

A289
A DISPERSED, REPETITIVE DNA ELEMENT FINGERPRINTS MYCELIAL
COMPATIBILITY GROUPS IN FIELD SAMPLES OF SCLETORINIA
SCLEROTIORUM. L.M. Kohn and J.B. Anderson. Dept. of Botany,
University of Toronto, Erindale College, Mississauga, Ontario, L5L 1C6.

Sixty-four strains of Scelerotinia sclerotiorum were obtained from
diseased plant tissues, and from soil samples, in Ontario. Mycelial
pairings of the strains were made, and then retested after 19 days. In
some cases, the strains remained distinct, while in others, they
merged. Among the 34 strains of the first field, 6
mycelial compatibility groups (MCGs) were recognized, the largest group
containing 19 strains. In the second field, 26 MCGs were identified. These
MCGs were characterized by unique combinations of restriction
endonuclease fragments. From these data, it was concluded that the
MCGs were composed of genetically distinct "individuals".

A290
AnalYsis of isoymes of two anatoMosis groups of
Rhizoctonia solani isolated from potato. J. Larocque, S.
Jabaji-Hare and P. M. Charest. Dépt. de Phytologie,
Université Laval, Ste-Foy, Québec, Canada, G1K 7P4.

Forty-eight isolates of Rhizoctonia solani belonging to anatoMosis
groups (AG) 3 and 9 and from different geographical regions were
analyzed for isozyme polymorphism. The activity of 20 enzymes was
screened using starch gel electrophoresis. Activity was detected for 7
enzymes, the scorable protein banding patterns of which were
analyzed using starch gel electrophoresis. The relative mobility of
values of electrophoretic bands were subjected to unweighted pair
mean average cluster analysis and principal coordinate anal-
ysis. Two major clusters were obtained matching the AG of 3 and 9
with some intermediate variation. Intra-group variation corresponded to
the isolates' geographic origins. The resulting correlations between
the present isolate grouping and the enzyme patterns reported here
reinforce the anatoMosis grouping concept and demonstrate that
enzyme patterns constitute a useful and practical taxonomic tool.

A291
protoplast formation and transformation of the
phytopathogenic fungus Ustilago hordei. K. E. Duncan
and D. D. Pope, Department of Plant Pathology, University of Georgia,
Athens, GA 30602.

Protoplasts of the barley smut fungus, Ustilago hordei, were generated by
treating spores for one hour in a potassium buffer (0.6M KCl, 50mM
MES) and the enzyme mixture Novozym 234® (20 mg/ml). Cell walls
were enzymatically removed from spores after this treatment and eight
percent of resulting protoplasts regenerated on Vogel's complete medium
amended with 1M sorbitol. Protoplasts stabilized in buffer (40% PEG
(w/v), 50mM CuCl2, 10mM Tris-HCl (pH 7.5), 10mM EDTA) containing
KCl (1M) were treated with pCMV DNA, containing a gene for
resistance to hygromycin. In one experiment, one hundred and forty-five
putative transfectants per µg of DNA were recovered after plating on
medium amended with sorbitol (1M) and hygromycin (50 µg/ml).
The transformation rate reported here is three- to fourteenfold higher than
previously reported.

A292
MycoParasitism of scelerotium rolfsii sclerotia by GlocIadium
Virens. D. J. Collins and G. C. Papavizas. USDA-ARS, Biocontrol of
Plant Diseases Laboratory, Beltsville, MD 20705.

MycoParasitism of S. rolfsii scelrotia by G. virens was studied by
light and scanning electron microscopy. To examine
mycoParasitism an agar plug with mycelia of G. virens was placed in the
center of a petri plate containing PDA. S. rolfsii was placed at the periphery
of the plate and incubated for 1 week at 25 °C. On PDA, G. virens
inhibited mycelial growth from sclerotia and prevented formation of secondary
sclerotia by S. rolfsii. Scanning electron micrographs of parasitized
sclerotia showed extensive colonization and sporulation on the
sclerotial surface and degradation of sclerotial walls. Cross
sections of sclerotia, examined by light microscopy, revealed
penetration of the sclerotial rind by hyphae of G. virens, and
extensive internal colonization.

A293
Two potential mechanisms by which atoxicogenic strains of
Aspergillus flavus prevent toxicogenic strains from contaminating
Cottonseed. F. J. Cotty, P. Bayman, and D. Bhattachary. USDA,
ARS, Southern Regional Research Center, 1100 Robert E. Lee
Blvd., New Orleans, LA 70124.

Atoxicogenic strains of Aspergillus flavus can reduce aflatoxin
production by toxicogenic strains when developing cotton bolls
are inoculated simultaneously with both strains. Relative
activities of toxicogenic and atoxicogenic strains to infect and
colonize developing seed were demonstrated with mutants unable
to reduce nitrate. Results suggest that competitive exclusion
may partially explain the efficacy of atoxicogenic strains.
Atoxicogenic strains similarly in vitro and in vivo. However, it did not
inhibit the growth of the toxicogenic strain on agar plates. The
atoxicogenic strain reduced toxin production even when mycelial
pods of the two strains were grown independently for 24 hrs
prior to co-cultivation. Thus, direct inhibition of toxin
production may be a second mechanism by which atoxicogenic
strains prevent contamination.

A294
Control of Soft Rot Erwinias with Bacteriophages.
Cynthia G. Fayre, Diane E. Conceolo, and J.A. Bartz, Plant Pathology
Dept., University of Florida, Gainesville, FL 32611.

Bacteriophages of Erwinia carotovora subsp. carotovora were isolated from
lake water by a standard enrichment technique. At least 16 different
phages were identified based on host range against 71 strains of soft rot
erwinas. One phage caused plagues in lawns of at least one strain of each of
the three major soft rot erwinas, e.g., E. c. atroseptica, E. c. carotovora, and
E. chrysanthemi. Certain phages infected up to 65% of the strains of E. c.
carotovora. However, none of the phages caused plagues in lawns of
E. herbicola. Bacterial soft rot erwinas pathogenicity was prevented when
12 µl of 10^6 cfu E. c. carotovora/ml. When the concentration of the
inoculum was 10-fold greater, some decay occurred, but lesion diameters
were reduced by the bacteriophage.

A295
The stimulation of pythium ultimum by seed volatiles and the
interaction of Pseudomonas putida. T.C. Faulk. Dept. of Plant
Sciences, Macdonald College of McGill University, Ste. Anne de
Bellevue, Quebec, Canada H9X 1C0.

Pseudomonas putida strain NR, applied to soils, increased
emergence of soybean and pea in soil infested with Pythium
ultimum. Germination and hyphal growth of soil-produced sporang-
Saprophytic Fusaria, isolated from rhizosphere of plants grown in soils suppressive to several forms of species of pathogenic Fusarium oxysporum, actively colonized rhizosphere of different hosts in steamed and raw soils. Fusaria densities were significantly higher at the root tip and at the base of the stems than in the midportion of the root. Highly rhizosphere competent Fusaria showed the best activity against carnation wilt when applied, at transplanting, by dipping rooted cuttings. Integration of dipping roots of rooted cuttings in conidial suspensions of benzimidazole resistant antagonistic Fusaria with soil application of benomyl (5-10 g/m²) improved wilt control. Use of these rhizosphere competent Fusaria eliminates the need of adding large amounts of inoculum to soil in order to induce suppressiveness.

A296


Vermiculite-wheat bran (3:1 w/w) colonized by G. roseum or G. catenulatum was added to nonsterile soil at rates ≤ 0.14 v/v. Microsclerotia of V. dahliae embedded in nylon mesh squares were buried in amended soil, recovered after 1, 2, 6, 10 or 14 days and plated on soil extract-polygalacturonase agar to assess viability. G. roseum reduced viability of V. dahliae by 80-90% even after 1 day of incubation. Six of seven isolates of G. roseum and both isolates of G. catenulatum used were effective for biocontrol at rates of 0.01-0.05% antagonist preparation in soil (F < 0.05). Algininate pellets were formulated with either vermiculite:bran colonized by G. roseum or bran plus a conidial suspension. Although colony-forming units per algininate pellet were 2-200 times greater with vermiculite:bran than with the conidial suspension, only pellets formulated with the conidial suspension were effective. This information is being used for field application of G. roseum.

A297

INFLUENCE OF SOIL EDAPHIC FACTORS ON SUPPRESSION OF TAKE-ALL BY PSEUDOMONAS FLUORESCENS 2-79. B. H. Owensley, B. W. Waller, and J. R. Alldredge. USDA, ARS, Washington State University, Pullman, WA 99164-6430.

Pseudomonas fluorescens 2-79 suppresses take-all of wheat caused by Gaumannomyces graminis var. tritici, but the level of disease suppression is variable from site to site. To assess the relative importance of soil edaphic factors on take-all suppression, seeds were treated with 2-79 (produces phenazine-1-carboxylic acid, Phz?), Phz? mutants, or mutants genetically restored to the Phz? phenotype, and sown in ten different steamed soils. Disease suppression by Phz? strains was positively correlated with (P<0.01) sulfate-sulfur, % sand; (P<0.05) pH, sodium, zinc, and (P<0.20) ammonium-nitrogen. Suppression was negatively correlated with (P<0.05) cation exchange capacity, exchangeable acidity, manganese, iron, % slit, % clay, and (P<0.20) % organic matter and total carbon. Regression models (R² > 0.95) for take-all suppression by Phz? strains included the soil characteristics: cation exchange capacity, sulfate-sulfur, magnesium, potassium, sodium, ammonium-nitrogen, total nitrogen, % organic matter, total carbon, % sand, copper, pH, zinc, and % slit.

A298

BACTERIAL COMMUNITIES IN SOIL AND ON SOYBEAN ROOTS, AND THE EFFECTS OF A BIOLOGICAL CONTROL AGENT. G. S. Gilbert, J. Handelman, and J. L. Parke. Dept. of Plant Pathology, Univ. of Wisconsin-Madison, WI 53706.

Communities of bacteria in bulk soil, on untreated soybean roots, and on roots from soybean seed treated with the biocontrol agent Bacillus cereus strain UW65n were distinguished by physiological attributes of the bacteria. The plants were grown both in the field and in a growth chamber with sieved, air-dried soil from the field site. Approximately 200 aerobic bacteria from each habitat were randomly selected by 10% trypticase soy agar. Each isolates was tested for a wide range of physiological characteristics, including extracellular enzyme activity, growth on single carbon sources, anaerobic growth, motility, and resistance to antibiotics and salts. Multivariate analyses of the physiological attributes of the field isolates showed that the three habitats contained distinct bacterial communities. Resistance to certain antibiotics was more prevalent on both treated and untreated roots than in the soil. Pectolytic activity was most common among isolates from the UW65n-treated roots. More soil isolates than root isolates produced various extracellular enzymes. Communities from the growth chamber experiment resembled those from the field in some respects but not others. Our results indicate that the introduction of a single organism for control of plant disease can have a significant impact on plant-associated bacterial communities.

A299

RULE OF HYGROSOPHIE COMPETITENONE ANTAGONISTIC ACTIVITY OF SAPROPHYTIC FUSARIA ISOLATED FROM SUPPRESSIVE SOILS. A. Saribaldi and M. Sulllina. Istituto di Patologia vegetale, Via Giuria 15, Torino, Italy.

A300


An isolate (TFI-np) which produced 45 times less glucose oxidase than the wild-type I. flavus TFI was selected by screening natural variants of TFI. In a greenhouse test in nonsterile field soil, TFI controlled Verticillium wilt of eggplant while TFI-np did not. No differences were found when the two isolates were compared for conidial or ascospore production, recovery from nonsterile field soil, or growth rate on agar. Comparison of extracellular protein profiles between the acrylamide gel electrophoresis also showed that TFI-np produced substantially less glucose oxidase than TFI. In addition, there were differences in quantity of other proteins produced. These data suggest the in situ involvement of glucose oxidase in biocontrol of Verticillium wilt of eggplant; however, other proteins may also be involved.

A303

THE ROLES OF INDOGENOUS FUSARIA OXYSPORUM AND VARIOUS OTHER MICROORGANISMS IN A SOIL SUPPRESSIVE TO FUSARIA WILT

Vol. 80, No. 10, 1990 995
Suppression to Fusarium wilt of watermelon developed through monoculture to cv. ‘Crimson Sweet’ (Phytophath. 77:607–611) was destroyed in soil exposed to microwave (MW) irradiation (2450 MHz, 700 watts) for 90s/kg soil at −0.01 MPa matric potential. This treatment eliminated F. oxysporum and most of the mycelium. There was only a slight effect on total numbers of bacteria and actinomycetes. Over 100 isolates of F. oxysporum and 250 isolates of miscellaneous bacteria, actinomycetes, and fungi isolated from the roots of watermelon plants grown in suppressive or conducive soils were tested for their ability to restore suppressiveness to a MW-treated soil. Successful isolates were also tested singly and in combination in a conducive field soil. Several isolates of F. oxysporum were most successful in reducing wilt (35–75% reduction) in both soils. Root colonization characteristics of selected isolates and mechanisms of suppression were also investigated.

A304
INCREASE OF TRICHODERMA POPULATIONS IN SOIL ASSOCIATED WITH ADDED RHIZOCONTIA SOLANI HYPAE. M. E. de la Fuente and C. A. Martinez, Plant Pathology, Iowa State University, Ames, IA 50011

Rhizocontia solani was grown on sterile beet seed; soil was amended with these infused seed (50/100g soil), seed sterilized with copolyene oxide to kill R. solani, uninfested sterile seed, or no seed. Soil was infested with T. viride spores to 2, 4, and 8x the natural population. Trichoderma populations increased to 1x10^6 in 4x the natural population, delved with living R. solani and with no added T. viride (X). Higher than in soils infested with T. viride (2, 4, 8x). Seed with killed R. solani hyphae and sterile seed had little effect on Trichoderma populations. Seed with living R. solani hyphae were excellent baits for isolation of Trichoderma from soil, even at very low populations. In soil repeatedly cropped to radish, wheat, or cucumber or uncropped, Trichoderma populations increased significantly when R. solani was infused into the soils; with added R. solani inoculum, Trichoderma populations increased from 3x10^3 to 2x10^6. Viable R. solani inocula stimulate Trichoderma activity in soil.

A305
TRANSFER OF HERBICIDE PRODUCTION GENES FROM STREPTOMYCES HYGROSCOPICUS INTO A PLANT PATHOGEN, XANTHOMONAS CAMPESTRIS pv. CAMPESTRIS. Virginia Joan Prange and R. Charudattan, Plant Pathology Dept., University of Florida, Gainesville, 32611.

Genes encoding the production of bialaphos, a potent glutamine synthetase inhibitor, have been cloned into a plant pathogen, Xanthomonas campestris pv. campestris (XCC), in order to create an experimental model to study horizontal gene transfers. These genes were originally isolated from Streptomyces hygroscopicus (ATCC 21705) and cloned into cosmid vector pBG9 (Murakami et al., 1986, Mol. Gen. Genet. 205:42–50). We have transferred these genes into pLaFR3, a cosmid functioning in both E. coli and XCC. The resulting cosmid, named pL1, was transformed into E. coli and incorporated into XCC by conjugation. Characterization of pL1 with regard to cosmid maintenance, generation time, and bioactivity detected in plant and microbial assays will be presented.

A306
A MODEL FOR THE EXPANSION OF COHORTS OF LESIONS ON CORNHOLDS OF LEAVES. R. D. Berger and A. Bergamini, Departments of Plant Pathology, University of Florida, Gainesville, FL 32611 and University of Sao Paulo/ESALQ, Piracicaba, Brasil 13400.

Six epidemiological parameters were incorporated into a modification of VanderPlank’s basic-infection-rate equation to obtain a model for disease progress with expansion of lesions. Daily cohorts of infections were initiated on age-defined cohorts of host area. A radial increase of >0.5 mm/day for the circular lesions resulted in the generation of the infection expansion. Expansion of lesions was an especially important component of total disease as latent period lengthened beyond 5 days. As the percentage of hypothetical susceptible sites increased above 1%, lesion expansion became a less important component of total disease; while the percentage of lesion area with sporeulation, the maximum basic infection rate, and initial lesion size were relatively insensitive parameters. Natural epidemics of Cercospora and Leptosporangia and rust of alfalfa and early blight of potato were used to validate the model.

A308
RELATIONSHIP OF EPIDEMIC POPULATIONS OF CITRUS BACTERIAL SPOT BACTERIAL STRAINS TO DISEASE DEVELOPMENT AND SAVVIVAL. T. R. Godbold, J. H. Graham, USDA/ARS, Orlando, FL 32803.

One end of each of five citrus nurseries was inoculated with one of three bacterial strains of Xanthomonas campestris pv. citri (Xcc) representing aggressive (AG), moderately aggressive (MG), and weakly aggressive (WAG) isolates of Xcc. Blowing rainstorms were simulated with a mist blower by spraying water down rows at high velocity over the inoculated plants toward receptor plants. Epiphytic populations of Xcc, sampled on receptor plants on day 1, correlated well (r = 0.679 to 0.960) with disease severity of these same plants 1, 20, 40 and 76 da following the event. Slopes of the bacterial dispersal gradients were directly related to seasonal disease development and strain aggressiveness. Disease severity (DS) decreased in all nurseries over time at ca. the same rate. Survival of Xcc strains was tested under simulated citrus grove conditions. Disease decrease was nearly linear for each strain with slopes of r = 0.0054, 0.0061, 0.0067/da for Swingle and r = 0.0067, 0.0059, 0.0018/da for grapefruit for AG, MG, and WAG strains, respectively.

A309

Field plots were established to investigate the efficacy of seed treatments with Pseudomonas fluorescens strain PRA2, P. cepacia strain AMMD, Corynebacterium spp. strain 5A, and captan on the epidemiology of root rot of pea caused by Aphanomyces euteiches. Emergence was evaluated 19 days after planting and disease incidence was determined every 2 to 3 days thereafter until harvest. Pre-emergence damping-off, caused by Pythium spp., was affected by the treatments with the bacterial strains having significantly greater emergence (P<.05) than the captan treatment. To determine the effect of treatments on disease progressing disease following emergence, treatments were divided into two halves. The change in the rate of disease progress was associated with changes in soil temperature and soil-water matric potential. The rates of symptom expression among treatments were not significantly different within the first or second half of the epidemic except the AMMD treatment had a significantly higher rate of symptom expression in the first half of the epidemic even though disease incidence was lower. Each bacterial treatment was also found to have significantly less area under the disease progress curve (AUDPC) and significantly less disease severity at 10% bloom than the captan treatment. Although the analyses indicated that disease progressed at similar rates regardless of the seed treatment and that the level of disease incidence may be determined by the effectiveness of the treatments against pre-emergence damping-off, the differences in disease severity indicate that A. euteiches is also controlled by the bacterial treatments.

A310
AN EXPANDED PARALOGISTIC MODEL OF RICE LEAF BLAST. Paul S. Tang and Jonathan E. Yuan, respectively, International Rice Research Institute, P.O. Box 933 Manila, The Philippines, and University of Hawaii, 3190 Maile Way, Honolulu, HI 96822, U.S.A.

The paralogistic equation of J.E. VanderPlank (1963), \( \frac{\text{d}X}{\text{d}t} \cdot R_e \cdot (R_o - X)^{1.1-X} \) was numerically simulated using a program called STELLA™ (High Performance Systems, New Hampshire) on an APPLE™ Macintosh microcomputer. The Equivalence Theorem was tested by incrementally building weather stochasticity (temperature, RH and rainfall) and biological relationships (latent period, infectious period, infectivity, receptivity) into the paralogistic. Stochastic weather was generated using WGEN developed by Richardson and Wright (1984). Epidemics of rice leaf blast caused by Pyricularia oryzae were simulated for Asian sites ranging from cool temperate to hot, tropical. The blast epidemiological potentials of different locations were calculated and then compared using stochastic dominance. This technique has potential use in guiding rice genotype deployment.
A311


An approach to link population models for leaf blast, panicle blast, sheath blight, leaf folder, stem borers, and plant hoppers to the IBSNAT CERES rice model was developed. Development of each disease or pest was modeled using a generic type model, derived from a paralogistic growth function. Pest effects were introduced into the crop simulator by mimicking effects at the physiological process level, following the 'coupling point' concept of BOOTE et al. (1983). The physiological processes and crop variables affected were: light use efficiency, photosynthesis, partitioning, amount and translocation of carbohydrates, evapotranspiration, leaf area index, stand density, senescence rate, grain filling, and panicle weight. Crop variables, such as leaf area index and stand density served as driving variables for pest development resulting in a method of handling pest competition.

A312

MICROCLIMATES IN TWO CROPPING SYSTEMS AND THE RELATIONSHIP TO INTENSITY OF ANGULAR LEAF SPOT OF BEAN. J. M. Lanter, J. G. Hancock, and J. W. White*. Dept. of Plant Pathology, University of California, Berkeley, CA 94720 and *CIAT, AA6703 Cali, Colombia.

Several microclimatic variables were continuously monitored and recorded in bean monocultures and bean-maize intercrops during three field trials (two rainy seasons, one dry season) in 1986-87 at the International Center for Tropical Agriculture. Temperature in- and outside the canopy, vapor pressure, saturation deficit, and duration of leaf wetness were compared by discriminant analysis. Significant canonical correlations of 0.834, 0.722, and 0.768 were found for the three trials when hourly data for the entire season were analyzed, and correlations improved as subsets of the data were analyzed separately. No single variable was consistently most important in distinguishing between treatments; however, duration of leaf wetness was the only variable that coincided with intensity of angular leaf spot (ALS). In each trial, the treatment with the longer duration of leaf wetness had the greatest ALS intensity. During the rainy seasons, monocultures averaged 0.84 and 1.44 hr/day longer with condensed moisture than intercrops, whereas during the dry season, the intercrop averaged 0.59 hr/day longer with condensed moisture than the monoculture.

A316

ASSESSING RISK WITH STOCHASTIC DOMINANCE. Jonathan E. Yuen and Paul S. Tang, respectively, University of Hawaii, 3190 Maili Way, Honolulu, HI 96822 USA and the International Rice Research Institute, PO Box 933, Manila, The Philippines.

The riskiness of different cropping strategies was assessed using stochastic dominance (Hadar and Russell, 1969; Meyer, 1977). A disease simulation model for potato late blight (Bruhn and Fry, 1981) was used with both simulated and historical weather data files to calculate net return from cropping strategies with different disease control measures. Probability density functions (pdf’s) for the net return resulting from different disease control measures were then estimated. These pdf’s were compared using first and second-order stochastic dominance which is a degree of stochastic dominance identified strategies with low return and which would not have been chosen by decision makers seeking to maximize return. Second degree stochastic dominance eliminated disease control strategies that would not have been chosen by decision makers that are risk-averse. Stochastic dominance has the ability to compare pdf’s without knowing the preferences of the decision maker.

A317

EFFECTS OF CLOVER YELLOW VEIN VIRUS ON EPIDEMIC COMPONENTS OF CERCOSPORA LEAF SPOT ON WHITE CLOVER. Scot C. Nelson and C. Lee Campbell, Department of Plant Pathology, North Carolina State University, Raleigh, NC 27695-7616.

Epidemic components of leaf spot caused by Cercospora zebrina were assessed on two clones of Trifolium repens that vary in response to infection by clover yellow vein virus (CYVV). Healthy and CYVV-infected plants of clones T7 (low virus titer, asymptomatic) and T117 (high virus titer, symptomatic) were grown at 28/23 C or 22/17 C and monitored for 17 days after inoculation with C. zebrina. Alterations of epidemic components for CYVV-infected plants of T117 were: diminished infection efficiency, shortened latent period, larger lesions, greater proportion of leaves with sporulating lesions, and reduced defoliation, disease incidence and severity. Incubation period (28/23 C) and latent period were shortened for CYVV-infected plants of T117. A clone x virus x temperature interaction was found for incubation period and lesion diameter. Incidence of infected leaves was greater at 22/17 C, regardless of clone/virus combination.

A318

PATTERNS OF ASSOCIATION AMONG PATHOGENS ON LEAFLETS OF ALFALFA. Jim A. Duthie and C. Lee Campbell, Department of Plant Pathology, North Carolina State University, Raleigh 27695-7616.

Presence of five pathogens on each of 5,800 diseased leaflets of Ralidor alfalfa from 19 sampling dates over 2 yr was recorded. For each of 10 pairwise combinations, strength of association between two pathogens was given by (A/B) when A was the odds that one pathogen was present given that the second pathogen was present, and B was the odds that the First was present given that the second was absent. On most dates, strong positive associations between Leptosphaeria trifolii and Stemphylium botryosum and between Cercospora medicaginis and Colletotrichum spp. were detected. Associations between L. trifolii or S. botryosum and C. medicaginis or Colletotrichum spp. usually were weak or negative. P. medicaginis often was associated negatively with L. trifolii and positively with Colletotrichum spp. Pathogens occurred in two distinct complexes, one of L. trifolii and S. botryosum and another of P. medicaginis, C. medicaginis, and Colletotrichum spp.
A319

QUANTIFICATION OF DISEASE PROGRESS AND DEFOATION IN THE POPULUS DELTOIDES-MELAMPISCA MEDUSAES PATHOSYSTEM. R. C. Hamelin, L. Shain, B. A. Thieges*, and R. S. Ferris, Departments of Plant Pathology and Forestry*, University of Kentucky, Lexington, KY 40546-0091.

Disease progress and defoliation were quantified in 11 half-sib families derived from natural stands of P. deltoides. Values for the area under the disease progress curve (AUDPC), the shape (m) and absolute rate (Rm) parameters derived from the Richards growth model and the final disease severity (Yf) ranged from 0.42 to 4.32, 0.67-3.59, 0.0012-0.0009, and 0.06-0.38, respectively. Values for the number of days before leaf fall (DLF) and the area under the leaf area curve (AUDLAC) ranged from 50.02-66.58 and 4.50-7.15, respectively. The AUDPC was strongly correlated with DLF (r = 0.86), Yf (r = 0.96) and Ra (r = 0.84); strong correlations also were found betwen DLF and Yf (r = 0.94) and AUDLAC and Ra (r = 0.84). These results confirm the causality of M. medusae on early defoliation of P. deltoides. A cluster analysis grouped families into 2 clusters: cluster 1 was composed of families from the northernmost locations and had larger AUDPC, Yf and Ra, and lower DLF and AUDLAC than cluster 2, which was comprised of families from the southernmost locations, indicating higher rust susceptibility in northern than southern families in the U.S. at the test site in Carlisle Co. KY.

A323

DETECTION OF TOMATO SPOTTED WILT VIRUS RNA IN THrips USING STRAND SPECIFIC PROBES. Thomas L. German and Yi Hu. Department of Plant Pathology, University of Wisconsin, Madison, 53706 and University of Hawaii, Manoa, 96822.

We have constructed a library of cDNA clones in the plasmid vector PBB322 and used these to develop a diagnostic dot-blot assay for tomato spotted wilt virus (TSWV). This assay will detect viral RNA in 16 ng of total RNA and is useful for detecting the presence of TSWV in all hosts tested and in individual thrips (Rice et al. Plant Disease, in press). One of these clones (pTSW80) was subcloned into the vector Blue Script II to take advantage of the TS and TG strand specific primers. Probes were generated and used to assay for the presence of each RNA strand in individual plant and insect samples. We were able to determine a time course for appearance of each strand in plants and to demonstrate the presence of both strands in thrips. Although we have not assigned polarity with respect to translation of the strands, these data indicate that both (+) and (-) sense RNAs occur in insects.

A324

TEMPORAL ACCUMULATION OF CAULIFLOWER MOSAIC VIRUS RNA, RNA, AND COAT PROTEIN IN RELATION TO SYMPTOM SEVERITY IN TURNSIPS. E. J. Anderson, A. T. Trese, and J. E. Scholz, Dept. of Plant Pathology, University of Missouri, Columbia, Missouri 65211.

Cauliflower mosaic virus (CaMV) strains W60 and CM014 produce markedly different symptoms when inoculated onto turnips (Brassica campestris L. "Just Right"). Strain W60 causes a prominent vein clearing and necrosis and severe stunting of both leaves and turnips, while infection by CM014 results in a prominent mosaic and only mild stunting. As part of our effort to correlate viral gene function with symptom development we have used ELISA and DNA dot-blot hybridization to measured viral coat protein and RNA accumulation in W60- or CM014-infected leaves. The inoculated, 1st, 2nd, 5th, and 10th systemically infected leaves were assayed independently, once available, from 0 through 56 days postinoculation. While strain W60 caused significantly more coat protein accumulation than did W60, only a slight increase in viral RNA accumulation was observed. Further experiments are in progress to determine whether increased accumulation of CM014 coat protein is due to increased synthesis of the 35S RNA.

A325

EVIDENCE FOR GENETIC RECOMBINATION IN THE 5' END OF THE COAT PROTEIN GENE OF A STRAIN OF SUGARCANE MOSAIC VIRUS. D.D. Shukla, M.J. Frenkel, N.M. McKern, P.M. Strike and C.W. Ward, CSIRO, Division of Bimolecular Engineering, Parkville, Victoria, 3052, Australia.

The 3'terminal 1343 nucleotides of the SC strain of the sugarcane mosaic virus (SCMV-S) genome have been compared with the sequence of maize dwarf mosaic virus B (MDM-B). The coat protein of SCMV-S shows high sequence identity (92%) with that of MDM-B except for the region between amino acid residues 27 and 70. This region is smaller (44 residues) than the equivalent region in MDM-B (59 residues) and shows a lower sequence identity (22%) to the MDM-B sequence. The sequence diversity in this region indicates the occurrence of a recombination event during the evolution of the virus. Sequence identities of both the major part of the coat proteins and the non-coding regions confirm that SCMV-S and MDM-B are strains of SCMV.

A326


A Carlavirus is associated with Sheep Pen Hill disease (SPHHD) of blueberries in New Jersey, and a similar Carlavirus causes blueberry scorch disease in the Pacific Northwest. Beginning with RNA extracted from virus that was isolated from blueberry tissue and from mechanically infected Chenopodium quinoa plants, we synthesized cDNA libraries representing the genome of SPHHD. Many of the cDNA clones from a library that was initiated by priming with oligo d(T) contained a poly (A) tract followed by residues that were identical for all clones examined. A cDNA clone, However, contained the 3' terminus. The sequence for approximately 2 kb at the 3' terminus has been determined and compared with published sequences for the Carlaviruses PSW and PVS. There was extensive conservation among the three genomes, and they appear to be organized similarly.
A332

ADAPTATION OF Puccinia recondita to SLOW-RUSTING WHEAT CULTIVARS DUE TO ARTIFICIAL SELECTION. L.S. Lehman and G.E. Shaver, Botany & Plant Pathology Dept, Purdue University, W. Lafayette, IN 47907

To study adaptation of Puccinia recondita to slow-rusting wheat cultivars, a wild-type population (WT) was reared and selected for reduced latent period (LP) for five generations on slow-rusher CI 13227. WT, first (C1), and fifth (C5) generation isolates were evaluated on fast-rusting cultivar Monon and slow-rusting cultivars CI 13227, L574, Suwon 85, and SW 72469-6 for differences in LP (greenhouse) and disease levels (field). C5 had a shorter LP and higher disease levels than WT. C1 did not differ from WT. CI 13227 and Suwon 85 had significantly longer LPs than Monon, while L574 and SW 72469-6 had intermediate values. Field comparisons of cultivars were less clear. Disease levels on L574 and Monon were similar despite L574 having a significantly longer LP. Also, SW 72469-6, with a shorter LP than CI 13227 and Suwon 85, had the least amount of disease. A significant cultivar X isolate interaction was detectable in the greenhouse and was mainly due to C5 on CI 13227. In the field, no interaction existed, but C1 and C5 on CI 13227, and C5 on L574, did differ significantly from WT on these cultivars. These results suggest isolate adaptation and some degree of race-specificity toward slow-rusting resistance, but not sufficient to overcome the resistance.

A333

RELATIVE RESISTANCE OF SOUTHERN RUNNER PEANUT TO STEM ROT IN COMPARISON TO OTHER CULTIVARS. T. B. Brenneman1, W. D. Branch2, and A. S. Campos1, Departments of Plant Pathology1 and Agronomy2, UGA, Coastal Plain Experiment Station, Tifton, GA 31793

The susceptibility of 16 peanut (Arachis hypogaea L.) genotypes (eight virginia and eight runner types) to southern stem rot (Sclerotium rolfsii Sacc.) was evaluated in the field for three years. The mean yield for the eight virginia types was 3287 kg/ha versus 3214 for the eight runner types. The mean disease incidence was 14.3 disease loci per 12.2 m of row for both market types. Of the virginia types, NG 6 and Florida 29 were the most susceptible with NC 9, VA 81B and Early Bunch being the most resistant. Most runner cultivars were quite susceptible except for Southern Runner and Langley which had about 50% less disease than the most susceptible entries. Southern Runner had the lowest disease incidence and highest pod yield of any cultivar. Compared to Florunner, the current industry standard, it had about 50% less disease and yields were 1346 kg/ha higher. There was a highly significant negative correlation (r = 0.01) between yields and disease incidence all three years.

A334

RESISTANCE TO KEY DISEASES IN SUB-SPECIFIC GROUPS OF RICE. J.M. Robinson, T.A. New, R. Koganesawa, T.J. Vergel de Oñez-Neu, C.M. Vera Cruz, E.S. Medalla, C.K. Kim, J.L. Notteghem, J.C. Glassmann, IRRI, P.O. Box 933, Manila, Philippines; IAS, Rural Dev., Adm., Suwon, Korea; CGIAR/IRRI, B.P. 5035, France

From the International Rice Germplasm Center, 263 accessions of Oryza sativa were selected to represent diverse geographic origin, cultivation type, and of six sub-specific groups. To ascertain if disease resistance is associated with sub-specific groups, the accessions were tested for resistance to 13 races of the blast (B1) pathogen, Pyricularia oryzae; 6 races of the bacterial blight (BB) pathogen, Xanthomonas campestris pv. oryzae, and rice tungro virus (RTV). The temperature accessions of group VI (japonicas) were the most B1 susceptible. Tropical accessions of group VI (bulu and upland rice), and groups II, III, IV, V and VI were less B1 resistant than group I (japonicas). Groups II, III, and IV were relatively resistant to BB, as were many endemic rice. Group V was the most resistant to RTV. Sub-specific groupings may help target resistance screening efforts.
**A335**

**The Effect of Age, Flowering, and Senescence on the Resistance of Tobacco to Blue Mold.** S. E. Wyatt and J. Kuc. Department of Plant Pathology, University of Kentucky, Lexington, KY 40546

Age-related resistance to blue mold in tobacco is correlated with flowering. Exposure to short day conditions and cool temperatures (16°C, 8-h light) 2-5 days after flowering caused plants to senesce and die. When flowering (14-h light, 23°C, 11-h dark with a 1 hr night break) flowered in 12 weeks f.s. and were 120 cm in height with 25-30 fully expanded leaves. These plants were resistant to blue mold caused by Pseudopeziza tabacinum, whereas tobacco inoculated to flower prematurely was highly susceptible. The prematurely flowering plants, however, became resistant as they aged. Senescence was correlated with the senescence of lower leaves. Thus, it would appear that the age of plants, not flowering, is the determining factor in age-related resistance to blue mold.

**A339**

**Expressions of Resistance to Extracellular Enzymes of Snow Mold Fungi in Another Culture Derived Callus Systems.** F. Meh dizadegan and J. H. McBeath. Agricultural and Forestry Experiment Station, University of Alaska Fairbanks, Fairbanks, AK 99775.

Calli derived from another culture of the winter wheat cultivar Roughrider were screened for expressions of enzymes associated with snow mold resistance. Chlorella borealis and sclerotial low temperature basidiospore (C. l. ST8) were used to screen 2,000 calli with green centers (leaves) primordia from 16 callus cultures (8 dark with a 11-h night break and 15 hr light) flowering at 5°C in darkness with 12 h light. Calli were harvested at 10°C for 4 weeks. Calli were evaluated weekly by their resistance to reactions recorded using a numerical rating (0-4). The responses of calli to snow mold enzyme treatments varied widely, ranging from no change (rated 0) to complete discoloration and death (rated 4). Among all calli treated, 2.4% and 3.4% showed degrees of resistance to enzymes of C. borealis and ST8, respectively.

**A336**

**Field Assessment of the Genetics of Phomopsis Resistance in Narrow-Leafed Lupins.** W.A. Cowling, Department of Agriculture, Baro-Hey Court, South Perth, Western Australia 6151.

Latent stem infections by Phomopsis leptostromiformis form lesions on narrow-leafed lupins (Lupinus angustifolius) during senescence. Resistance to field emergence, growth, and senescence of lesions in Phomopsis stem ratings in plots before seed harvest. Over 3 years of testing, parent lines and cultivars were screened as well as derived (F1 and F2) progeny. Protein (R) and completely resistant (MR), susceptible (S) and very susceptible (VS). Random F2-derived lines from crosses among parents belonging to these categories were tested for Phomopsis resistance in the F4 and F5 generations. The derived parental genotypes were those of the F2. These derived lines were 233 plants from 20 crosses among 363, 61 MR, and only 11% of progeny were susceptible in 123 plants from 13 crosses of MR R with S or VS. More than one resistant gene for resistance may exist, one of which may be epistatic to another, but more MR and S progeny are predicted by these hypotheses then were identified in these experiments.

**A337**


The coffee leaf rust was detected in the Western Hemisphere in 1970. The development of a composite cultivar was considered the best breeding strategy to cope with specific resistance and to confront a variable pathogen. The variety Colombia was used as a perennial crop such as coffee. After 20 years of research such cultivar was selected in Cenicafe, Colombia. The Caturra variety was used as the base leaf rust and the Timor Hill as the donor parent, with more than fifty genes for specific resistance and other genes for partial resistance. About fifty progenies (F5) were used, selected that display high yield, extreme resistance to the rust, phenotype uniformity and good bean and liquor quality. Since 1983 more than 200,000 hectares have been planted with a mixture of such progenies, which has been named Variety Colombia. Until now its resistance has remained stable.

**A338**

**Comparison of Field Screening Methods to Evaluate Resistance of Soybean Cultivars to Frogeye Leaf Spot.** C.N. Aker, K.E. Dashiel, and A.M. Emchebe. Grain Legume Improvement Program, International Institute of Tropical Agriculture, Ibadan, and Institute for Agricultural Research, Zaria, Nigeria.

Different methods were compared in field plots at two locations in Nigeria, to evaluate 4 soybean cultivars (cvs) for resistance to Cercospora sojina. Entries were arranged in a completely randomized block design with 4 replications. At both locations, plants were inoculated by spraying susceptible soybean cv as a border row 2 wks before establishing the main plot, which was most effective in obtaining early and maximum disease build up. This method resulted in a significantly (p<0.05) higher disease severity and disease incidence (DI) of frogeye leaf spot at the R8 growth stage. At the R7 stage, however, there was significant difference in disease among, but not within cvs, suggesting possible interplot interference. This test demonstrates the effectiveness of using infected border rows established early, to increase disease pressure needed in screening for disease resistance under field conditions.

**A340**

**The Effect of Flusilazole on the Germination of Conidia of Flusilazole-Resistant and -Sensitive Isolates of Venturia inaequalis.** Framing D. Smith and Wolfram Köller, Cornell Univ., NYSAES, Geneva, 14456.

The effect of flusilazole on germination and appressorial formation by conidia of flusilazole-sensitive (S92) and -resistant (W10) isolates of V. inaequalis was studied. In this study, resistance to flusilazole was expressed at an early stage of conidial germination and the degree of resistance was transient. Also flusilazole acted as an appressorial inducer. Germination of W10 was not affected at 1 µg/ml flusilazole and not fully inhibited at 10 µg/ml by 16 h germination and through 38 h it was inhibited only 12% at 0.001µg/ml. Germination of S92 was completely arrested 6 h after germination at 0.001µg/ml and full inhibition occurred after 4 h perossmulation at 1µg/ml. The 50% value of W10 based on germtube elongation decreased over time. At 8 h the ED-50 value was >10 µg/ml and between 32 and 38 the ED-50 value was 0.006 µg/ml. The greatest decrease in ED-50 occurred between 24 and 36 h. On agar only 5% of the untreated conidia formed appressoria at 44 h, but when conidia were treated with flusilazole, appressoria were differentiated between 24 and 36 h. Appressorial differentiation was doce dependent but did not exceed 80%.

**A341 Withdrawn**

**A342**


Valencia and Hamlin sweet orange, Ruby Red and Marsh grapefruit, and Orlando tangelo fruit were inoculated biweekly in the field at different stages of growth with an aggressive strain of X.c. citri meiro (F1). Various concentrations of F1 were applied as pressurized sprays (ps) to watersoak the rind tissue and were compared 2 and 4 days after the last spray (gs) at 10® µf/ml. The gs and ps at 10® µf/ml yielded ergotic infection within the watersoaked zone whereas the ps at 10® and 10® consistently produced lesions within and outside the treated area. Disease rating decreased as fruit size increased more rapidly at ps 10® than at ps 10®. The period of susceptibility was greater for grapefruit than for orange, with tangelo intermediate. Lesions did not expand in size after 2 wk and bacteria did not multiply in lesions, i.e. citrus fruit was not susceptible to Xc. citri meiro. A similar relationship between fruit size and susceptibility was shown for X.c. citri in Argentina.

**A343**

**A Semiselective Medium for the Isolation and Preliminary Identification of Xanthomonas campestris pv. vescicatoria from Tomato Seeds.** K. Siman, C. J. Chang, and E. D. Galtis. Department of Plant Protection, Universiti Pertanian Malaysia, 43400 Serdang, Selangor, Malaysia, and Department of Plant Pathology, University of Georgia, Georgia Station, Griffin, GA 30223 and Coastal Plain Station, Tifton, GA 31793.

A semiselective medium was developed for the isolation and preliminary identification of Xanthomonas campestris pv. vescicatoria from tomato seeds. Selectivity was afforded through the use of cycloheximide, bacitracin, neomycin, cephalixin, 5-fluorouracil and t Bowman, while TWEEN 80 was included for colony differentiation. All X.c. pv. vescicatoria isolates can be distinguished from other contaminating...
bacteria in the seeds by the formation of clear rings around their colonies. *X. c. pv. vesicatoria* was found in 13 of 23 seed lots which previously tested negative on other selective media. Recovery of up to 100% was achieved compared to TWEEN B medium.

**A344**

**COLONY HYBRIDIZATION WITH TI PLASMID PROBES TO AGROBACTERIUM ISOLATES FROM APPLE ROOTSTOCK TUMORS.** M. L. Garrard, M. D. Kavalek and L. W. Moore. Department of Botany and Plant Pathology, Oregon State University, Corvallis, OR 97331-2902

Crown gall in apple rootstocks has been difficult to study due to the inability to isolate tumorigenic strains from galls. A new method for identifying putative A. tumefaciens strains was therefore attempted. Isolates were made from tumors on Emila 7, Mark and Domestic Seeding rootstocks from two nursery sites in Washington state. Nineteen tumors were sampled and dilution plated onto KERR 1A, KERR 2E and Roy and then replica-plated to several selective media including biovar 2, 3 and respectively and onto a rich mannitol-glutamate medium supplemented with several auxins, trace elements and yeast extract. Two hundred and fifty isolates were collected from each tumor and tested for the presence of Ti plasmid DNA using 32P labeled probes. Colony hybridizations were carried out using a 7.5 kg fragment of *PTII805* including trnI and trnL loci and a 25.2 kb fragment of *PT118* spanning the *virA*, *virB*, *virF* and *virG* loci. Isolates of one tumor from Domestic Seeding rootstock showed hybridization to both probes in 79% of the colonies tested, while isolates of other tumors from Mark rootstock had no colonies which hybridized to either probe.

**A349**

**EFFECT OF Xanthomonads on Aroids.** A. R. Chase, University of Florida, IFAS, Central Florida Research and Education Center-Apopka, FL 32703.

Approximately 150 strains of *Xanthomonas campestris* pv. *syngonii* and *X. c. dienfencchia*ae, obtained from ornamental and agronomic Aroid plants were characterized by pathogenic and physiologic reactions. These strains were from plants in the following genera: *Aglanaema, Anthurium, Colocasia, Difenphicha, Euphorbijum, Philodendron, Syngonia, and Xanthosoma*. Pathogenicity tests with *Aglanaema, Anthurium, Difenphicha, Philodendron, and Syngonia*, groups of strains were more virulent on their host of origin, but were not host specific. Strains from some host genera showed different characteristics based on minimum pH for growth, copper and streptomycin resistance, pectolytic activity, and starch utilization on four media, although no single characteristic or combination of characteristics could be used to separate one group of strains from another.

The degree of differences in pathogenic and physiologic reactions indicate the heterogeneous nature of *X. c. dienfencchia*ae but does not support separation into more than one pathovar, at this time.

**A350**

**EFFECT OF SUGARCANE CULTIVAR SUSCEPTIBILITY ON SPREAD OF RATOON STUNTING DISEASE BY THE MECHANICAL HARVESTER.** K. Dammann, Dept. of Plant Pathology, LAES, LSU Agricultural Center, Baton Rouge, LA 70803.

Disease-free sugarcane cultivars susceptible (L 62-96), intermediate (CP 65-357), and resistant (L 60-25) to ratoon stunting disease (RSD) were planted in the fall of 1986 and harvested in the fall of 1987. Inoculum was provided by diseased plants planted each year. Stalk samples were collected every 6 months from a 12.5m of each row in first ratoon and assayed for RSD in the fall of 1988. Sampling was repeated in second ratoon in the fall of 1989 and incidence determined. RSD incidence in L 62-96 preceded by 1, 2, or 4 diseased plants was 30, 60, and 75% respectively, in 1988 (after 1 harvest) and increased to 84, 98, and 99% in 1989 (after 2 harvests). Incidence in CP 65-357 was 10, 11, and 9% in 1988, and increased to 25, 24, and 44% in 1989. No spread was detected in L 60-25. Gradients were apparent when incidence in consecutive 3.05m row segments was determined. Little spread occurred beyond the first segment in CP 65-357. Fourth segment incidence in L 62-96 preceded by 1, 2, or 4 diseased plants averaged 4, 38, and 43% respectively.

**A351**

**A METHOD FOR THE DETECTION OF SEEDBorne BACTERIAL PATHOGENS IN TOMATO...** D.A. Madder and J.P. Hubbard Agraw Seed Co. San Juan Bautista, CA 95015

Residual chlorine (Cl) was detected when calcium hypochlorite (CaOCl) treated tomato seed lots were assayed, using the Stomacher blender and liquid plating technique. Cl levels greater than 20 ppm interfered with the recovery of bacterial pathogens in the assay. To eliminate the effects of residual Cl, 0.25 sodium thiosulfate (STS) was added to the 0.03M phosphate extraction buffer (pH 7.2). STS amended buffer reduced residual Cl of treated seedlots to undetectable levels. When *Clavibacter michiganense* subsp. *michiganense* *Xanthomonas campestris* pv. *vesicatoria* and *Pseudomonas syringa* pv. *tomato* were added to treated seed lots and assayed with STS amended buffer, all three bacteria were recovered at expected levels. The recovery of all three pathogens was better with STS amended buffer than with unamended buffer, when artificially infested seeds were added to CaOCl treated seedlots. Assays of CaOCl treated tomato seed lots with STS amended buffer may help in determining the efficacy of CaOCl to control seedborne bacterial pathogens.
A352

Storage roots of cv. Beausreege were either not treated (control), disinfested with 1% NaOCl for 10 min, or inoculated with *Erwinia chrysanthemi* (strain 1842) in 10 min. Each treatment was punched with a flamed needle. They were then incubated submerged in distilled water in plastic bags for 5 days at 25 C. Souring symptoms were present in 93 and 83% and 87% of the punctured and non-punctured roots from the control, disinfested, and Ech-2-inoculated treatments, respectively. *E. chrysanthemi* was isolated only from the inoculated storage roots. However, at least two pectolytic spore-forming, sulfate-reduction negative, gelatin hydrolysis positive, anaerobic bacteria resembling *Clostridium* spp. were found in most lesions, regardless of treatment isolates of the two clostridia were isolated from sweetpotato, potato, carrot, and onion, but symptoms were distinct from the sticky rot symptom reported previously. Among other characteristics, the two clostridia can be differentiated by the diameter (1 and 2.5 mm) of their colonies and by their ability to form the pigment, 25 days after 2 days at 32 C. Further characterization of the clostridia is in progress.

A355
IMPORTANCE OF WOUNDS AS INFECTION COURTS FOR POSTHARVEST DECAY OF PEAR BY PHALOPHORA MALARUM. Davis, E.D., Sugars, and R.A. Gots. Oregon State University, Medford 97502.

Bosc pears were not infected by *Phalophora malarum* via lenticels or intact intercellular surface areas following 6 mo exposure to various inoculum concentrations at 0°C. Punctured lenticels (0.75 mm wounds) dissected were consistently infected by the inoculum, whereas none of lenticels by impact of a 142 g steel bolt dropped from 5-20 cm prior to inoculation did not result in infection, regardless of immersion depth in inoculum solution. Incubation of bruised lenticels dissected, following soaking in methylene blue solution, only 4 were penetrated by dye solution. Tissue beneath lenticels was penetrated by dye solution following impact from 15 and 20 cm. Germination of conidia of *P. malarum* was enhanced in water in which wounded or wax-removed but not intact pears had been soaked.

A356
ANTHRACNESE OF CITRUS FRUIT CAUSED BY COLLETOTRICHIUM LIMONIUM. ISOLATION IS ENHANCED BY POSTHARVEST HANDLING. C. Eldon Brown, Florida Department of Citrus, Citrus Research and Education Center, Lake Alfred, FL 33850.

Anthracnose develops postharvest mostly on early season Florida citrus fruit which have to be ethylene-degreed. The disease is most prevalent on the Robinson tangerine cultivar, particularly on those fruit with the poorest color following degreewing. Last season, significant commercial losses from anthracnose also occurred on grapefruit. To assess the extent of handling procedures on anthracnose, Dancy tangerines were subjected to different treatments of degreewing, washing and waxing. Higher than standard degreewing temperatures (35 vs. 30°C) and anthracnose development. Disease incidence and severity were increased by higher than recommended ethylene concentrations (50 vs. 5 ppm), washing, and by waxing with resin solution water wax rather than with solvent wax or no wax. The greatest stimulation occurred on degreewed (50 ppm) and washed fruit, on which the surface area affected by anthracnose was increased from 1.5 to 44% by applying water wax.

A357

An isolate of *F. culmorum* (W.G. Smith), Sacc. HM-8 from a scabby wheat kernel sample from England produced a toxin in culture when grown on rice grains in the laboratory. This new toxin, given the trivial name of HM-8, caused food refusal, weight loss in rats, cytotoxic effects to mouse and human fibroblast cells at concentrations of 2.5 μg/ml and 7.5 μg/ml, respectively, and mortality to chick embryos (10 to 70%) at a concentration range of 0.5 μg to 4 μg per egg. HM-8 showed UV absorption maxima at 210 and 285 nm and does not fluoresce under UV light. It exists as powdery white crystals with a melting point range from 167 to 182°C. HM-8 showed bright blue fluorescence after its reaction with 20% sulfuric acid in MeOH or acetic anhydride. Elemental and accurate mass determinations in both electron impact (EI) and chemical ionization (CI) indicate an empirical formula of C8,H10, O5. Carbohydrate composition and a hemiacetyl group. This is the first report of this compound, HM-8, produced by *F. culmorum*.

A358

We are interested in identifying and characterizing genetic variations in regulation of aflatoxin biosynthesis among wild-type *Aspergillus* isolates. Aflatoxin production by previously described isolates has shown to be very low or lacking when grown in a peptone/minimal salts medium without sugar (PMS), and delayed more than two days in a malate/minimal salts medium (MMS). We have identified two wild-type isolates of *Aspergillus flavus*, NRRL 6554 and SRRC 26, which make significant amounts of aflatoxin on PMS, as well as within two days of growth on MMS. We have also used mutagenesis on several wild-type aflatoxin non-producers or low producers to determine if any of these strains can be induced to produce large amounts of aflatoxin. Of seven strains tested, one of the low producers was induced by mutation to produce large amounts of aflatoxin. We are using parasexual analysis, with protoplast fusion when necessary, to determine dominance and allelic relationships among the phenotypes described above. Auxotroph isolation, required for this analysis, has been facilitated by selecting for mutants in nitrogen metabolism by the use of chlorate-containing growth medium.
A360

COMPARISON OF HARD WHITE AND HARD RED WINTER WHEATS IN STORAGE. D. M. Trigo-Stockli, J. R. Pedersen, and D. B. Sauer, Food and Feed Grains Institute, Kansas State Univ., and USDA-ARS, U. S. Grain Marketing Research Laboratory, Manhattan, KS 66506

Research on the production and utilization of hard white winter wheats has increased in recent years due to their known susceptibility to storage fungi. Samples of hard white and hard red winter wheat were stored in plastic bags at 14, 16, and 18% moisture content and 25°C. They were tested over a 16-week storage period for moisture content, germination, and mildew invasion. When stored at moisture contents of about 16 and 18%, the hard red winter wheat had lower percentages of surface disinfected kernels yielding Aspergillus flavus compared to hard white winter wheat. Reduction in germination percentage was generally similar in the two wheat types, but at 16% moisture the hard red wheat decreased more than the hard white wheat.

Pell and R.R. Hill, Jr., Penn State Univ. and USDA-ARS, U.S. Regional Pasteurization Lab., Univ. Park, PA 16802.

The effect of V. albo-strum on net photosynthesis (Pn), stomatal conductance, stomatal limitation of Pn, and activity of Rubisco-1,5-bisphosphoribulose-carboxykinase (Rubisco) was determined in two greenhouse-grown alfalfa clones. A/C response curve analysis and leaf protein extraction and assimilation of 14C were used to determine the in vivo and in vitro activity of Rubisco, respectively. Rubisco activity, Pn, and stomatal conductance were reduced in the infected susceptible clone but stomatal limitation of Pn was not affected. Reduced Rubisco activity was attributed not to a decrease in carboxylating capacity. The pathogen had no effect on Pn/ stomatal limitation of Pn or Rubisco activity in the resistant clone. Activity assays confirmed the in vivo finding that Rubisco activity was significantly reduced in the infected susceptible clone but not in the resistant clone.

A365

EFFECTS OF LIGHT ON THE GERMINATION OF SPORANGIOSPORES OF PERONOSPORA TABACINA AND DEVELOPMENT OF BLUE MOLD IN TOBACCO LEAVES. H. Feng and J. A. Sc, Department of Plant Pathology, University of Kentucky, Lexington, KY 40546.

Experiments were conducted to determine whether a period of darkness (18-24 h at 18-22°C) was necessary for development of Peronospora tabacina Adam in tobacco leaves. Light (blue fluorescent, 86.3 μW·cm-2·s-1) inhibited the germination of sporangiospores 50-70% and spore germination 10-70%. Sporulation and disease development of blue mold were markedly inhibited when plants (Nicotiana tabacum L. cv. Ky-14) were directly exposed to light; however, light was not effective when plants were exposed to light or not. Exposure of two expanded leaves to light reduced blue mold development and sporulation not only on the leaves exposed to light but also on unexposed leaves. This suggests a factor is transported from leaves exposed to light which is either inhibitory to P. tabacina or elicits resistance in leaves kept in the dark.

A366

MOLECULAR BASIS THAT RULES THE HOST RANGES OF PATHO-TYPES OF ALTERNARIA ALTERNATA. K. Kohmoto, Y. Itoh, M. Kodama, I. Otani, and S. Nakatsuji, Plant Pathology Lab., Fac. of Agric., Tottori University, Tottori 680, Japan and *Organic Chemistry Lab., Fac. of Agric., Nagoya University, Nagoya 464, Japan.

The collective species of A. alternata contains many pathotypes affecting certain genotypes of diverse plants. Of these, Japanese pear, strawberry and tangerine pathotypes produce highly potent host-specific toxins (HST) which destroy epoxy-8-hydroxy-9-methyldehydrorhizinic acid. Although the structures are very similar each other, their toxicity is definitely selective. AK-toxin from Japanese pear pathotype is toxic to only susceptible pear. AF-toxin from strawberry pathotype is toxic to certain pear as well as susceptible genotypes of strawberry. ACT-toxin I from tangerine pathotype is toxic to tangerines and mandarins susceptible in the field, and toxin II, the 5'-deoxy derivative of toxin I, is harmful to certain pear that are experimentally found to be susceptible to each pathotype. Release of HST on germination can induce the accessibility to penetration at infection sites in susceptible genotypes.

A367

REVERSIBLE INHIBITORY EFFECTS IN VITRO AND IN VIVO OF A RESISTANCE COMPOUND FROM MAIZE. Frank A. Cantor and Larry D. Dunkle, USDA-ARS, Department of Botany and Plant Pathology, Purdue University, West Lafayette, IN 47907

Resistance of maize to Helminthosporium carthorum race 1 is induced by prior inoculation with race 2, a nonpathogen. Appressorium formation, penetration, and hyphal growth by the pathogen, as well as development of large lesions typical of the susceptible reaction are decreased in induced tissue. The induced reaction is consistently associated with the presence of a compound(s) in diffusates on the leaf surface which inhibits conidial germination and germ tube elongation, prevents infection, and the formation of labeled precursors into protein and RNA, and substantially reduces respiration. The inhibitory effects are reversed when conidia are washed with water or when organic or amino acids are added to the conidial suspension. The addition of sodium acetate to race 2 or challenge race 1 inoculum on the leaf negates the inhibitory activity of the diffusates, abolishes induced resistance, and results in formation of susceptible lesions. Resistance as well as production of the inhibitory compound(s) is induced by other fungi and in other maize lines inoculated with H. carthorum. The results suggest that a general resistance mechanism is activated upon contact of the maize leaf with a potential pathogen.

Vol. 80, No. 10, 1990 1003
A372
CORN STALK SENESCENCE VERSUS CORN STALK ROT. J. E. Partridge, B. L. Dougnik, and D. S. Wyong. University of Nebraska, Lincoln, NE 68583-0722.

Corn hybrids were selected to provide a range of maturities from short to full season. Fourteen hybrids were evaluated in each of four tests over a span of 10 years. Crushability, determined by hand squeezing, second internode above the brace roots, was used as the criterion for evaluation. Data collection was begun prior to any loss of green color in the stalks, and continued weekly until three weeks past killing frost. Conclusions drawn include: 1) there is little or no host resistance to Fusarium spp., 2) the rate curve for stalk senescence is hybrid dependent and predictable, 3) stalk senescence is often reported as stalk rot, and 4) stalk lodging vulnerability can be predicted and managed to reduce losses due to "stalk rot".

A373
FIELD STUDIES ON SUDDEN DEATH SYNDROME OF SOYBEAN. S. B. Belmar and H. W. Kirby, Department of Plant Pathology, University of Illinois, Urbana, IL 61801.

A three year field study was conducted in Illinois to evaluate soil applied pesticides for control of sudden death syndrome (SDS) of soybean. Plots treated with methyl bromide lacked disease symptoms, i.e., interveinal chlorosis and necrosis of the leaflets. Application of Ridomil 50G, Teraclor 10G, and Imazalil 50G did not consistently reduce foliar symptom development compared to a non-treated control. Populations of soybean cyst nematode were reduced with Temik 15G; however, control of SDS was not observed. Disease symptoms were more severe with an "early" planting than with a "late" planting of soybeans. Regardless of planting date, symptoms consistently appeared in Illinois when plants were in the early pod growth stage. Seed harvested from plants with severe SDS symptoms were planted in methyl bromide treated soil; however, no SDS foliar symptoms were observed. Therefore, the causal organism did not appear to be transmitted by seed in this study.

A374
OBSERVED SPREAD OF SOYBEAN STEM CANKER DISEASE. B. L. Keeling, USDA-ARS, Janie Whitten Delta States Research Center, Stoneville, MS 38776.

The spread of soybean (Glycine max) stem canker disease, caused by Diporthe phaseolorum var. caulivora, in a susceptible cultivar (377-339) from a point source of inoculum was measured. Data was taken from field plots during two growing seasons. During the first season, plots were subjected to rain and wind from the remnants of two hurricanes. The disease was spread in diaspores; a maximum of 6.4 m. Spread of the disease reflected influence of the weather pattern. During the second year, plots were subjected to several periods of rain without strong winds. Diseased plants occurred randomly dispersed in all directions from the source of inoculum. In both years, most diseased plants occurred within 2 m of the source of inoculum.

A375
RHIZOPOUS ROOT ROT AND PHYTOPHTHORA ROOT ROT OF SUGAR BEET IN WYOMING. E. C. Vincelli, C. M. S. Beaufre, and W. F. Wilcox*, Dept. of Plant Soil & Insect Sciences, Univ. of Wyoming, Laramie, 82071 and *Dept. of Plant Pathology, New York State Agric. Exp. Stn., Geneva, 14456.

Two root diseases previously unreported from Wyoming were identified in sugar beets from commercial production fields in Washakie County during 1988-89. Rhizopus root rot, caused by Rhizopus arrhizus, was observed in a field in 1988. The disease was most severe in a portion of the field with poor surface drainage, where over 50% of the plants were affected. Poor drainage, temperatures well above normal, and insect injury were thought to have promoted development of the disease. Phytophthora root rot was diagnosed in sugar beet tap roots submitted to the UW Plant Disease Clinic following the 1989 harvest. Although Phytophthora drechsleri has been reported as causing root rot of sugar beet in other states, the isolate obtained in Wyoming more closely fits the description of Phytophthora cryptogeae based on morphology of sporangia and sporangiospores and temperature growth relations. Excessive soil moisture, possibly caused by overirrigation, was thought to have promoted disease development. Although Rhizopus root rot is the most common root rot pathogen of sugar beet in Wyoming, these previously unreported growth patterns may occasionally be responsible for root rot, as well.

A376
POPULATION DYNAMICS OF FUSARIUM OXIDIUM AND PYTThON SPP. IN TiTOM ATASYMPTOMATIc PLANTS IN A SOUTH DAKOTA SOYBEAN FIELD. C. M-S. Beaufre, M. W. Ferguson*, and G. W. Buchenau**, Dept. of Plant, Soil & Insect Sciences, Univ. of Wyoming, Laramie,
Under field conditions in Arkansas, septoria nodorum blotch is favored by two or more consecutive cool, wet, and windy days. Laboratory and greenhouse studies determined the effect of free moisture on lesion development on moderately resistant (cv. Calvert 302) and susceptible (Calwell) cultivars. On Calwell seedlings, the percent leaf area diseased increased as the post-inoculation dew period increased from 12 to 30 hr (6 hr increments, 12, 17, 22). However, on Florida 302 seedlings, the percent leaf area diseased remained low for 24 hr (20° air temperature), but lesions enlarged slowly on Florida 302 for all dew periods. At the flowering stage, the rate of lesion enlargement with increasing dew period was similar on both cultivars.

A381

In a survey of a total of nine winter wheat crops in the years 1987, 1988 and 1989, the predominant Fusarium species isolated from stem bases was Fusarium oxysporum. Fusarium avenaceum, F. culmorum and F. graminearum were also isolated. The highest incidence of F. nivalis occurred during April, 1989 in the cultivar Brock when the fungus was isolated from 65% of the tillers sampled. The highest incidence of F. avenaceum was 60% (August 1988, cv. Sleipner) and F. culmorum 37% (August, 1989, cv. Mercia). A delay in onset of infection during 1987 was attributed to the low January temperatures and an upsurge of F. culmorum during the warm dry summer. The incidence of F. nivalis fluctuated during the 1988 and 1989 seasons, particularly during spring. The ability of F. nivalis to produce acospores and complete its infection cycle relatively rapidly in spring temperatures accompanied by the natural death of late-formed tillers of winter wheat between March and the end of May is discussed as possible contributory factors to this.

A382
DEVELOPMENT OF COLLETOTRICHUM GRAMICICOLA ON WOUNDED MAIZE STEM TISSUES AS AFFECTED BY "WOUND HEALING" AND MAIZE GENOTYPE. A. Mumba, Kankongo and G. C. Bergstrom, Dept. of Plant Pathology, Cornell University, Ithaca, NY 14853.

This study was conducted to determine the effects of two types of resistance, maize genotype and wound healing, which each reduce anthracnose stalk rot, on early stages of fungal development at wound sites. Five tissue cylinders (2 mm diam.) from the first internode above the brace roots of Cornell 281 (susceptible) and B 37 x LB 31 (resistant) plants at anthesis were sliced into 15 ± 30 μm thick discs and placed on glass slides in moist chambers. The wounded surfaces were inoculated with 10^5 conidias/cm^2 either immediately or 2 hr after the slices were made. Conidial germination at 6 hr after inoculation was 98% on freshly wounded Cornell 281 vs. 84% on aged wounds, and 63% on freshly wounded B 37 x LB 31 vs. 19% on aged wounds. Germ tube and appressorium development as well as cell penetration were delayed on aged wounds and in the resistant hybrid. "Wound healing" and genotypic resistances are expressed at early stages of the host/pathogen interaction and may involve a diffusible, antifungal substance.

A383
RICE DEAD TILLER SYNDROME IN ARKANSAS. Fleet N. Lee, University of Arkansas, P.O. Box 351, Stuttgart, AR 72160.

"Dead Tiller Syndrome" (DTS) was observed in rice cultivars in Northeast Arkansas. Symptoms first appear 6 to 10 days after the permanent flood and progress over 8 to 14 days until the flood water equilibrates to ambient soil and air temperatures. The initial symptom is slight discoloration of plants with some wilting. Advanced symptoms include severe wilting. Occasional plants have yellow to orange chlorosis along older leaf tips or leaf margins. Plants have a rotting culm which frequently produces a distinct odor when crushed. Decay begins internally at a node and can be well advanced before detection. The plant dies in 24 to 48 hours. The main culm in a hill is usually displaced. Tillers or nearby plants are not affected. DTS continues to develop throughout the growing season in areas where cold flood water is added to the field. A fungus from diseased tissue inoculated into 3 to 4 leaf stage rice seedlings caused culm rotting and death.
A385
STUDIES ON RHYNCHOSPORIUM SEGALIS AS A SEEDBORNE PATHOGEN. A. D. Martinez-Espinosa, M. E. Bjarko and J. H. Rieszman. Dept. of Plant Pathology, Montana State University, Bozeman 59717.

Seed to seedling transmission was examined for Rhynchosporium segalii, the cause of scald on barley. Infected seed was obtained using a new artificial inoculation method. One ml of spore suspension containing 150,000 spores was injected into the boot. Infected seed had the characteristic double-eyed shape of naturally infected seed. This method was highly effective in greenhouse and field tests. Transmission of the pathogen from seed to seedling was demonstrated in greenhouse tests. Between 33% and 41% of infected seed gave rise to infected seedlings. Symptoms were detected mainly in the first leaf (65%) or second leaf (35%). Conspicuous symptoms were observed seven days after emergence. These data conclusively demonstrate the importance of seedborne inoculum in the initiation of barley scald.

A389
SEVERITY OF WHEAT STREAK MOSAIC (WSM) ON WHEAT INOCULATED AT DIFFERENT PHYSIOLOGICAL MATURITIES. R. M. Hunger, J. L. Sherwood, and C. E. Evans. Dept. of Plant Pathology, Oklahoma State University, Stillwater, OK 74078-9947.

Hard red winter wheat cultivars ('Chisholm' and 'Rally') are considered susceptible and resistant, respectively, to WSM. These cvs were planted on 12 Sep 88, 12 Oct 88, and 09 Nov 88, and were inoculated with wheat streak mosaic virus on 24 Mar 89 as described previously (Phytopath. 1988. 78:1503). At inoculation, plants of both cvs at each planting date were at Zadoks growth stage 31, 30-31, and 30 (Weed Res. 1974. 14:415-421). Development of WSM was monitored by symptomatology and enzyme-linked immunosorbent assay, and yield and thousand kernel weight were determined. Wheat which was physiologically young at inoculation showed severe WSM and yield reductions. Wheat inoculated at a more mature growth stage (Sep and Oct planting dates) exhibited mild WSM and inconsistent yield reductions. This suggests that planting date affects WSM severity on wheat infected in the spring.

A390

We determined genotypes at the isozyme loci Gpi1 and Ppol in collections of Phytophthora infestans from central and northern Mexico, the United States, Japan, The Netherlands, and Poland. The central Mexican sample had the highest diversity, as measured by the number of dilocus genotypes and the average number of alleles per locus. All collections had the same most common allele for both Gpi1 and Ppol, but there were several alleles unique to particular regions. Non-random associations, consistent with a lack of sexual recombination, were found in the United States, where only the A1 mating type occurs, and also in northwestern Mexico and Japan, where both mating types occur. A comparison of samples from the early and late 1980's suggests that the genetic makeup of P. infestans in Europe has changed significantly during the last decade.

A391

Two dispersed, moderately repetitive clones have been isolated from Phytophthora infestans. Each of these clones reveals 15 or more bands when hybridized to Southern blots of genomic DNA cut with the restriction enzyme Eco RI. Most of the bands are highly polymorphic, segregate as Mendelian markers, and do not appear to be tightly linked. Furthermore, individual banding patterns are stable through single-locus inoculations. These clones may therefore provide true genotypic fingerprints that are specific to particular individuals. Over 200 isolates collected from several P. infestans populations worldwide have been probed with these two clones, and differences were found among isolates that were identical for other markers. In some populations, where both mating types occur, reproduction is exclusively asexual, while in central Mexico almost every isolate is unique.

A392

Ploidy of isolates of Phytophthora infestans with a variety of isozyme genotypes and A1, A2, and self-fertile mating types from central and
northern Mexico was tested with a simplified DAPI (4',6-diamidino-2-phenylindole) staining method. The isolates are mainly diploid, but great variation in levels of the ploidy, including apparent diploid, triploid and tetraploid, were found in both central and northern Mexico populations. In northern Mexico the genotype Gpi 100/111/122, Pep 92/100 was mainly diploid, while the genotype Gpi 100/111/122, Pep 100/100 included different ploidy levels. In central Mexico, the isolates primarily are diploid, but some are of higher ploidy.

stability over sites and years. The 12% reduction in percent of needles infected, which is predicted on the basis of genetic theory, may be conservative because of the effects of epidemiological effect of planting only resistant trees in a stand. In the presence of high Dothistroma infection, wood volume production has been greater for the resistant bred than for improved populations which have not been isolated for resistance.

A397
GENETIC FINE STRUCTURE OF A COMPLEX RUST RESISTANCE LOCUS IN MAIZE. S. H. Hubbert and J. L. Bennetzen. Kansas State University, Manhattan KS, 66506 and Purdue University, West Lafayette IN, 47907.

Chromosome 10 of maize carries a cluster of genes which control resistance to the rust species Puccinia sorghi and P. polysora. RFLP markers which closely flank this region were mapped and used to study the genetic arrangement of these genes. The positions of the resistance factors mapped within about 0.2 map units of each other. Others, such as Rpg1 and Rpg5, mapped up to three map units away from the cluster. Reassociation frequencies between Rpg genes depended heavily on the parents used in the cross. Changes in resistance of progeny from some Rpg heterozygotes arose by mechanisms other than simple recombination. Possible mechanisms are being investigated.

A398
LENGTH HETEROGENEITY OF rDNA CODING REGIONS IN RHOCYLIA SOLAN. Okkio Gonzalez and Ryusuke Yagishita. Department of Botany, Duke University, Durham, NC 27706.

Length variation within the non-transcribed regions of rDNA is frequently observed both at the species and population level in many fungi. We employed sequence data and restriction analyses using the polymerase chain reaction to examine length variation within genic regions coding for both ITS1 and 25S RNA. The length polymorphisms were detected among different anastomosis groups of the R. solani complex for almost every region of the 25S RNA examined. Additional survey of 12 isolates from AG 1 also detected 25S RNA length variation between individuals. Several AG isolates were found to possess up to 3 rDNA length variants. Sequence data and restriction analysis showed that most of the major length variation is located about 600 bp from the 5' end of the 25S RNA coding region, and extends up to 500 bases inside the gene. In contrast to the 25S RNA coding region, sequence analysis of 1200 bp from the 5' end of the 18S RNA gene showed no length variation and only base substitutions. The presence of genic rDNA length polymorphisms among and within isolates adds a new level of subgenomic variation that needs to be considered in comparative analysis of this gene.

A399
IN VITRO INDUCTION OF PSEUDOTHIA, ASCOSPORE RELEASE AND VARIATION IN FERTILITY AMONG GEOGRAPHIC ISOLATES OF LEPTOSPHAERIA MACULANS. A. Mangold, S.R. Rimmer, and P.H. Williams. Department of Plant Pathology, University of Wisconsin, Madison, WI 53706.

The sexual compatibility and reproductive capacity in Leptosphaeria maculans from Europe, Canada, and Australia was assessed. To induce sexual mating pairs of single ascospore isolates were placed 1 cm apart on V8 agar in plastic petri plates, sealed with parafilm and incubated at 24 C under continuous fluorescent light (60-75 µE sec^-1 m^-2). After 7 days, the developing fungal colonies were removed, and a layer of 2% water agar cooled to 55 C is poured over the V8 agar layer. Plates are resealed and incubated for 3-4 weeks at 16 C under black lights (Sylvania 40 watt, BLB) with a 12 h photoperiod. Release of ascospores was facilitated by placing a drop of 5% 1-glucuronidase and water over the agar. There was a significant variation among isolates in the number of perithecia. Isolates from the different geographic areas were able to intermate. Isolates PWH 1275, 1276, and 1306, all from Australia, had the highest number of perithecia and were the most fertile of the isolates listed.

A400
EXPRESSION OF THE TRANSPOSABLE ELEMENT Tc1 IN THE NEMATODE CANCROHABITANS ELEGANS. Anthony Radice and Scott Emmons, Department of Molecular Genetics, Albert Einstein College of Medicine, Bronx, New York 10461.

In order to understand the mechanisms underlying Tc1 excision and transposition, we are studying transcription and translation of the Tc1 open reading frame. In Northern blot hybridization experiments, we have shown that a Tc1-open reading frame probe hybridizes to a large number of RNA species, including ribosomal RNA. To further characterize Tc1 transcripts,
we have carried out PCR amplification experiments after first strand cDNA synthesis from polyA+ RNA preparations. We determined that there was a region of Tc1 that could not be amplified in this way, suggesting the presence of a 3' end. So far, the largest region we have amplified spans from nucleotide 304 to 1548 (putative polyadenylation signal). To study expression of the Tc1 ORF protein, we have three antisera that recognize the putative transposase, TcA. The antisera react with the TcA portion of a Tnp1-TcA fusion protein isolated from E. coli, but not to a protein of the expected molecular weight in preparations of nematode proteins. Therefore, the Tc1 ORF protein, if it is expressed, is a rare protein.

A402

RELATIONSHIP AMONG THE VASCULAR WILT FUSARIA OF THE CHENOPODIACEAE. R. D. Shrey, D. H. Kim, C. M. Rush, and E. A. Dillard, Department of Plant Pathology and Microbiology, Texas A&M University, College Station 77843.

Isolates of Fusarium oxysporum causing vascular wilt in the Chenopodiaceae plants sugar beet (Beta vulgaris), spinach (Spinacia oleracea), and redroot pigweed (Amaranthus retroflexus) were compared using host pathogenicity, isozyme relatedness, and miDNA RFLP patterns. Pathogenicity tests indicated a range of host specificity among the isolates, i.e. some were specific to their original host, a few were primarily pathogenic to their original host but caused some wilt on other hosts, and two isolates were highly pathogenic to both sugar beet and spinach and moderately pathogenic to pigweed. Isozyme profiles and miDNA hybridizations correlated with the pathogenicity results. Isolates specific to sugar beet had similar isozyme matching in between. RFLP analysis revealed three main polymorphic groups and two subgroups: isolates specific to sugar beet and spinach separated into two distinct groups while two cross-over isolates were in a third group. These data suggest that while most isolates display a high degree of host specificity, there exists within the Chenopodiaceae isolates that cross-over to other species within the Chenopodiaceae.

A403

CHARACTERIZATION OF ANASTOMOSIS GROUPS OF BINUCLEATE RHIZOCTONIA FUNGI USING RESTRICTION ANALYSIS OF RIBOSOMAL RNA GENES. M.A. Cubeta, E. Echandi, R. Vigilias, and T. Abernethy, Dept. of Plant Pathology, North Carolina State University, Raleigh, NC 27695 and Dept. of Botany, Duke University*, Durham, NC 27706.

Seven US and 15 Japanese binucleate Rhizoctonia anastomosis tester isolates were characterized by restriction analysis of ribosomal RNA genes. Total genomic DNA was extracted and a 1.4 kb fragment coding for 25S RNA was amplified using the polymerase chain reaction. Amplified DNA was digested with six different restriction enzymes to determine restriction fragment length polymorphisms. Restriction patterns of AG1 and AGD were similar to each other with all enzymes tested, but distinct from all other isolates. Isolates belonging to CAG2, CAG3, CAG6, AGA, AGF and AGG had very similar restriction patterns but could be differentiated with a specific restriction enzyme. These findings support previous separation of binucleate Rhizoctonia fungi based on hyphal anastomosis by Burpee and Ogoshi.

A404

FUNGISTIC COMPounds FROM SOIL INHIBIT GERMINATION OF COCHLIODIUS VICTORIACE Conidia. J. A. Liebman and L. Epstein, University of California, Berkeley, CA 94720.

Conidia of the fungus Cochliobolus victoriae do not germinate on soil but do germinate on sterile distilled water. We investigated the cause of this soil fungistasis. To quantify fungistasis, we counted percentage germination of conidia. Conidia were incubated on agarose blocks which were placed on soil. The blocks were kept sterile and separate from soil by polycarbonate membranes with 0.2-µm pores. Thinner agarose

blocks became fungistatic more quickly than thicker blocks, and blocks became more fungistatic with increasing time on soil. When removed from soil, blocks remained fungistatic, but only for a few hours. Agarose separated from soil by a glass cover slip and placed in a light-tight chamber did not become fungistatic. Four soils gave similar results. The data suggest that many soils contain a fungistatic compound which diffuses through agarose and which is not highly volatile.

A406

A NOVEL DIFFERENTIAL MEDIUM FOR THE QUANTIFICATION OF PHOMA TERRESTRIS IN ORGANIC SOILS. N. L. Strube and J. W. Lorbeer, Department of Plant Pathology, Cornell University, Ithaca, NY 14853.

Phoma terrestris, causal agent of pink root of onion, colonizes and stains cotton fibers a distinctive pink to red color. Cheesecloth, therefore, was employed as the basis of a novel differential medium for P. terrestris. Twenty ml of medium containing 3 g NaNO3, 1 g MgSO4.7H2O, 0.5 g chlamyaphencol, and 20 g agar, per liter of H2O was poured over a 9-cm diameter single layer of sterilized cheesecloth in a plastic petri dish. Pink to red areas appeared on the cheesecloth in dilution plates of organic soils naturally infested with P. terrestris. The presence of the pathogen in these areas was confirmed by resiotation and pathogenicity tests on onion seedlings. This medium has been found to be useful for monitoring the influence of soil fungation on populations of P. terrestris in the organic soils of commercial onion fields in New York.

A407


Field grown asparagus preinoculated with a commercial peat containing vesicular-arbuscular mycorrhizal (VAM) fungi Glomus intraradices (Gl) and G. versiforme (GVR) previously exhibited improved survival and growth compared to non-inoculated plants. To determine if the VAM effect was nutritionally mediated, we evaluated the response of asparagus to different levels of applied P when grown in the greenhouse in a peat-based mycorrhizal inoculum. Dry weight, tissue P concentration and root colonization were assessed at 10 and 14 weeks. Plant growth increases by Gl and GVR were positively correlated with root colonization. There was no significant response of plant dry weight to 100 ml of P solution applied to 10 cm pots weekly at 0, 50, 100 and 150 ppm for either inoculated or uninoculated plants.

A408


Pathogenicity of 59 isolates of Rhizoctonia from various geographical and host sources was determined on potato at 10, 15.5, and 21.1°C. Isolates representing 11 anastomosis groups of R. solani and other multinucleate and binucleate of Rhizoctonia were included. Isolates of R. solani AG-3 killed significantly more sprouts per plant at all temperatures than isolates of any other group. Damage to sprouts attacked by isolates of AG-3 was greater than damage caused by any other groups at 10 C, but isolates of AG-6 and AG-8 caused similar damage to sprouts at 10 C. At higher temperatures isolates of many groups were more virulent, particularly AG-6 and AG-4, but virulence of isolates of AG-6 and

1008 PHYTOPATHOLOGY
AG-8 did not increase with temperature. Although isolates of AG-8 were as damaging as isolates of AG-3 to roots at all temperatures, AG-8 caused only minor damage to shoots. No groups were more virulent to shoots or roots at any temperature than AG-3. However, isolates of AG-8 cause severe damage to roots and may be able to cause economically significant reductions in yield.

USE OF A HIGHLY SUSCEPTIBLE WATERMELON CULTIVAR TO SELECTIVELY RECOVER FUARISUM OXYSPORUM F. SP. NIVENUM FROM SOIL FOR RACE DETERMINATION.

D. L. Hopkins and R. J. Lobinske, Central Florida Research and Education Center, University of Florida, Leesburg, FL 34788.

Florida Giant, highly susceptible to all races of Fusarium oxysporum f. sp. niveum, was used to selectively recover the pathogen from soil. The three vegetative compatibility groups (VCG) for this Fusarium were found, corresponding with the highly aggressive race 2, were mixed in various proportions in steam sterilized soil. The pathogen was then transferred from Florida Giant seedlings that wilted when planted in this infested soil. The proportions of the three VCG isolated from wilted seedlings were very similar to the proportions of F. oxysporum f. sp. niveum in the soil that is the highly aggressive race 2. Using this technique, the resistant cultivar Calboun Gray was shown to select for race 2 of the pathogen.

SUPPRESSION OF APHANOUMES DAMPING-OFF OF SUGAR BEET BY INCORPORATION OF GREEN PLANT RESIDUE INTO SOIL.

Carol E. Windels and Donna J. Naben-Schindler, Northwest Experiment Station, University of Minnesota, Crookston 56716.

Soils naturally infested with Aphanomoses cohnii were collected from fields and planted in the greenhouse (18°C) to 18 crops representing the Chenopodiaceae, Cruciferae, Gramineae and Leguminosae families. After 4 wk, plants were cut at soil level, dried at 38°C, cut into pieces, and incorporated into the same soil from which the crop had been removed, and incubated for 3 wk. Soils were then planted with sugar beet (24-27°C). Incorporation of green oat residue resulted in an increase in sugar beet yield in both races (58 and 83%) compared to sugar beet after sugar beet (45% and 55%) or sugar beet after fallow (22% and 23%). Root rot indices (0-100 scale) were decreased in both soils treated with green oat residue (17 and 16) compared to sugar beet after sugar beet (57 and 51) and sugar beet after fallow (98 and 82). Incorporation of green oat residue into soil may be useful in managing Aphanomoses damping-off of sugar beet.

THE DISTRIBUTION OF 14C IN PLANT TISSUE AND ROOT EXUDATE OF CITRUS INCUBATED WITH FUARISUM SOLANI, PHYTOPHTHORA CITROPHORA, AND BOTH F. SOLANI AND P. CITROPHORA L.-M. Damurdar and J. A. Menge, Dept. of Plant Pathology, Univ. of California, Riverside, CA 92521.

Heat-stressed and non-stressed citrus seedlings were labelled with 14C for 48 h, 6 wk after inoculating with Fusarium solani (Fs), Phytophthora citrophora (PC), or both Fs and Pc. At harvest, the distribution of 14C among leaf, stem, root, and root exudate was measured by liquid scintillation. The percentages of 14C in the leaf and stem fractions of plants incubated with Pc were significantly greater than for non-inoculated plants. The percentages of 14C in roots were significantly less for plants inoculated with Pc. Fs did not have a significant influence on partitioning of 14C. The percentage of 14C in the leaf and roots tissues of heat-stressed plants was not significantly different than those of the non-stressed plants. Heat-stressed plants co-inoculated with Fs and Pc had a significantly lower percentage of 14C in the root exudate than non-stressed, co-inoculated plants.

PSORALEN COMPOUNDS ACTIVATE DISEASE RESISTANCE RESPONSE GENES IN PEA.

A. Parsons, D. Horozov, and L. A. Hadwiger, Department of Plant Pathology, Washington State University, Pullman, WA 99164-0350.

Natural plant psoralen compounds applied to pea pods and subsequently cross-linked to pea DNA with 366 nm U.V. light have been previously shown to be elicitors of piaxin in pea. This enhanced susceptibility of major flowering, one very similar to the pattern of proteins synthesized when peas are induced to resist the pathogen Fusarium solani f. sp. piax. We now report that a synthetic psoralen, trisoxalen, 4-amino methyl HCl (AMT), induces two protein patterns in pea seedlings. DRG49 and DRG20. Pea seed was treated with AMT (60 mg/kg) for 45 minutes and subsequently cross-linked by U.V. 366 light for 12 minutes to initiate the induction observed. The DNA specificity of the psoralen binding in relationship to the mode of action of AMT and a biotic DNA-specific elicitor, chitinase, will be discussed.

MOLECULAR CLONING OF THE TOX LOCUS FROM COCHLIIOBOLUS CARBONUM.

J.D. Walton, J.S. Scott-Craig, and J.A. Pocارد, DOE Plant Research Laboratory, Michigan State University, East Lansing, MI 48824.

We previously described the purification of two enzymes involved in the biosynthesis of HC-toxin, the host-selective toxin produced by race 1 but not races 2 or 3 of Cochliobolus carbonum. Genomic DNA sequences homologous to a cDNA clone for HTS-1 are found only in race 1 isolates of the fungus. By chromosome walking in both directions from the HTS-1 coding region, we have identified and cloned a 22-kb region of DNA that is unique to race 1. Cloned sequences on either side of the race 1-unique region contain elements that are moderately repeated (ca. 20 to 100 copies/clone) in both races. Left and right border sequences have homology with one another. We anticipate that the 22-kb, race 1-unique region is a gene cluster encoding HTS-1 and additional enzymes involved in HC-toxin biosynthesis.

CHARACTERIZATION OF THE TOX LOCUS OF COCHLIIOBOLUS CARBONUM.

D.G. Panaccione, J.S. Scott-Craig, J.A. Pocارد, and J.D. Walton, DOE Plant Research Laboratory, Michigan State University, East Lansing, MI 48824.

HC-toxin synthetase I (HTS-1) is an enzyme involved in the biosynthesis of HC-toxin, the host-selective toxin produced by race 1 but not races 2 or 3 of Cochliobolus carbonum. Genomic DNA sequences homologous to a cDNA clone for HTS-1 are found only in race 1 isolates of the fungus. By chromosome walking in both directions from the HTS-1 coding region, we have identified and cloned a 22-kb region of DNA that is unique to race 1. Border sequences on either side of the race 1-unique region contain elements that are moderately repeated (ca. 20 to 100 copies/clone) in both races. Left and right border sequences have homology with one another. We anticipate that the 22-kb, race 1-unique region is a gene cluster encoding HTS-1 and additional enzymes involved in HC-toxin biosynthesis.

Aspects of pathogenesis and host resistance in the interaction between Venturia inaequalis and apples. C. Valsangiacomo, M. Müller, B. Koller, and C. Gessler. Institute of Phytopathology, 8092 ETH-Zürich, Switzerland.

V. inaequalis and apple establish a peculiar relationship where the fungus develops between the cuticular membrane and cells of the upper epidermis without penetrating any cell. TEM studies showed local degradation of host epidermal cell walls. Enzymes potentially involved in this phenomenon, such as cellulases and polygalacturonases, were found in fungal liquid cultures. Polygalacturonase was purified to homogeneity and characterized both biochemically and immunologically. The activity of cellulytic enzymes was detected and further purification and characterization of the enzyme were made. A polygalacturonase inhibitor (PGI) from uninfected apple leaves was purified and partially characterized. The role of cell wall degrading enzymes and of PGI in the interaction between V. inaequalis and Malus will be discussed.

ELICITATION OF SESQUITERPENOID CYCLASE AND SUPPRESSION OF SQUALENE SYNTHETASE ACTIVITY IN POTATO TUBER TISSUE.

M. N. Zook and J. A. Kuc, Department of Plant Pathology, University of Kentucky, Lexington, Kentucky 40546.

Arachidonic acid (AA), an elicitor of sesquiterpenoid phytoalexins from Phytophthora infestans, causes a 20-fold increase in sesquiterpene cyclase activity in potato tuber tissue 48 h after application, as compared to untreated tissue. Squalene synthetase activity decreased by 90% in the same elicitor-treated tissue 12 h after AA application. Elicitation of sesquiterpene cyclase activity and suppression of squalene synthetase activity were also observed after inoculation with Helminthosporium carbonum, a non-pathogen of potato, and two incompatible races of P. infestans. These results indicate that elicitation of phytoalexin accumulation in potato involves coordinate regulation of two important enzymes in the acetaldehyde-mevalonate pathway.

GLYCOSYLATED ELICITORS INDUCE MAJOR AND DISTINCTLY DIFFERENT RESPONSES IN LOCAL AND DISTAL SOYBEAN CELL POPULATIONS.

Vol. 80, No. 10, 1990 1009
A418
THE EFFECT OF MYCOTOXIN FUMONISIN B1 ON THE GROWTH AND DEVELOPMENT OF MAIZE CALLUS. M.A.J. van Asch, F.H.J. Rijkenberg, and T.A. Coutinho. Department of Microbiology and Plant Pathology, University of Natal, P.O. Box 375, Pietermaritzburg, 3200, South Africa.

The phytotoxic effect of Fumonisin B1, a mycotoxin of Fusarium moniliforme, was tested using callus from the scutella of immature cobs of maize, Zea mays. The callus was grown on modified MS medium with the toxin in different amounts (0.0, 1.0, 10, and 100 mg/g per liter). Callus growth decreased as the concentration of toxin increased, resulting in a significant growth reduction at the highest toxin level (100 mg/g). Transmission electron microscopy studies showed an increased level of activity in the treated cells resulting in thicker cell walls and the occurrence of starch grains. It is postulated that this increased activity led to cell disorganisation and, finally, death of many cells. At the 100 mg/g level, callus cells ceased to divide and were dead, but growth studies revealed that most of the callus pieces had retained their viability, even though the growth rate of the callus was significantly slower than in all the other treatments. Callus, grown at all other concentrations, recovered fully, and at the end of the regrowth period, no difference could be demonstrated between the other treatments.

A419
HPR MUTANTS OF Pseudomonas syringae pv. tabaci ACTIVATE THE TRANSCRIPTION OF GENES ASSOCIATED WITH DISEASE RESISTANCE IN BEAN. J. L. Jakobek, and P. B. Lindgren, Department of Plant Pathology, North Carolina State University, Raleigh, NC, 27695.

We have been studying the induction by bacteria of genes in bean (Phaseolus vulgaris L.) associated with disease resistance. Slot blot and Northern analyses were conducted using RNA isolated from inoculated bean leaves and cDNA probes for phenylalanine ammonia-lyase (PAL), chalcone synthase (CHS), chalcone isomerase (CHI), or chalcone synthase. Transcripts corresponding to all four cDNAs are rapidly induced in the bean cultivar ‘Red Kidney’ after inoculation with Hrp mutants of Pseudomonas syringae pv. tabaci (Pst), even though visible hypersensitive responses are not seen after inoculation with these mutants. This induction is very similar to that which occurs after bean is inoculated with wild-type Pst with respect to the timing and level of gene transcription. The expression of PAL after inoculation by Hrp strains is not transient, as this transcript is still detected 120 hours post-inoculation. PAL and CHI are also induced in bean after inoculation with P. fluorescens and heat killed Pst cells, but not after inoculation with Escherichia coli.

A420
INOCULATION OF BEAN WITH HRP MUTANTS OF PSEUDOMONAS SYRINGAE PV. TABACI ALTERS SUSCEPTIBILITY TO P. S. PV. PHASEOLICOLA. P. B. Lindgren and J. L. Jakobek, Department of Plant Pathology, North Carolina State University, Raleigh, NC, 27695.

Bean plants (Phaseolus vulgaris L.) that are inoculated with P11528X1, a Hrp mutant of Pseudomonas syringae pv. tabaci (Pst), may be more resistant to infection by P. s. pv. phaseolicola (Psp) than are plants which have not been inoculated with this mutant. Although P11528X1 did not elicit a visible hypersensitive response on bean, certain genes which are associated with plant disease resistance are induced after bean is inoculated with this strain. The bean cultivar ‘Red Kidney’ was simultaneously inoculated with Psp and P11528X1, or inoculated with Psp eight hours after being inoculated with P11528X1. In both situations the population of Psp increased by at least 3 log units, compared to an increase of 5 to 6 log units when plants were inoculated with Psp alone. Disease symptoms were also variable on plants inoculated with bacteria, but were reduced when compared to symptoms seen when bean was inoculated with Psp alone.

A421
RECOGNITION MECHANISM IN MYCOParasitic SYSTEM. M. S. Manocha, Y. Chen, N. Rao, Department of Biological Sciences, Brock University, St. Catharines, Ontario, Canada, L2S 3A1.

Recognition by the mycoparasite, Piptoploecis virginiana, of its hosts (compatible and incompatible) and nonhosts occurs at least at two different levels, i.e., cell wall and proteoplast surface. At the cell wall level, the mycoparasite recognizes the differences in sugar distribution pattern between the host and nonhost species and it attaches to the former and not to the latter. Attachment of mycoparasite to its host surface could be inhibited by N-acetylglucosamine, glucose and arabinose. These three sugars are the major components of the agglutinin present as two distinct bands of glycoproteins, observed in SDS-PAGE of the cell wall extract of host and not of nonhost. These sugars, however, do not affect the appressorium formation which probably is affected by the protein component of the agglutinin. At the proteoplast level, the mycoparasite recognizes the differences between the compatible and the incompatible host species. The exact nature of recognition at the proteoplast level is not clear, but it seems to involve the rejection of self in the incompatible interaction and the acceptance of non-self in the compatible interaction.

A422
ISOLATION OF CDNA SEQUENCES FOR 3-HYDROXY-3-METHYLLULURYL-COENZYM E A REDUCTASE FROM POTATO TUBER. Choi, B. L., Ward, W. L., Bostock, R. M., Department of Plant Pathology, University of California, Davis 95616.

Induction of potato HMGR activity precedes the accumulation of the phytolxoxas, ubiquinone, and riboflavin, which accumulate following treatment of tuber tissue with the fungal elicitor, arachidonic acid, and are associated with hypersensitive response of potato cultivars to incompatible races of Phytophthora infestans. Southern blot hybridization of potato genomic DNA with a highly conserved sequence from the 3' region of the HMGR gene from Arabidopsis thaliana detects four EcoRI restriction fragments each present in one to several copies per haploid genome. Forty-seven positive clones were isolated after screening 670,000 plaques of Yg11 potato tuber cdNA library by hybridization with the Arabidopsis probe. Eleven independent cdNAS were subcloned and mapped. Northern blots of total RNA from potato tuber tissue and young tomato fruit detected a similar size transcript in both plants after hybridization with the Arabidopsis probe.

A423
CROSS PROTECTION INDUCED BY FUSARIUM OXYSPORUM AGAINST VERTICILLIUM WILT IN FUSARIUM RESISTANT TOMATO. P. E. Jorge, W. R. Chaney and R. J. Green, Dept. of Forestry and Natural Resources, Purdue University, West Lafayette, IN.

The occurrence of cross protection (induced resistance) was tested with near isolines of Roma tomato cultivars that differed in absence or presence of a gene(s) for resistance to vascular wilt pathogens. Fusarium oxysporum f. sp. lycopersici (inducer) and Verticillium dahliae (challenger) interactions were studied. Tomato seedlings in their 3'-true leaf stage were field inoculated in suspensions of 7 x 10^5 propagules/ml of fungal suspension. Plants were inoculated for 21 d under controlled soil temperature at 28 C. Fusarium oxysporum induced resistance against V. dahliae in Fusarium resistant Roma seedlings when inoculated simultaneously with both fungi. Resistance was expressed as a statistically significant higher fresh weight, lower disease severity index and lower stem isolation rate of Verticillium for plants inoculated with both fungi (protected) compared to plants inoculated only with V. dahliae (unprotected).

A425

Growth chamber studies were conducted to determine the potential of Apophaea amarranthi as a biocontrol agent for tumble pigweed (Amaranthus albus L.). Seedlings at the 4-6 true leaf stage were killed within two days when inoculated with conidial suspensions of 1x10^6 spores/ml when given a 12-hr dew period at 28 C. Conidial concentrations as low as 1x10^5 spores/ml were sufficient for conidial control when the dew period was increased to 24 hr. Dew temperatures ranging from 16 to 28 C were conducive for disease development. The onset of the dew period following inoculation could be delayed for 24 hr without an apparent decrease in disease severity. Field inoculations in 1989 resulted in 75% mortality of tumble pigweed seedlings. Results from these studies suggest that A. amarranthi may have potential as a bioherbicide for tumble pigweed.


Hirsutella rhosiospsis, an obligate parasite of nematodes, produces mummified spores which adhere to and infect passing hosts. A natural selection (loss of adhesive properties) of spores was quantified in vials containing 17 cm^3 loamy soil (heated to 60 C for 2 hr, adjusted to -60 mbar matric potential) at 20 C. Vials contained two nematodes, and 1.1 x 10^6 spores at day 0. Vials were assayed periodically for spore production by adding hosts (juveniles of Heterodera schachtii). Hosts were exposed for 3 days, and number of spores/host and infection of hosts were determined. Spore mortality was inferred from changes in spore detection. Each vial was assayed once (five vials/date). Some spores died within 21 days and others were viable for at least 200 days. Over 90% of the hosts with spores were infected regardless of date. The relative rate of spore mortality was 0.072 ± 0.003/day.

A428 ENVIRONMENTAL FITNESS OF SELECTED ENDOPHYTIC BACTERIA: A POTENTIAL BIOCONTROL FOR OAK WILT. E. H. Gehring, D. N. Appel, C. F. Gonzalez, Department of Plant Pathology and Microbiology, Texas A&M University, College Station 77843; and T. H. Filer, USDA-ARS Forest Service, Stoneville, MS 38776.

Three endophytic bacterial isolates with antibiotic resistance markers were introduced into mature live oaks (Quercus fuscifolia) to evaluate their environmental fitness as potential biocontrol agents for oak wilt, caused by Ceratocystis fimbriata. Bacterial isolates previously isolated from Pseudomonas demantisica, strain 1-15 suppressed oak wilt development in containerized trees. Distribution of the introduced bacteria was determined by dissection of an infected tree at 1 wk. Strain 1-15 was recovered in 12% of the root, bole, and branch samples, while Bacillus pumilus (1-1) was found in only 3% of the samples. P. putida (Y-20) was not recovered from a dissected tree. In a previous test, the bacterial species were determined by taking samples at 1 day, 1 wk, 1, 2, 3, and 6 mo after introduction and plating tissue samples onto a medium amended with the appropriate antibiotic. At branch samples Y-20 (bole sample) were isolated at 1 and 3 mo, respectively, while all other samples remained negative. A429 DEVELOPMENT OF PUCINIA CARDUORUM AND ITS IMPACT ON MUSK THISTLE IN VIRGINIA. A.B.A.R. Soudain, Dept of PEMS, R.A. Abe, S. A. Sok, Division of Entomology, USDA-ARS, VA 24061, and M.L. Bruckart, USDA-ARS, Frederick, MD 21701.

A Puccinia carduorum strain from Turkey was evaluated as a biological control agent for musk thistle (Carduus thornellii) in a 2-yr field trial. Musk thistle plots were inoculated successfully with the rust fungus in the fall and spring. To control the pathogen, the disease was inoculated (slightly susceptible in greenhouse trials), artichoke, and selected Cirsium spp. were planted between the inoculated plots. One rust species was inoculated in 1989; all other plots remained rust-free despite severe disease on surrounding musk thistles. On musk thistle, pathogen spread was limited during the rosette stage; disease became severe only when the plants bolted. The rust spread only a few hundred meters in 1988, but in 1989 traces of rust were detected at naturally occurring musk thistle stands up to 7 km away. Rust had little effect on plant size, but reduced seed production and accelerated senescence significantly in 1989.

A430 BIOLOGICAL CONTROL OF PUSARIUM MONILIFORME IN RICE BY ANTAGONISTIC BACTERIA. T.N. Hew and A.M. Rosales. The International Rice Research Institute, P.O. Box 933, Manila, Philippines.

Bakanae disease of rice caused by Pusarium moniliforme is a seedborne disease. The potential of antagonistic bacteria from paddy water, rhizosphere soils and rice plants, to control this disease was assessed by dual culture, filter paper and seed germination techniques. Out of 441 isolates, 113 were inhibitory to mycelial growth of the pathogen. Bacterization of naturally-infected IR422 seeds reduced bakanae incidence from 5-88% and 67-84% in seedbox and seedbed tests, respectively. Bakanae incidence in IR8 was reduced by 33-84% in seedbox test. Using the blotter and seed germination test, isolates were divided into three groups: 1) those that promoted germination and enhanced seedling vigor 2) no effect on germination and 3) those that were deleterious which inhibited germination.


In greenhouse pot experiments, seeds of cowpea (Vigna unguiculata var. Blackeye) were planted in pasteurized sand-soil infested with Phytophtora viagnae. Seeds were treated or not with suspensions of bacterial biocontrol agents (10^6 cfu/ml) known to inhibit the pathogen in vitro: some were isolated from cowpea field soils in Sri Lanka where the pathogen was present but the disease was absent. After 2 weeks, several bacteria had significantly increased plant survival and prevented disease symptoms. Though most bacteria increased plant dry weight compared to the control (pathogen only), the increases were only significant for two of the Sri Lanka isolates. These findings suggest that seed treatments with bacterial biocontrol agents could provide control of Phytophtora root rot of cowpea.


Several root pathogen bacterial biocontrol agents inhibited several species of Phytophtora in vitro, including P. cinnamomi, P. cactorum, P. sycogaeae, P. cambivora, and P. viagnae, due to agar-diffusible substance. Production of volatile inhibitors in vitro was substrate dependent, and no volatile inhibitors were produced by effective bacteria added to sterile soil unless some substrate was also added. These results demonstrate that production of volatile inhibitors by biocontrol agents should be considered as a potential mechanism of biocontrol of root pathogens.

A433 THE HYPERPARASITE ANPELOCLYMNA QVISICOLARIS INCREASES VIABILITY AND VIABILITY OF HOST Fungi. T. P. Cooper and C. Crow, Dept. of Plant Pathology and Microbiology, Faculty of Agriculture, Beni-Ben, Israel.
Effective biocontrol of cucumber (C) and zucchini (Z) powdery mildew (FM) was obtained by applying a second application of G. catenulatum (A), which failed to produce fruits in a field trial, when treated with FM. The disease severity was reduced by 50% in all treatments. In a greenhouse trial, application of AQ alone to CPM significantly increased yield over untreated controls, while in Z, yields of AQ alone were significantly higher than those of untreated controls. In a field trial, when treated with ZFM, the yield of AQ alone was significantly higher than that of untreated controls. In CPM, the yield of AQ alone was significantly higher than that of untreated controls. In ZPM, AQ alone resulted in a 17% higher yield than that of untreated controls, respectively. The yield of AQ alone was significantly higher than that of untreated controls. In CPM, the yield of AQ alone was significantly higher than that of untreated controls. In ZPM, AQ alone resulted in a 17% higher yield than that of untreated controls, respectively. In greenhouse experiments, Guanidine hydrochloride (GHC) reduced larval populations of root-knot nematode (Meloidogyne incognita) and cyst nematodes (Heterodera glycines) in soil and in root tissue of soybean. Larvae of cyst nematodes were less sensitive to GHC than those of root-knot nematode. At concentrations between 0.1-0.15 mg/kg soil, GHC was not phytotoxic and was highly effective in nematode suppression. GHC also stimulated plant growth at concentrations below 0.5 mg/kg soil in the presence of nematodes.}

**A434**

**SURVEY OF PLANT PARASITIC NEMATODES ASSOCIATED WITH TURFGRASS DAMAGE IN WEST VIRGINIA.** J. B. Kotcon. Div. of Plant and Soil Sci., West Virginia Univ., Morgantown, WV 26506-6057.

One hundred and twenty soil samples from fairways and greens of 8 WV golf courses were assayed for nematodes using centrifugal flotation techniques. Twelve species of plant parasitic nematodes and 5 predaeous nematode species were identified. Tylenchysisagri and Heterolaimus galeatus were the most frequently associated with unthrifty growth and decline of turfgrass. In a few sites, Tylenchysisagri dubius, T. proximus and Longidorus brevianulatus were associated with damage, particularly on bentgrass greens. Symptoms were most severe during July and August when soil temperatures combined with stunted root systems to induce drought stress. Population densities of T. agri and H. galeatus were not reduced significantly by ecohoprew at 27 kg a.i./ha 7 weeks after treatment and Cephalomiellus curvata populations were greater than in untreated plots.

**A439**

**SOYBEAN CYST NEMATODE RACES IN TENNESSEE.** Lawrence D. Young, USDA-ARS, G.A. Adams Blvd., Jackson, TN 38301.

A survey of soybean cyst nematode (SCN), Heterodera glycines, races present in Tennessee soybean fields was conducted in 1993. One sample was taken for each 20,000 hectares of soybeans grown in each county. SCN race determination based on reproduction on standard differentials plus reproduction on the race 14 resistant cultivar PI 88786 and Cordell (race 9 resistance from PI 90765) soybean cultivars. SCN populations were measured in a greenhouse for populations from 21 fields. Races 2, 3, 4, 5, 6, and 9 represented 148, 54, 104, 396, 194, and 144, respectively, of the total population of P. Bruchus pisi (female index) on race differentials PI 89786 and PI 90763 was not a good indicator of the reproduction occurring on cultivars Bedford and Cordell. The female index for eight populations were 61% of the From the PnR and Cordell populations. Recomendations of cultivars to be planted should be based on SCN reproduction on the cultivars instead of reproduction on the race differentials.

**A440**

**POPULATION DYNAMICS OF THE POTATO ROTT NEMATODE ON SNAPBEAN.** A. E. MacGowin and R. M. Wintter. Department of Plant Pathology, University of Wisconsin, Madison, WI 53706.

Ditylenchus destructor, the potato rot nematode (PRN), is usually detected only in potato tubers. To improve the detection of PRN in plants, we developed a population dynamics of PRN on snapbean, a crop rotated with potato. PRN reproduced on intact plants grown in heated-treatment and untreated field soil and in monospecific root explant cultures. Nematodes functioned as both eco- and endoparasites of roots and infected stems of intact plants, producing elongate lesions as high as 10 cm above the soil line. No organisms besides PRN were recovered from lesions on stems. Six wks after planting, the population of the population of the lesion stems varied from 2 to 10 in a series of experiments. Tubers showing dry rot symptoms typical of PRN were identified in soil grown in infested with infected bean stems. In general, equal proportions of PRN populations were recovered from soil and roots.

**A441**

**LOCATION AND SIZE OF SYNCYTIA INDUCED BY HETERODERA GLYCINES IN THE ROOT TISSUES OF TWO SOYBEAN CULTIVARS.** Yum, K. J., Park, E. W., and Kim, Y. H. Department of Agricultural Biology, Seoul National University, Suwon, 440-744, Korea.

The soybean cultivars Bangsa and SNUA were compared in terms of location and size of syncytia within the root tissue after penetration of the soybean cyst nematode. The ratio of syncytia in Bangsa and SNUA after inoculation in the greenhouse were 336 cysts (range: 177-513 cysts) and 253 cysts (range: 197-360 cysts), respectively, suggesting that these cultivars were equally susceptible. However, syncytium development in the root tissues incited by the soybean cyst nematode differed in the two cultivars. Transverse sections of roots at the sites of nematode...
penetration showed that considerably larger area of the stelar region of root tissues of Banga (33.2%) was displaced by synctia that than of SNUA (6.3%). Thus, nutritional transport of water and nutrients would likely be more severely inhibited by synctia in Banga than in SNUA. Although both Banga and SNUA were susceptible to H. glycines, SNUA may be more tolerant to damage caused by the soybean cyst nematode than Banga.

A442
HOST RESISTANCE MANIFESTED IN EGRESSED GLOBODERA ROSTOCIENSI S JUVENILE INFECTIVITY. L. L. Porier and B. K. Horst, Department of Plant Pathology, Cornell University, Ithaca, NY 14853.

Potato cultivars with the H$_2$ resistance gene to Globodera rostochiensis produce a greater emergence of second-stage juveniles (J$_2$s) than their susceptible counterparts. An in vitro root assay was devised to determine the ability of H$_2$-egressed J$_2$s to invade subsequent host roots. Roots of susceptible and resistant cultivars on Hussey and Stacey medium were inoculated with J$_2$s collected from resistant (R) and susceptible (S) plants. Freshly hatched J$_2$s from cysts maintained in an incubator (I) served as a control. Penetration of roots by J$_2$s were examined 3 days after initiation. The treatment represented the highest occurrence of penetration in susceptible roots. J$_2$s from the R treatment retained some ability to penetrate susceptible roots. Egressed J$_2$s from the R treatment did not penetrate either susceptible or resistant roots. These results suggest that H$_2$ resistance produces a population of egressed J$_2$s with reduced ability to invade potato roots.

A443

Several native grasses, sorghum varieties and maize genotypes were tested for susceptibility to Peromisosporospora sorghi using artificial inoculations at two locations in the South-West and North-Central parts of Nigeria. In the South-Western location, none of the native grasses or the sorghum varieties were infected, but most maize varieties were susceptible. In the North Central zone, sorghum genotypes TX41, TX430, ICMPE5-28, ICMPE7-928, TEN, and most maize varieties were systemically infected. Among the native grasses, only Rothbuehelia coccinellensis and Vactocyclus nigrum were partially susceptible. Dormancy was observed in the North-Central savannah ecology while only conidial sporulation of donny mildew was detected in the South-Western forest ecology. The susceptibility of differential sorghum hosts to the North-Central but not to the South West donny mildews may suggest the presence of two pathotypes in Nigeria. New maize varieties developed at IITA are highly resistant against donny mildews in both areas.

A444
Maison, M., Pearson, R.C., Scaphoides tianus Ball, a possible vector of Grapevine Yellows Disease in New York. Dept of Plant Pathology, Cornell University, New York State Agric. Exp.Srn., Geneva 14456

Scaphoides tianus is the vector of Flavescence doree (FD) of grape in Southern Europe. It was collected in New York vineyards of Vitis vinifera affected by Grapevine Yellows Disease (GYD), which has identical symptoms to FD, and in adjacent wild Vitis riparia. None of the wild grapes exhibited symptoms of GYD. Scaphoides tianus is a new species and is similar to Scaphoides tianus from Italy. Scaphoides tianus feeds on wild grapes and is a possible vector of the disease. Feeding of collected leaffoppers on Vicia faba in a greenhouse resulted in symptoms typical of infection with mycoplasma-like organisms (MLO) in 29% of the host plants within three to four weeks. Symptoms of GYD have not yet been detected in potted grapes which were fed upon by the same collection of leaffoppers. Individual leaffoppers were tested by ELISA using polyclonal antibodies to FD (supplied by Boudon-Padieu and Caudwell, Dijon, France). A positive reaction of 13.3% of the tested insects from commercial vineyards and wild grapes indicates a serological relationship between the pathogen of FD and the MLOs in New York leaffoppers. MLOs were observed by ISEM in individual preparations of ELISA-positive leaffoppers.

A445
PURIFICATION AND CHARACTERIZATION OF ANTIGENIC FUNGAL GLYCOLIPIDS FOR SPECIFICALLY DETECTING Botrytis cinerea IN JUICE FROM INFECTED GRAPE. R.W. Ricker, R.M. Bostock, & J.J. Marois. Department of Plant Pathology, University of California, Davis, CA 95616

Immunization of rabbits with crude extracts from Botrytis cinerea stimulated production of polyclonal antibodies that bind proteins and various glycocoujugates, with early immune response directed primarily against the carbohydrate portion. ... If an extremely antigenic glycolipid (ABGL). ABGL is unique to B. cinerea and apparently integrated into membranes but readily solubilized in acidic detergent. A decrease in disease severity correlated by immunoassays (ELISA) and correlated to the degree of infection by B. cinerea in individual berries and grape clusters. ABGL is small (M, 2 700 daltons) and appears to have only one epitope available for binding with antibodies. The small size, univalent nature, and uniqueness make ABGL an ideal candidate for direct competitive immunoassays to specifically detect B. cinerea. In a highly purified state, however, ABGL cannot be adsorbed onto plastic and measured directly on the surfaces of microtiter wells using heterogeneous assays. Details concerning the structural modification of this molecule for use in immunoassays will be presented.

A446
EFFECT OF EARLY-SEASON POWDERY MILDEW ON FORMATION AND SURVIVAL OF TILLERS OF WINTER WHEAT. K. L. Everts, S. L. Sorensen, USDA-ARS, Department of Plant Pathology, North Carolina State University, Raleigh 82014-7616.

Early-season powdery mildew severity and tiller formation was studied on three winter wheat cultivars differing in susceptibility and with or without triadimenol seed treatment. Plants in one-half meter row of plot were destructively sampled from emergence through tillering to determine the correlation and survival of tillers at different nodes. Disease severities ranged from 0 to 110 on the last fully expanded leaf during tillering. Analyses of variance indicated that cultivar and seed treatment and the interaction of cultivar x seed treatment influenced disease severity and tiller formation. Previously reported yield increases associated with triadimenol seed treatment may be in part due to increased tillering or tiller survival. The correlations between mildew severity, tiller initiation and survival are presently being examined.

A447
PLANT GROWTH AND YIELD DEVELOPMENT OF SOYBEAN UNDER THE INFLUENCE OF PHAKOSPOROSpora PARCHYRHIZI. X. B. Yang, T. A. Tschanz, and W. M. Dowler. USDA-ARS, Frederick, MD 21701 and USDA-APHIS, Hyattsville, MD 20782.

Effects of Phakosporospora parchyrisi (causal agent of soybean rust) on growth and development of soybean were evaluated to integrate a disease management strategy for soybean. Disease reduced potential yield by decreasing the maximum pods/plant up to 40% at plant growth stage R6. From R6 to R7, abortion of pods increased significantly in severely diseased plants than protected plants. Potential number of seeds/pod at R6 was not affected. Rates of seed growth (g/day) from R6 to R7 were reduced as much as 60% in diseased plants compared with protected plants. Dry matter was partitioned more to seeds in diseased plants than protected plants during the period from R6 to R7. The periods required for plant growth from R1 to R6 and R6 to R7 were reduced up to 9 and 3 days, respectively in diseased plants.

A448
COMPARING EFFECTS OF PHAKOSPOROSpora PARCHYRHIZI ON INDIRECT AND DIRECT YIELD COMPONENTS, PLANT YIELD, AND PLOT YIELD OF SOYBEAN. X. B. Yang, W. M. Dowler, and T. A. Tschanz. USDA-ARS, Frederick, MD 21701 and USDA-APHIS, Hyattsville, MD 20782.

To determine the optimum coupling point to integrate a soybean rust model with a soybean simulation model, relationships of disease progression to yields of 6 soybean cultivars in two years were quantified at four levels: indirect yield components, direct plant yield, plot yield and total yield. Indirect yield components were poorly correlated with disease progression. However, direct yield components were consistently correlated with disease progression for all cultivars. Disease did not affect number of plants per plot. Plant yield and plot yield were significantly affected by disease progression for all cultivars. Plot yields were accurately predicted using green leaf area duration from plant growth stages R6 to R7 and area under disease progress curve as a predictor. Results indicate that the disease effects were best determined at the plot yield level.

A449

Three soybean (Glycine max) cv., Samsoy 1 (susceptible), Tg2105-128 (moderately resistant) and Tg 996-246 (resistant), were evaluated for yield losses resulting from frogeye leaf spot caused by Cercospora sojina. Replicated field plots were established at 2 locations in Nigeria that are naturally infected with C. sojina. The cv. were either not sprayed, sprayed once or twice during the growing season with the fungicide benomyl. Disease spread was early in the season from border rows of a susceptible soybean plot to the plots. Mean disease severity (DS) and disease incidence (DI) for unsprayed
cultivars ranged from 0.6 to 4.5 and 6.3 to 95%, respectively. Plots receiving 2 sprays had lower DS and DI values, ranging from 0.5 to 2.4 and 5.0 to 42%, respectively. Differences between unsprayed and double sprayed plots for yield and 300-seed weight, ranged from 2.5 to 58.8, and 0.6 to 28.6%, respectively. Seed weight was negatively correlated with DS and DI.

minimal sporulation occurred at 10, 15, and 35 C. Slight or no growth of lesions was observed when either intact stems or excised stem pieces of rough lemon were inoculated with P. citrophthora or P. parasitica and incubated at 5 and 30 C or 10 and 30 C, respectively. The suppressive effect of cooler temperatures on sporulation and disease development could be useful for determination of optimum times for application of fungicides for disease control.

A454

Anthraxnose, caused by Colletotrichum dematioides, is a destructive disease of spinach in the Arkansas River Valley where over 4,000 acres of spinach are grown annually. Anthraxnose can occur as a primary infection or as an infection on white rust lesions caused by Albugo species. Isolates recovered from both primary and piggy-back infections were compared. Sixty-two isolates were examined for both vegetative compatibility, using nitrate non-utilizing mutants (NIM mutants), and virulence, using a standardized greenhouse pathogenicity test. Colony morphology, sporulation and spore germination rate were also compared. Two distinct vegetative compatibility groups (VCGs) were identified when NIT and NIM mutants, recovered from each isolate, were paired in all combinations. Sixty-two percent (16/29) of isolates in VCG1 were recovered from primary lesions and 64% (21/33) of isolates in VCG2 were recovered from white rust lesions. Significant differences (P > 0.05) in virulence were observed among isolates within each VCG; virulent and weakly virulent isolates were identified in each VCG and from each infection type (primary and piggy-back). Significant differences (P > 0.05) in spore germination rate were observed among isolates in VCG2. Colony morphology was considerably more variable among isolates in VCG1; several isolates in VCG1 had apparently lost their ability to produce acervuli.

A455

The influence of soil moisture on the number of pycnidia and perithecia produced, duration of production, and conidial and ascospore release in Diaporthe phaseoli var. caulivora (Dpc) was studied. Dpc-inoculated stem pieces of susceptible soybean 'Bedford' were incubated at 25 C on the surface of soil samples adjusted to moisture potentials of 0, 0.1, 0.2, 0.4, 0.8, 1.6, and 3.2 bar. The number of pycnidia and perithecia produced and the number releasing conidia and ascospores, respectively, were counted on 1 cm² area of the stem pieces at 15-day intervals. Pycnidia and perithecia were produced after 15 days incubation in 0 to 0.08 bar while in the drier treatments (0.4 to 3.2 bar), they were produced after 30 days incubation. Maximum production of pycnidia and perithecia occurred between 30 and 45 days after incubation in 0 to 0.8 bar. Conidia and ascospores were released from the spore-bearing structures in a gelatinous matrix. Spore release occurred only in treatments 0 to 0.8 bar. These observations indicate that Dpc requires different soil moisture regimen for the production of spore-bearing structures and spore release.

A456

A monthly disease survey of 275 willow clones in a woodgrass biomass plantation (one year cutting cycle) was conducted to assess disease incidence and its effects on growth. Shoot blight (Colletotrichum gloeosporioides and Polyaella salicicola) incidence increased during August to 10% and declined in September. Septoria spp. and Marsonorina spp. (brown spot) incidence increased throughout the growing season to 70% in September. Melampsora rust incidence increased from 5% to 18% from August to September. Clones with these diseases grew as high or higher than undiseased clones. Chrysopora lugens caused localized mortality in one clone. Shoot and foliage pathogens appear to have had minimal annual impact on growth, but long term and intraclonal impacts were not assessed.

A457
SPATIAL ANALYSIS OF VIRULENCE VARIATION IN EUROPEAN POPULATIONS OF ENYSIPHE GRAMINIS F. SP. HORDEI. J. M. Hembert and E. Limpert. Institut für Pflanzennwissenschaften, Phytomedizin/Pathologie, ETH-Zentrum, CH-8092 Zürich Switzerland.

Powdery mildew of barley is the most important foliar disease of barley in Europe. Considerable effort is being expended in monitoring the air-spore throughout Europe, generating a large spatially-structured data base of variation for virulence and fungicide sensitivity. These data are yielding critical
information on regional differentiation and dynamics of barley mildew populations. Spatial statistical analyses are crucial to investigate genetically distributed polymorphisms and to develop post hoc explanations for existing genetic variation. This should provide an improved conceptual basis for the analysis of current strategies for disease control and the development of new approaches.

A458
EFFECTS OF IMAZAQUIN, CHLORIMURON ETHERYL, AND GLYPHOSATE ON IN VITRO GROWTH AND DEVELOPMENT OF CALONECTRIA CROTALARIAE. D. K. Berner, G. T. Bergren, and J.P. Snow. Department of Plant Pathology and Crop Physiology, Louisiana Agricultural Experiment Station, Louisiana State University Agricultural Center, Baton Rouge, LA 70803.

Commercial herbicide formulations of imazaquin, chlorimuron ethyl, and glyphosate were evaluated for their effects on Calonectria crotalariae, the causal agent of red crown rot of soybean and Crotalaria black root of peanut. Isolates of the fungus from soybean were grown on a medium amended with either water or volumes of the respective herbicides at 0.5, 1.2, and 4X labeled rates for weed control in soybeans. Glyphosate, in both surfactant and non-surfactant containing formulations, significantly reduced colony area compared with the water control. Simulated repeat applications of imazaquin and chlorimuron ethyl also reduced colony area. Imazaquin at rates of 1X or greater significantly inhibited microcrystalline production. Additions of amino acids to herbicide amended media failed to prevent observed herbicide effects. When used in combination, 0.5X imazaquin and 0.5X glyphosate significantly reduced fungal growth, indicating the potential of these materials as economical and simultaneous preplant weed and disease control agents.

A459
DNA POLYMORPHISM AND PATHOTYPE VARIATION IN THE RICE BLAST FUNGUS. Morris Levy, M. A. Marchetti1 and J. E. Hamer. Dept. of Biological Sciences, Purdue University, West Lafayette, Indiana and USDA-ARS, Box 995, Beaumont, Texas.

A repetitive DNA family (named MGR sequences) diagnostically marks the genomes of the rice blast pathogen, Magnaporthe grisea (Pyricularia oryzae). Southern hybridizations with an MGR probe (pGCM56) produced a monomeric type of DNA fingerprint. EcoRI restriction fragment length profiles) among all field isolates tested. A blind-test experiment now shows that these DNA fingerprints distinguish the major pathotypes in the USA (New races IB-1, IB-45, IB-49, IB-54, 1C-17, IB-13, IB-1, and HM-1), reliably index pathotype diversity among USA field isolates collected from 1959-1994, and indicate the phylogenetic relatedness among pathotypes. e.g., IB-49 is composed of two distantly related lineages, one of which shares recent common ancestry with IB-54. These results resolve lingering questions about the stability in M. grisea and illustrate new opportunities for determining population dynamics and evolution of this important crop pathogen.

A460
POLIAR AND SOILBORNE ASPECTS OF PATHOGENICITY OF COCCODIDES ON RUSSET BURRANK POTATO. A.W. Barkdoll and J.R. Davis, Univ. of Idaho Research and Extension Center, Aberdeen, ID 83210.

Pathogenetic variation and symptom expression of C. coccodes on Russet Burbank potato were evaluated with root inoculations in the greenhouse and foliar inoculations in the field and the greenhouse. Potato foliage was treated with air-blown sand (40 Kph air speed) to induce wounding and stimulate rapid tissue damage and sprayed with conidial suspensions of each isolate. Plants were misted to establish infection. In the greenhouse, leaf lesions resembling those produced by Alternaria solani and wilt resembling Verticillium wilt were observed. All isolates reduced tuber yield. Wilt incidence was negatively correlated with tuber yield and specific gravity (p<0.001). In the field, one isolate reduced total tuber yield and yield among larger tubers (280g). Pathogenicity from soil inoculum was studied by mixing ground, colonized oats to produce soil inoculum levels (100-180 cfu/g soil) comparable to those observed in a soil survey of potato production areas. Most isolates significantly reduced root dry weight and some reduced tuber yield. Root cortical damage ranged from slight to extreme.

A461
PHENOTYPIC AND GENOTYPIC MARKERS IN HOST SPECIALIZED ISOLATES OF SPOROSIRIUM REILIANUM. G. Naikoo, R. A. Frederiksen, D. G. Bai, and C. W. Magill, Department of Plant Pathology and Microbiology, Texas A&M University, College Station, Texas.

Both maize and sorghum are hosts of Sporosirium reilianum, the causal organism of head smut. Some isolates colonize only maize, while others colonize sorghum predominantly and sudan grass and maize to a lesser extent. In the USA the latter type has been further delineated with the use of a sorghum host differential series. Light and scanning electron microscopy reveal that teliospores from sor on maize and sorghum are morphologically indistinguishable. Differences in optimal conditions for germination and growth were detected. Starch gel electrophoresis of sporidial extracts did not provide any distinctive isozyme marker. Differences found between maize and sorghum isolates using IEF-PAGE separations were not consistent for all isolates. IEF-PAGE separations and RFLP's are being examined in order to obtain unique isozymic and genetic markers.

A462
PREPARATION OF DRY, VIALABLE MYCELIUM OF SCLEOROTINA MINOR. H. A. Melolou and C. Bowen, USDA-ARS and Department of Plant Pathology, Oklahoma State University, Stillwater 74074-9947.

Pifty ml of potato-dextrose broth with streptomycin sulfate (SS), 100 Mg/ml, in 250 ml bottles were each inoculated with a plug (15 mm diam) from a 2-day old culture of S. minor grown on potato-agar with SS (SPDA). Bottles were placed on a shaking (15 rpm) at 25 C for five days. Mycelia were collected by centrifugation (2000g), 20 min at 4 C, suspended in 20 ml of 5 to 15% polyethylene glycol (MW 6000), and then collected by centrifugation followed by filtration on a Millipore filter (0.45 um). Filters with mycelia were dried at 25 C for 2 days in a desiccator with CaSO4. Viability of mycelial fragments was determined by plating on SPDA. Dry mycelia were stored at 40 or 80% relative humidity (RH) for up to 10 wk with periodic determination of viability. A significant loss of viability of mycelia occurred after 4 wk storage at 80% RH and continued to increase until the experiment was terminated at 10 wk. Storage of mycelia at 40 RH up to 10 wk did not affect viability.

A463
AFLATOXIN CONTROL IN PREHARVEST CORN (ZEA MAYS): EFFECTS OF CHITOSAN AND TWO MICROBIAL AGENTS. R.G. Cuerda, E. Duffus, G. Gouji, and R. Pettit*. Prairie View A&M University, CMC, Prairie View, Texas 77446, and *Texas A&M University, Department of Plant Pathology and Microbiology, College Station, TX 77843.

Aspergillus flavus growth and aflatoxin B production in preharvest corn kernels was determined after treatment with chitosan, Bacillus subtilis, and trichoderma harzianum. Individual or combined control treatments were applied at the milk stage of ear development, 48 hr before or after kernel inoculation with aflatoxicogenic A. flavus isolate. Both single and combined treatments reduced A. flavus growth and aflatoxin production, but there was not consistent correlation between fungal growth and aflatoxin production. Untreated kernels, inoculated with A. flavus, produced 1104 ppb of aflatoxin B1. Toxin accumulation was significantly (p<0.05) reduced, in same instance to non detectable (ND) levels, by single treatments applied 48 hr before A. flavus inoculation inhibited aflatoxin production (ND and 2 ppb, respectively).

A464
THE CORRELATION OF RHIZOMOBILITY AND BIOLOGICAL CONTROL POTENTIAL OF RHIZOBACTERIA. I.J. Misagi and M.W. Olsen, Department of Plant Pathology, University of Arizona, Tucson, AZ 85721.

Determination was made of the importance of rhizomobility (a term used here to denote the ability of a bacterium to move along
roots in the absence of percolating water) of rhizobia in their ability to control bacterial wilt of tomato caused by *Pseudomonas solanacearum*. Ten rhizobial isolates were compared for rhizomobility and biological control potential. All ten rhizobial isolates and only two of ten rhizomobility isolates provided statistically significant (P=0.05) reduction in disease incidence. Moreover, rhizomobility isolates reduced disease incidence at a significantly higher level than non-rhizomobility isolates. Ten rhizobial isolates and only five of ten non-rhizomobility isolates colonized tomato roots. These results point out the importance of rhizomobility and root colonizing ability in biological control activity of tested rhizobia.

**A465**


A nonfluorescent *Pseudomonas* sp (PSJN222) isolated from onion rhizosphere strongly promoted "in vitro" growth of potato plantlets. On micropropagated plants, the bacteria elicited significant increases in root and haulm dry weight, stem length and adventitious root number when compared to unincoculated controls. Bacterization promoted secondary root branching as well as increased root, stem, and leaf biomass. Total bacteria on plantlets was significantly augmented. The mechanisms of growth enhancement are not yet known but the involvement of a 90 Kb mega-plasmid is being investigated. Various mutagens were used in order to produce a non-growth promoting mutant; and to eliminate the plasmid from PSJN222. The effects of bacterization on field survival, growth and yield of micropropagated potato plantlets was also studied. In order to facilitate the identification of the bacteria in colonization studies, a recombinant plasmid encoding luciferase activity was constructed and incorporated into PSJN222.

**A466**

THE USE OF SURFACTANTS TO FACILITATE INFECTION OF LEAVES BY BACTERIAL PLANT PATHOGENS: IMPLICATIONS FOR BIOLOGICAL WEED CONTROL. N. K. Ziedack and P. A. Backman. Dept. of Plant Pathology, Auburn University, Auburn, AL. 36849.

Bacteria have been largely ignored as potential bioherbicides due to their sensitivity to environmental effects or their inability to directly penetrate plant tissue. Their application has been limited due to requirements for wounds, vectors and/or free water for the invasion of nectaries, stomata and hydathodes. Reports indicate that surfactants capable of reducing the aqueous surface tension below 25mN m⁻¹ can cause wetting of submatal cavities. A stomate-flooding surfactant has been shown to greatly enhance infection when sprayed with plant pathogenic bacteria. *Pseudomonas syringae* pv. *phaseolicola* (P.s.p.), a pathogen of zucchini (Luffarina lobata) and common bean (*Phaseolus vulgaris*) was applied to greenhouse grown bean plants with and without surfactant. When applied with surfactant, high levels of disease development and infection was independent of leaf surface moisture. Without surfactant, no disease developed on inoculated plants.

**A467**


Glidiocladium virens (GL-21) produced the antibiotic, gliotoxin, in soilless mix (0.42 µg/cc), composted soil (0.36 µg/cc), loamy clay soil (0.20 µg/cc), and sandy loam (0.02 µg/cc) when added (0.12 µl/well) as a bran-alginate formulation (W. R. Grace & Co.-Conn.). Gliotoxin (extracted with chloroform and detected by HPLC) quadrupled when 0.45 (w/v) prill was added. Gliotoxin in vitro of *P. ultimum* was completely inhibited by extracts contained at least 1 µg gliotoxin/cc of soil media. Biocontrol of damping-off of zinnia caused by *P. ultimum* and *E. solani* was effective with both of the above amounts of GL-21 prill. Gliotoxin was detected in soilless mix 2 and 7 days after amendment with prill, but not at zero time or after 14 days. Aqueous extracts from Glidiocladium-amended soilless mix was effective in reducing damping-off caused by *Pythium*. These results indicate a role of gliotoxin in biocontrol.

**A468**

REDUCTION OF DETERMINANT EFFECTS OF APHID HONEYDROD BY YEASTS ON WHEAT LEAVES. A.J. DíKe, N.J. Pökkema and R. Rabbijn. Willie Comm-
were monitored using semi-selective and differential media. Population levels of B1 detected on blossoms of B1-treated plants exceeded log 4 colony-forming units over 14 days. Treatment with strain B1 resulted in disease severity which was significantly lower than that of the nontreated control and similar to that with benomyl. In contrast, B0 did not establish on blossoms in appreciable numbers and also failed to inhibit white mold. None of the treatments provided any improvement in yield over the control.

Honey bees were used to deliver apple pollen treated with a suspension of antagonistic bacteria (10^8 cfu/ml). The bees successfully delivered bacteria to newly opened flowers. The bacteria subsequently multiplied on the flowers and survived for at least 2 weeks. Biological control of fire blight is dependent on the antagonist used, the concentration of bacteria deposited on the stigma, the environmental conditions and the inoculum level of Erwinia amylovora.

Subcloning of 500 bp and 1400 bp Hinc II fragments derived from a 2000 bp cDNA of SPFM (strain RC) containing the coat protein gene in vitro transcription vector plasmid pEM 42 (Promega) and pEM 32L+ (Promega) generated plasmids pG4PMC4.05 and pG4PMC4.14 respectively. These plasmids served as templates for phage T7 RNA polymerase to synthesize 32p labeled cRNA probes (Riboprobes). Slot-blot hybridization assays under high stringency conditions showed that pG4PMC4.05 Riboprobe detected all known strains of SPFM (RC, YV, 835, and C). In contrast, pG4PMC4.14 did not detect strain C which is distantly related serologically. Sequence analysis of these cRNAs revealed that pG4PMC4.05 contains 509 bp cDNA near the 3' end of the gene, whereas pG4PMC4.14 encodes for the predicted putative amino acid sequence for proteolytic cleavage typical of Potyviruses. The complete analysis of the coat protein gene is in progress.

An infectious cDNA clone of a strain of tobacco mosaic virus that produces attenuated systemic symptoms on Nicotiana tabacum cv. Xanthi has been constructed. Through fragment swapping experiments, where portions of a cDNA clone from a green mosaic producing strain have been switched with portions of the attenuated cDNA sequence, the cause of the attenuation has been mapped to the ORF encoding the 126kd protein. Sequence analysis shows multiple base changes that would result in amino acid changes. Since the attenuated strain produces normal necrotic lesions and normal virus accumulation in inoculated leaves, virus replication and spread in inoculated and systemically infected leaves over time will be analyzed by tissue and RNA blots. These results will help determine how the base changes in the attenuated strain result in the masked phenotype.

Various cucumber mosaic virus (CMV) satellite (sat) RNAs can induce chlorosis on either tobacco or tomato plants. On tobacco, B2- and W3-sat RNAs only induced chlorosis with subgroup II CMV helper strains, whereas with B3-sat RNA on tomato. Sequence comparisons of a number of chlorosis-inducing satellite RNAs indicated a single nucleotide position that might dictate whether a given satellite RNA could induce chlorosis on either tobacco or tomato. This position was mutated in a cDNA clone of a sat RNA which only induced chlorosis on tomato, and the resulting transcripts were inoculated onto tomato and tobacco. The mutant satellite RNA no longer induced chlorosis on tomato, but instead, induced chlorosis on tobacco that was phenotypically identical to that induced by B2- and W3-sat RNAs. These results indicate that a single domain is responsible for chlorosis induction on both tomato and tobacco, but that the specificity is controlled by a single nucleotide. The induction of chlorosis on tobacco and tomato plants by CMV satellite RNAs therefore appear to be mutually exclusive pathogenic effects.

Biological control of fire blight can be achieved by protecting pear and apple flowers with antagonistic bacteria. However, orchard spraying may not be the most efficient application method. The distribution of antagonistic bacteria by honey bees for biological control of fire blight, G. V. Thomson and K.M. Shortwell (Dept. of Biology), J.D. Vandenberg (USDA-ARS Bee Lab), Utah State Univ., Logan, UT 84322-5305.

Biological control of fire blight can be achieved by protecting pear and apple flowers with antagonistic bacteria. However, orchard spraying
Tomato spotted wilt virus (TSWV) is the sole member of the TSWV group. The virus has a single strand RNA genome consisting of three RNA molecules, 3.3 kbp (RNA-S), 5.2 kbp (RNA-M), and 8.3 kbp (RNA-L). The complementary DNA was synthesized by random priming to the RNAs of the BL isolate of TSWV purified from Datura stramonium and cloned into plasmid vector pUC18. Several clones were found containing cDNA specific to RNA-M of the virus after Northern blot hybridization. The largest size of the inserts is about 1.6 kbp. A full length clone (3.4 kbp) of RNA-S was identified by the same type of assay. These TSWV clones do not react to healthy plant materials when tested by Northern blot hybridization with purified D. stramonium nucleic acid or by dot blot hybridization with crude or purified D. stramonium extract. The sequences of these clones are being determined.

**A481**

Immunological detection of the red clover necrotic mosaic virus polymerase expressed by ribosomal frameshifting. M. Zong, T. L. Kendall, and S. A. Lommel, Dept. of Plant Pathology, North Carolina State University, Raleigh, NC 27695-7616.

Sequence data suggests that the red clover necrotic mosaic virus (RCNMV) RNA-1 encoded polymerase is translated and read by a ribosomal frameshifting mechanism which is structurally and functionally identical to the retroviral pol gene frameshift element. Two distinct open reading frames (ORFs) are identified in RNA-1 in addition to the 3' terminal caped protein ORF. A pre-frameshifted 27 kDa protein and a lesser amount of an 86 kDa protein (p86) are observed p88. Translation from ribosomal frameshifting at the end of the first ORF with continued translation through the second ORF. Oligopeptides were synthesized to an internal region of the post-frameshift, and the C-terminus of p88. Oligopeptides were conjugated to KLH and used as an antiseraum. Oligopeptides were synthesized on an oligo-peptide affinity column. The polymerase antibodies immunoprecipitated p88 but not the pre-translation p86 translation products. In addition, a viral encoded 57 kDa protein was also recognized by the antibody. These data suggests that the p88 is expressed at catalytic levels controlled by the frameshift element.

**A482**

TRANSGENIC NICOTIANA BISELOVIETI THAT EXPRESS THE GENE VI PRODUCT OF CALIFLOWER MOSAIC VIRUS (CAMV) COMPLEMENT A CAMV STRAIN THAT IS DEFICIENT IN SYSTEMIC SPREAD. J.E. Scholz, K.-B. Goldberg, and J.M. Kternan, Dept. of Plant Pathology, University of Missouri, Columbia, MO and Dept. of Plant Pathology, University of Kentucky, Lexington, KY 40504.

Gene VI of CAMV recombinant virus H12 was transferred into the genome of N. biselovitii through the use of the Agrobacterium vector pGAD7. Virus H12 was chosen as the source of gene VI because previous work demonstrated that the gene VI product of this virus determined systemic infection of N. biselovitii. In order to create complementation lines which expressed the CAMV gene VI product and nontransformed controls were inoculated with recombinant virus H31, a virus which could not systemically infect nontransformed N. biselovitii. Transgenic N. biselovitii inoculated with H31 developed systemic symptoms while the nontransformed N. biselovitii controls remained symptomless. In order to show that no changes had occurred within the H31 genome that would affect host specificity, the H31 was isolated from systemically infected leaves of the transgenic plants and re-inoculated to both nontransformed and transgenic N. biselovitii. Again, the transgenic N. biselovitii developed systemic symptoms while the nontransformed controls remained healthy.

**A483**


Nucleotide sequences of RNA II of wild type and a deletion mutant of soil-borne wheat mosaic virus were determined from cloned cDNA. Next to the 5' untranslated region (ca. 330 nt long), there is an open reading frame (ORF) for a 19.3 kbp polypeptide. The 3' untranslated region is organized into eight open reading frames (ORFs). Based on size, position, and homology, seven of these putative genes appear to correspond to similar genes of other caulimoviruses. However, the null mutant shows evidence of the intact gene sequences is considerably less than that between polytomovirus in other caulimoviruses. In addition, a novel ORF, occurring between ORF II and III is observed in the PCISV genome. Like most caulimoviruses, PCISV's genome contains a large intergenic region between ORF II and V. However, the small intergenic region which occurs between ORF V and VI in the genomes of caulimovirus mosaic virus, pimpelid virus mosaic virus, and nematode vectoring virus is not present in the genome of PCISV.

**A484**

COMPARISON OF THE 3' TERMINAL SEQUENCES OF TOMATO SPOTTED WILT VIRUS ISOLATES. M. D. Law and J. W. Moyer, Department of Plant Pathology, Raleigh, NC 27695-7616.

A tomato spotted wilt-like virus (TSWV-1) isolate with a serologically distinct N protein has been identified which predominantly infects ornamental crops. Complementary DNA (cDNA) was synthesized to both RNA-I and RNA-II by artificially polyadenylating genomic RNA. cDNA probes specific for either TSW-1 S RNA or TSW-1 M RNA did not cross-hybridize with TSW-1 S RNA or TSW-1 M RNA. Sequence analysis of the TSWV S cDNA confirmed the TSWV consensus sequence in our TSWV isolate (common genotype). The 3' terminal RNA sequence of TSWV-1 S and M RNA was determined from the cDNA clones and has been deposited in the GenBank database. The TSWV-1 S terminal consensus sequence is identical to the TSWV consensus sequence. In contrast, the TSWV-1 S 3' terminal sequence did not contain the entire TSWV consensus sequence.

**A485**

USE OF CHIMERIC COAT PROTEIN CONSTRUCTS AND DELETION MUTANTS TO EXAMINE POTYVIRUS STRUCTURE AND COAT PROTEIN MEDIATED RESISTANCE. J. Hammond, R. L. Jordan and K. K. Kamo, USDA-ARS, Beltsville, MD 20705.

Several monoclonal antibodies (MAbs) prepared to potyviruses react with a fusion protein expressed in E. coli from a CDNA clone containing the complete coat protein (CP) gene of bean yellow mosaic virus (BYMV). Some of the MAbs also react with CPs of other potyviruses. Chimeric CP genes were constructed between BYMV and pepper mottle virus or zucchini yellow mosaic virus. Carboxylterminal BYMV CP deletion mutants were provided termination codons from an oligonucleotide. The chimeric and truncated constructs were expressed in E. coli, E. coli were analyzed by Western blotting. MAb reactivity was compared to amino acid sequences of the CPs. Constructs are being expressed in Nicotiana benthamiana to examine the contribution of different domains to the protective effect reported in plants expressing viral CP.

**A486**

PRODUCTION AND INITIAL CHARACTERIZATION OF MONOCLONAL ANTIBODIES TO HELP COMPONENTS OF POTATO VIRUS Y AND TOBACCO VEIN MOTTLING VIRUS. Ramon Jordan, David Thornbury and Tom Pirome, USDA-ARS, Florist & Nursery Crops Lab., Beltsville, MD and Dept. of Plant Pathology, Univ. of Kentucky, Lexington, KY.

Helper component (HC) preparations purified from potato virus Y (PVY) and tobacco vein mottle virus (TVM) infected plants by oligo (dT)-cellulose chromatography were used to generate a panel of monoclonal antibodies (MAbs). HC-specific MAb-secreting hybridomas (102) were selected using sucrose gradient purified HC preparations in antigen-coated plate and triplle-antibody-sandwich ELISAs. Fifty-one MAb were specific to TVM-HC, 25 were specific to PVY-HC and the remaining 26 reacted with both TVM- and PVY- HC. Some of the TVM- HC, PVY-HC-specific and cross-reactive MAb also reacted with crude sap preparations of bean yellow mosaic virus, pepper mottle virus, and/or aphid and non-aphid transmissible strains of potato vein stunt virus. These MAbs may be useful probes in experiments designed to examine the role of specific HC protein sites in HC-mediated aphid transmission of potyviruses.

**A487**


Peanut chlorostreak virus (PCCSV) is a caulimovirus which infects peanuts (Arachis hypogaea) as well as a variety of solanaceous plants of India. An infectious clone, pPICSV-K1, was prepared by ligating Kpn I digested PCCSV DNA into pUC19. Subclones of pPICSV-K1 were sequenced by the Sanger dideoxynucleotide method. The 5.2 kbp ORF was organized into eight open reading frames (ORFs). Based on size, position, and homology, seven of these putative genes appear to correspond to similar genes of other caulimoviruses. However, the null mutant shows evidence of the intact gene sequences is considerably less than that between polytomovirus in other caulimoviruses. In addition, a novel ORF, occurring between ORFs II and III is observed in the PCISV genome. Like most caulimoviruses, PCISV's genome contains a large intergenic region between ORFs V and VI. However, the small intergenic region which occurs between ORF V and VI in the genomes of caulimovirus mosaic virus, pimpelid virus mosaic virus, and nematode vectoring virus is not present in the genome of PCISV.

**A488**

DIRECT RNA SEQUENCING FOR IDENTIFICATION AND DETERMINATION OF GENETIC RELATEDNESS OF POTYVIRUSES INFECTING WHEAT. N. L. Robertson, R. F. Suckling, and W. G. Longley, USDA-ARS, Department of Plant Pathology, University of Nebraska, Lincoln NE 68583.

The genetic relatedness among four isolates of wheat streak mosaic virus (WSMV), hordeum mosaic virus (HMV), and agropyron mosaic virus (AMV) infecting wheat was compared by direct RNA sequencing. Like definitive potyviruses, these cereal viruses contain poly(A) tails. Synthetic dT14A, dT14C, or dT14G primers specifically initiated
cDNA synthesis on polyadenylated RNAs; other than the presence of a poly(A) tail, no prior sequence information is necessary. At least 200 3' terminal bases were determined for WSMV and HMV. Comparison of the profiles showed that four WSMV isolates ('Wyoming', 'Sidney', 'Type', and 'Com') were over 96% identical, while no sequence similarity existed among WSMV, HMV, and AMV. Determination of the easily accessible 3' terminal sequences is a rapid procedure for precise identification of distinct viruses and their strains, and is a practical alternative to time consuming plant host range and vector tests, serological and electron microscopy methods, and molecular cloning for many purposes. Non-polyadenylated RNAs can also be sequenced by this method after "tailing" with poly A-polymerase.

A489
DELETION ANALYSIS OF BROMOVIRUS 2A PROTEIN: EFFECTS ON RNA REPLICATION AND SYSTEMIC SPREAD
P.-L. Trauner, B. M. Young, and P. G. Ahlquist, Institute for Virology, Univ. of Wisconsin, Madison, WI 53706 USA
Brome mosaic virus (BMV) 1a and 2a proteins are required for viral RNA replication, GC of a 2a-containing polyprotein-like domain, and shows extensive similarity with noncapsid coat proteins in other viruses, such as nsP4 in Sindbis and the 183 KD protein in tobacco mosaic virus (TMV). The 2a protein contains additional amino- and carboxy-terminal domains which have no counterparts in the BMV and Sindbis proteins. Frame shift and deletion mutants of BMV RNA2 were used to investigate the role of these flanking domains in directing RNA replication in protoplasts, and possible involvement of RNA2 or 2a protein sequences in systemic spread of BMV in barley.

A490
CHARACTERIZATION OF AN INFECTIOUS TWO-BASE DELETION MUTANT OF POTATO SPINDLE Tuber VIREOID (PSTV). D.K. Lakshman and S.M. Tawfik, Dept. of Botany and Plant Pathology, Univ. of Maine, Orono, ME 04469.
A two-base deletion mutant (ST4) of PSTV cDNA (bases 339 and 340) was isolated from wild-type PSTV RNA. cDNA clones were used to inoculate potato cv. Rutgers. Symptoms resulting from infection with ST4 DNA or (+)RNA diners appeared later and were considerably milder than those induced by the parental severe strain (ST4-2), indicating that PSTV RNA replication in potato plants was hybridized to radiolabeled in vitro transcribed (-)RNA, treated with ribonuclease and resolved in an 8% polyacrylamide 7M urea gel. Two bands of 251 and 108 bases, but only one band of 359 bases, were associated with extracts of ST4- and ST4-infected plants, respectively. These results suggest that ST4 RNA progeny possess the two-base deletion. Sequencing of this progeny is currently underway.

A491
TOXICITY AND TOXIN PRODUCTION OF Fusarium ISOLATES FROM SAMPLES COLLECTED IN ALASKA. Weiping Xie, Chester J. Mirocha, Xiaoling Wang, Department of Plant Pathology, University of Minnesota, St. Paul, MN 55108, USA.
One hundred and seventy Fusarium isolates were obtained from 84 samples (soil, oat and barley stems) in Alaska in 1986 and 1987. Among 91 isolates checked for toxicity in rats by feeding test, 67 were toxic. The characteristic symptoms of the toxic isolates caused hematuria, diarrhea, hemorrhage in intestines and enlarged uterus in the rats. Fifty-eight isolates produced fusarochromanone (TOD-1) in culture filtrate. In 143 isolates, LOD, fusaric acid, and 53 μg/g, ranging from 82 to 3760 μg/g. All the fusarochromanone-producing isolates were identified as F. equiseti. Six isolates produced wormtin (MI) and were identified as F. sambucinum. This was the first time that wormtin-producing isolates were found in Alaska. It is most likely that fusarochromanone accounted for the toxicity of F. equiseti. F. sambucinum was found to include zearalenone and small amounts of trichothecenes.

A492
BIOSYNTHESIS OF TWO FATTY ACID DERIVATIVES OF FUSAROCHROMANONE BY Fusarium equiseti: ACASE STUDY. Weiping Xie, Chester J. Mirocha, Yechuan Wu, Won Joo Chang, and Robert V. Pawlosky, Department of Plant Pathology, University of Minnesota, St. Paul, MN 55108, USA.
Two fluorescent compounds with trivial names TOD-9a and TOD-9b were isolated from rice cultures of Fusarium equiseti (Alaska 2-2). Analysis by mass spectrometry indicated that TOD-9a had a molecular weight of 596 with a molecular formula of C36H56N4O4 and that TOD-9b (molecular weight 596) had a molecular formula of C36H56N4O4. Both compounds were synthesized from acetoacetic acid by decarboxylation, and had the same IR, UV, and NMR spectra as those described for fusarochromanone (TOD-1), 3'-acetyl-2-[(3-acetamidophenyl)oxy]-6-acetoxy-2,4,6(1H,3H,5H)-octatetraen-3-ol. The latter two compounds were resolved by gas chromatography with a capillary column. TOD-9a was identified as 3'-acetyl-4'-0-deactecondienioic acid fusarochromanone, and TOD-9b was identified as 3'-acetyl-4'-0-deactecondienioic acid fusarochromanone. The locations of the double bonds in the fatty acyl moieties of these two compounds were not identified.

A493
MOISTURE VARIABILITY OF INDIVIDUAL SEEDS OF SOYBEANS AND ITS ROLE ON STORAGE FUNGI DEVELOPMENT. F.A. Lazzari and R.A. Meronuk, Dept. of Plant Pathology, University of Minnesota, St. Paul 55108.
The moisture content (MC) of individual seeds and percentage (%) of seeds infected with storage fungi were determined from six soybean samples stored two years at 75% RH and 25 C. The MC was determined by oven-drying 16 single seeds at 110 C for 20 hours. The % of seeds infected with storage fungi was determined by plating 60 soybean seeds on T-6 media.

A494
PRODUCTION OF ZEARALENONE SULFATE BY Fusarium spp. Javier Plasencia and G. Mirocha, Dept. of Plant Pathology, University of Minnesota, St. Paul, MN 55108.
A water-soluble compound related to zearalenone was isolated from a culture of Fusarium graminearum #30 grown in rice. Negative ion Fast-Atom Bombardment mass spectrometry of the metabolite gave a molecular ion with m/z ratio of 397 which is coincident with the mass of the conjugate zearalenone sulfate. Acid hydrolysis yielded zearalenone and a water soluble compound which was reacted with barium chloride precipitating into presumably barium sulfate. The presence of the metabolite in several Fusarium cultures grown in rice was determined using thin layer chromatography. F. graminearum, F. equiseti and F. sambucinum were found to produce it. In the rat uterine bioassay, the metabolite was found to have the estrogenic activity characteristic of zearalenone. Natural occurrence of this previously undescribed conjugate might be significant because analytical methods devised for zearalenone in grain may not detect this compound. It retains all the biological properties of the mycotoxin when ingested by animals.

A495
A PRELIMINARY STUDY OF POSSIBLE MECHANISMS OF UVAIREREDUCED RESISTANCE TO POSTHARVEST ROTTS. C. Stevens, J. Y. Liu, V. A. Khan, C. L. Wilson, M. K. Kubbe and H. Zhong, Dept. of Agricultural Sciences, Tuskegee University, AL 36088 and USDA/ARS/APPalachian Fruit Research Station, Kearneysville, WV 25430.
Resistance to fruit ripening (increase in firmness, acidic-acidic decrease in titratable acids) was observed following increased postharvest rot resistance of peaches, apples and tangerines after the application of numerical doses of ultraviolet irradiation (254 nm UVC) for 20, 30 and 40 days respectively. The increase in firmness and low soluble acids of Elberta peaches was associated with UVC irradiation resistance to brown rot (Monilinia fructicola). Higher antioxidant content of UVC-irradiated Golden Delicious apples and nancy tangerines was inversely correlated with bacteria (Erwinia spp.) and green mildew (Pendilicum digitatum) resistance, respectively. Exposure of peach sweetpotato, apples and tangerines to certain horticmic UVC level promoted increases in phylactine amine house (PAH) activity. A close association between increases in PAL and increase in resistance of sweetpotato Fusarium storage rot (Fusarium solani) was observed.

A496
ACTIVITY OF TOXINS FROM CERESCOYA SOLANICA AGAINST SOYBEAN FUNGAL PATHOGENS AND EMBRYONATED EGGS. Roca K. Velechich and J. B. Sinclair, Dept. of Plant Pathology, Univ. of Illinois at Urbana-Champaign, Urbana, IL 61801-4709.
Three chromatographic fractions were recovered from Cercospora solani, cause of frogeye leafspot of soybeans. A deep red frac-
tion was identified as cercosporin, and a yellow fraction as a mixture of fatty acids. A red fraction was not studied. The radial growth of the following soybean fungal pathogens was inhibited by filter paper discs with 5.0 μg cercosporin: Alternaria alternata, Colletotrichum coccodes, Cercospora sojina, Phytophthora sojae, and P. insidiosa, but not of Cercospora beticola, C. kikuchii, C. nicotianae, C. sojina, Colletotrichum truncatum, or Phoma striata when incubated under 24 hr light. Cercospor-in was of no inhibitory action against any fungus grown under continuous light. A ν of 50.0 μg cercosporin was recorded with embryonated chicken eggs incubated under continuous light. The yellow fraction did not inhibit growth of any fungus under light or dark, or was toxic to embryonated eggs.

A497

POPULATION DYNAMICS OF CRYPTOCOCCUS LAURENTI IN WOODS AND APPLE PEAR STORED UNDER AMBIENT OR CONTROLLED ATMOSPHERIC CONDITIONS. P. A. Sheftelbine and R. G. Roberts. USDA-ARS, Tree Fruit Research Laboratory, Wenatchee, WA 98801.

Ripe ‘Golden Delicious’ apples and ‘Anjou’ pears were each wounded twice, then 10 μl of a buffered, washed cell suspension of C. laurentii (PMB-108) at 5 x 10^5 cfu/ml were placed in each wound. Fruit was stored at 1.2°C in a controlled atmosphere (CA; CO2:2%, O2:15%). Four replicates of apple and pear fruits were sampled 3 times weekly for 20 days for population determinations. C. laurentii colonized wounds in both apples and pears, reaching a maximum by day 10 in pears (5 x 10^6 cfu/ml) and by day 7 in apples (1.8 x 10^6 cfu/ml). Populations remained constant after reaching a maximum and were significantly (P<0.05) greater in pears. In a subsequent experiment populations (10μl of a 5 x 10^5 cfu/ml suspension in each wound) were followed for 60 days in apples (PMB-108) stored under ambient and controlled atmospheric conditions. Populations reached a maximum (4.3 x 10^6 cfu/wound) by day 4 under both storage conditions and remained constant. These results establish the microaerophilic nature of C. laurentii. Because C. laurentii exhibits biological control of fungi, preharvest pathogens under ambient atmospheric conditions, it should be equally as effective under the cold temperature, low-oxygen conditions typical of commercial storage.

A498

EFFECT OF ATOXIGENIC STRAINS OF ASPERGILLUS FLAVUS ON PREHARVEST AND POSTHARVEST AFLATOXIN CONTAMINATION OF MAIZE KERNELS. R. L. Brown, P. J. Gotty, and T. E. Cleveland.

USDA, ARS, Southern Regional Research Center, 1100 Robert E. Lee Blvd., New Orleans, LA 70124.

Field inoculations of developing maize ear kernels with an aflatoxin-producing strain of Aspergillus flavus produced high levels of aflatoxin at maturity. Simultaneous inoculation with a naturally occurring atoxigenic and a toxigenic strain reduced toxigenic levels by 72% at maturity when compared with ears inoculated with the toxigenic strain alone. Inoculations done with the toxigenic strain one day prior to inoculation with the toxigenic strain reduced toxigenic levels by 95%. The atoxigenic strain also reduced postharvest aflatoxin contamination by both the applied toxigenic strain (by percentages similar to those in preharvest tests) and strains resident on the kernels when inoculated onto kernels after harvest. These results indicate that atoxigenic strains of Aspergillus flavus have potential use as biological control agents of both preharvest and postharvest aflatoxin contamination of corn.

A499

SURVIVAL OF ASPERGILLUS FLAVUS IN CORN DERRIS AND SOIL FROM 106C C. FIELDS. L. F. Sweetman, W. J. Fox, H. L. Tiffany. Iowa State University, Ames, IA 50011.

Samples of soil and of stalk and cob debris were collected from 60 corn fields in eight Iowa counties following the 1988 harvest. Additional samples were collected from these fields in the spring of 1989, fall 1989 and spring 1990. Soil samples were tested for the presence of Aspergillus flavus by sprinkling approximately 0.5 g of soil onto the surface of M3510B agar plates. Pieces of cob and stalk pith approximately 2 cm in diameter were pulled out of freshly broken cob and stalk samples and plated on M3510B agar plates. Plates were incubated at 37°C for 3 days and visually examined for presence of A. flavus colonies. Aspergillus flavus was detected in soil samples from all fields at the end of the first three sampling times. About 70% of the cob pieces from both fall 1988 and spring 1989 samples were positive for A. flavus. However, with the stalk pieces 42% of the fall 1988 samples and 84% of the spring 1989 samples were positive for A. flavus.

A500

THE BIOCONTROL ACTIVITY OF A YEAST STRAIN US-7 AGAINST POSTHARVEST DISEASES OF FRUITS AND VEGETABLES: POSSIBLE MODE OF ACTION. S. Brophy, E. Chalut*, C. L. Wilson,** A. A. Strydom, E. J. F. Bradley. The Volcani Center, Bet-Dagan 50250, Israel; and **Appalachian Fruit Research Station, USDA, ARS, Kearneyville, WV, USA.

The yeast strain US-7 was found an effective antagonist of several postharvest diseases of fruits and vegetables. It effectively inhibited the development of decay caused by Penicillium digitatum, P. italicum and Geotrichum candidum on citrus fruits. It also inhibited Botrytis cinerea and Rhizopus stolonifer on apples, peaches and grapes. Studies on the mode of action of US-7 revealed that it does not inhibit the pathogen through production of antibiotic substances. Other possible modes of action investigated were competition with the pathogen for nutrients and/or space, induction of the host resistance mechanisms and direct interaction between the antagonist cells and the pathogen. Possible involvement of the yeast cell-wall material in the inhibition of spore germination and mycelial growth will be discussed.

A501

ANTIFungal EFFECT OF CHITOSAN ON TWO FUNGAL PATHOGENS OF STRAWBERRIES IN Vivo AND IN vITRO. Ahmed El Ghaouth, Rathy Ponnamapla, Joseph Arul and Marcel Boulet. Dept. of Food Science and Technology, Laval University, Ste-Foy, Quebec, G1K 7P4, Canada.

The effect of chitosan coating on strawberries inoculated with Botrytis cinerea or Rhizopus stolonifer stored at 15°C under high humidity – regular atmosphere was investigated. Chitosan coated berries were significantly less decayed after 20 days of storage than the uncoated berries. Chitosan (pH 5.6) at concentrations of 3000 μg/ml and 6000 μg/ml increased protein and other U.V. absorbing materials in suspension culture of B. cinerea and R. stolonifer, with a greater effect at the higher concentration. Furthermore, the excessive leakage caused by chitosan at 6000 μg/ml suppressed the ability of fungal tissue for regrowth during 48 hours. It appears that one possible mechanism by which chitosan inhibits fungal infection in strawberries is due to its ability to induce intracellular leakage in fungi.

A502

THE EFFECT OF CHITOSAN ON GROWTH AND MORPHOLOGY OF RHIZOOPUS STOLONIFER. Ahmed El Ghaouth, Joseph Arul and Rathy Ponnamapla. Dept. of Food Science and Technology, Laval University, Ste-Foy, Quebec, G1K 7P4, Canada.

The effect of chitosan at pH of 5.6 and 7.2, chemically modified chitosan (O, N, carboxymethylated) and gluconate on spore germination, germ tube growth, mycelial growth and morphology was studied. Chitosan (pH 5.6) at concentration of 3000 μg/ml was significantly more effective in inhibiting spore germination, germ tube length and radial growth than either chemically modified chitosan, or chitosan (pH 7.2) or gluconate. Only chitosan (pH 5.6) at 3000 μg/ml induced significant morphological changes such as lateral branching and slower expanding colonies. This work indicates that the antifungal effect of chitosan is attributable to its polycationic nature and polymeric size.

A503


Spores of Mucor piriformis, Botrytis cinerea, and Phytophthora parasitica were exposed to 1.5 - 3.8 μg/ml ozone or 400 μg/ml chlorine solution. Ozone in aqueous solution was prepared by passing pure oxygen through an ozone generator and bubbling gaseous ozone through a column of water. Concentration of ozone was determined with an ozone test kit. Spores of these organisms in a water suspension were inactivated by exposure to 1.5 μg/ml and 3.8 μg/ml ozone. Spores of B. cinerea on the surface of non-injured tomato fruit were inactivated when exposed to 3.8 μg/ml ozone solution for 10 min. The addition of 0.1% of a joany alcohol solution did not affect the ability of ozone to inactivate spores. Spores placed in surface injuries of tomato fruit, however, were not inactivated. In all trials, except injured fruit treatments, 2 min exposures to a chlorine solution inactivated spores of these fungi. Mycelial fragments of P. parasitica were more sensitive than Rhizopus stolonifer and M. piriformis to ozone and chlorine treatments.

A504

DETECTION OF ERGOLINE IN FESCUE SEED BY ELISA USING A MONOCLONAL ANTIBODY. S. B. Heuwerk, A. A. Strydom, C. E. Kelby, and G. E. Ruttehsold. Departments of Plant Pathology and Botany and Microbiology, Auburn University, AL 36849 and College of Veterinary Medicine, U. of Missouri, Columbia MO 65211.
Ergovaline (EV) is one of the ergo-peptide alkaloids of fescue infected with the endophytic fungus, _Acremonium coenophialum_, associated with the grass bloat syndrome. A serum antibody to EV was used in a competitive indirect (CI) ELISA of 41 fescue seed lots. Percent fungal infection determined by microplate CV varied from 0.05 to 25.0%. Accuracy of the CI ELISA in determining EV content was evaluated by HPLC. EV levels determined by both methods were positively correlated with percent infection and ranged from 0-10 mg/g. CI ELISA agreed in 97% cases with HPLC in terms of the presence or absence of EV, indicating no false positives or negatives, but tended to underestimate EV by about one half in most samples. The CI ELISA provides a rapid screening method for presence and relative concentration of EV, to be used in conjunction with HPLC when more accurate quantitative data are needed.

**A509**

ONSET OF MAIZE DWARF MOSAIC IN NORTHERN OHIO. B. J. Knoke, USDA/ARS and The Ohio State Univ., Wooster, OH 44691.

Trap plant (TP) plots with and without diseased source plants (SP), successive plantings, and grass weeds in tile plots were used to monitor maize dwarf mosaic disease (MDM) onset in northern Ohio. TP plots normally detected MDM in late-August and early-September. TP plots with SP detected MDM beginning in late-June, when SP were placed in the plots. The average incidence of MDM in TP increased from 44 to 52% as the number of SP placed at 0.6 m distance from the TP increased from 25 to 100 plants. At a constant level of 100 plants, the average incidence of MDM decreased from 52 to 33% as the distance between SP and TP increased from 0.6 to 4.9 m. Successive plantings detected MDM onset 31 and 12 days earlier in 1986 and 1987, respectively, than did TP plots. MDM was not recovered from either the 832 weed samples collected from the field or the six grass weeds grown in tile plots. _Rhopalosiphum maidis_ (Fitch) was significantly related to MDM onset. Aphid migration, seed transmission, and infected weed host hypotheses were evaluated as initial sources of MDM, but a weed host hypothesis best explained MDM onset in northern Ohio.

**A511**

ASSOCIATION OF TOBACCO STRIPE ILLINOIS (TISV) SEED TRANSMISSION AND ANOTHER TISSUE INFECTION IN BEANS. M. H. Walter, W. J. Kaiser, R. E. Klein and S. D. Wyatt. Washington State University, Pullman. 99164.

TISV seed transmission was investigated by antigenicity and infectivity assays of flower parts from beans systemically infected with either TISV isolate Me 40 (Kaiser, Wyatt and Pesho. Phytopathology 72:1508-1512) or TISV isolate Me F E and through reciprocal pollinations. ELISA results indicated that antigen levels of the two virus isolates were similar in flower petals and in ovaries of beans infected with either virus. However, antigen levels in stamen tissues were much lower for TISV than for TISV Me 40. The amount of infectious virus (as measured by infectivity assays of flower parts on local lesion host Chenopodium quinoa) was also less in stamens of TISV F-infected plants than in stamens of TISV Me 40-infected plants. Healthy Black Turtle Soup (BTS) bean (Phaseolus vulgaris L.) plants were pollinated using anthers from plants systemically infected with either Me 40 or Me F. Infected plants were also pollinated using anthers from healthy plants. When Me 40-infected anthers were used to pollinate healthy plants, 25.28% of the resulting progeny seedlings were infected. Healthy anther X infected ovary pollinations produced 3.36% seedling infection. Me F isolate was seed transmitted in BTS at less than 0.5%. Seed transmission of TISV in beans may depend on early movement into and replication in pollen-associated tissues.

**A512**

LEAFHOPPER PROBING PATTERNS ASSOCIATED WITH MAIZE CHLOROTIC DWARF VIRUS (MC DV) TRANSMISSION. A. G. Wayandadane and L. R. Nault, Dept. of Entomology, Ohio State University, GARD, Wooster, OH 44691.

Female _Griminella nigricornis_ leafhoppers were electronically...
A513

VIRION MORPHOLOGY DIFFERENCES BETWEEN TWO TOMATO SPOTTED WILT VIRUS ISOLATES. L. L. Urban, Pi-Yo Huang and J. W. Moyer, Department of Plant Pathology, Box 7616, Raleigh, N.C. 27695.

Infection of Nicotiana benthamiana by two isolates of tomato spotted wilt virus (TSWV), the common type (TSWV-D), and the impatiens type (TSWV-I), was investigated. Transmission electron microscopy of TSWV-D infected samples revealed electron dense areas, single, spherical enveloped virions, and non-enveloped particles. TSWV-I samples also contained morphologically similar electron dense areas, but rarely exhibited single enveloped particles and had no membrane bound particles. TSWV-I samples uniquely contained pacocrystalline arrays. All structures of both isolates appeared to be associated with endoplasmic reticulum. Immunogold labeling with whole virus antisera of the electron dense areas of TSWV-I infected plants have shown these areas to be associated with viral protein. These observations suggest that TSWV-I is defective in virus particle assembly. However, this defect does not affect the disease severity.

A514

PURIFICATION AND PROPERTIES OF CLOSTREROVIRUS-LIKE PARTICLES ISOLATED FROM A CORKY BARK DISEASED GRAPEVINE. S. Namba, D. Boscia, O. Azzam, M. Maixner, J. S. Hu, D. Golini1 and D. Gregorius, Plant Pathology, Cornell University, Geneva, NY 14456. 1 USDA-ARS, Dept. of Plant Pathology, Univ. of California, Davis, California, 95616.

Clostrerovirus-like particles were purified from petioles of Viitis vinifera cv. Semillon affected with grapevine corky bark disease. Electron microscopy of the purified preparation revealed the presence of flexuous rod-shaped virus particles, which were about 13.1 x 1,400-2,000nm with a helical pitch of 3.4 nm. The mean weight of the coat protein was 5.2 x 10^6 daltons. Transformation analysis using specific antisera. A large dsRNA molecule (ca. 10.4 x 10^6) and lower molecular weight species were isolated from cork phloem of corky bark affected Semillon. In ELISA and ISEM tests, antisera produced to the virus did not react to clostreroovirus-like particles associated with grapevine leafroll disease (Types II, III, IV) or grapevine virus A (GVA). Reciprocal tests confirmed these results.

A515

GEOGRAPHICAL ISOLATES OF MAIZE STRIPE VIRUS DIFFERING IN EFFICIENCY OF TRANSMISSION BY, AND TITER IN, THE PLANTHOPPER PEREGRINUS MAIDIS. E. D. Ammar1, R. E. Genginger1 and L. V. McDonald2. 1Dept. of Economic Entomology, Facultly of Agric., Cairo Univ., Egypt, USA. 2ARS-DEP. of Plant Pathology, The Ohio State Univ.-Ohio Agricultural Res. and Dev. Center, Wooster, OH 44691.

Isolates of maize stripe virus (MSV) from Florida (US), Costa Rica (CR), and Africa (AF), were transmitted to plants by Peregrinus maidis (from Hawaii) with respective frequencies of 0.18, and 60% after 3 days in the presence of MSV-AF. Two isolates were transmitted 80% after a 7-day AAP. The isolates were transmitted transovarially to progeny with respective frequencies of 21, 32, and 44%. ELISA of infective planthoppers that had acquired virus orally wk earlier indicated a significantly lower titer of MSV-AF compared to MSV-SE and MSV-RN. These results suggest that, compared to the US isolate, the AF and CR isolates reach higher levels in and are transmitted at higher frequencies by P. maidis from Hawaii.

A517

CORN LETHAL NECROSIS IN HAWAII. S. G. Jensen, United States Department of Agriculture, Agricultural Research Service, Department of Plant Pathology, University of Nebraska, Lincoln, NE 68503-0722. J. J. Ooka, University of Hawaii, H. E. Lockhart, University of Minnesota, S. A. Lommel, North Carolina State University, L. C. Lane, D. E. Wysong, and B. Doupnik, Jr., University of Nebraska, Lincoln, NE 68503-0722.

Coral lethal necrosis (CLN), a serious disease of maize, has been identified as the synergistic action between maize chlorotic mottle virus (MCMV) and a true virus, which has been positively identified from several dent and sweetcorn fields along the west coast of the island of Kauai, Hawaii. The presence of MCMV was confirmed by serological tests. The presence of this second virus was confirmed by serological tests. The second virus was identified as the true virus component of the Hawaiian CLN infection. The finding is important to the feed industry of Hawaii.

A518

1989 CORN VIRUS SURVEY IN SOUTH CENTRAL NEBRASKA AND CORN LETHAL NECROSIS UPDATE. B. Doupnik, Jr., University of Nebraska, Clay Center, 68933, and L. C. Lane and S. G. Jensen, University of Nebraska and USDA-ARS, Lincoln, 68583.

Following the severe outbreak of corn lethal necrosis (CLN) in 1988, an intensive corn virus survey was conducted in July and August, 1989, in SC Nebraska. Of the 361 fields surveyed, symptomatic plants were observed and leaf samples collected from 92 fields. These samples were assayed for maize chlorotic mottle virus (MCMV), maize dwarf mosaic virus (MDMV), and wheat streak mosaic virus (WSMV) using gel electrophoresis and host reaction. Of the 92 samples, 38 were positive for MCMV, 12 for WSMV, 5 for MDMV-B and 2 for MDMV-B and 1 for MDMV-B. None were positive for MCMV and MDMV-B (the synergistic combination that is most commonly associated with CLN) and 34 had no viruses detected. MCMV was widespread in 1989; but, the incidence of MDMV was low and of CLN extremely low. This is attributed, in part, to the low incidence of greenbugs, the primary vector of MDMV throughout the 1989 growing season. CLN county records now stand at 9 in SC Nebraska and 7 in NC Kansas.

A519

DEVELOPMENT OF A TRANSFORMANTATION SYSTEM FOR PEANUT. T. E. Clemente1, E. A. Neissinger1, J. W. Yoyer1, and K. K. Beute1. 1Department of Plant Pathology and Crop Science, NC. State University, Box 7616, Raleigh, NC 27695-7616.

Transformation of peanut (Arachis hypogea) via high velocity microprojectiles to deliver transforming DNA is being investigated. To date, a transformation assay has been developed utilizing marker genes, it will be employed to introduce gene(s) for potential disease control into the cultivated peanut. Explants from the cultivars 'trench', 'bluff' and 'NC 7', have been tested with two different plasmids, pBI 922, and pBI 221, both of which carry the β-glucuronidase (β-GUS) marker sequence driven by the 35S promoter. Transient expression in transformed plates was detected by the β-GUS histochemical assay in mature embryo, mature embryonic leaves, callus cultures derived from stem and leaf explants, and mature leaf tissue. Embryonic leaves had the highest degree of transient expression, ranging from 0-12 expressing cells per leaflet.

A520

INDUCTION OF PHENYLPROPANOID METABOLISM DURING A HYPERSENSITIVE RESPONSE IN ARABIDOPSIS THALIANA. Keith R. Davis and Farida Shaeen, Department of Botany and the Biotechnology Center, Ohio State University, Columbus, OH 43210

1022 PHOTOPATHOLOGY
Recent studies by several groups have established the infection of Arabidopsis thaliana with phytopathogenic pseudomonads as a model system for studying plant disease resistance. The goal of these studies is to use a combination of biochemical, molecular, and genetic approaches to analyze the complex responses associated with a resistance reaction. Our initial studies have focused on the induction of defense-related genes in cell cultures treated with elicitors, or in leaves infiltrated with virulent or avirulent Pseudomonas syringae pathovars. These studies demonstrated that genes involved in phenylpropanoid metabolism, including phenylalanine ammonia-lyase (PAL) and 4-coumarate:CoA ligase (4CL), are induced in elicitor-treated cell cultures and in leaves expressing a hypersensitive response. We have isolated genomic clones of Arabidopsis PAL and 4CL genes and are currently analyzing their structure and expression. The results of these studies will be discussed in the context of utilizing genetic approaches for identifying factors involved in activating plant defense responses.

A525


Bacterial spot of peach caused by Xanthomonas campestris pv. pruni (Xcp) is strongly influenced by water congestion of leaves. The effect of water congestion and bacterial infection on leaf proteins was studied. Water congestion was induced by placing plastic bags over cuttings in a dew chamber for 48 h. The plants were spray-inoculated and the bags placed over them for another 24 h. Bacterial spot was observed 3, 5, 6, and 9 days after inoculation. Protoplasts were isolated in phosphate-citrate buffer (pH 2.8) and separated by SDS-PAGE. Water congestion caused a decrease or disappearance of some proteins between 21.5 kD and 42.7 kD and below 16 kD as compared to the non-water-congested plants, while new proteins between 16 kD and 42.7 kD appeared in the plants infected by Xcp. Changes that accompany water congestion may predispose the plants to infection by Xcp.

A526

DETECTION OF PROTEASE PRODUCED BY Xanthomonas campestris pv. zinniae IN ZINNIA LEAF TISSUE USING THE SUBSTRATE B-CASEIN. X.K. Suh and D.F. Ritchie, Department of Plant Pathology, North Carolina State University, Raleigh, 27695.

Zinnia (Zinnia elegans) leaves were infiltrated with suspensions of two wild type strains of X. xanthii pv. zinniae (Xz1 and Xz2 24) and a protease-impaired mutant generated by adding orange (G) treatment of wild type strain Xz1. Leaf tissue samples were taken 6-72 h after infiltration, ground in 0.1 M Tris-HCl buffer (pH 8.0), and centrifuged. Supernatants were subjected to RNAse-resuspended in 0.1 M Tris-HCl buffer (pH 8.0). Protease samples were incubated with B-casein (5 nmol) at 37°C for 3 min and assayed by 16% SDS-PAGE. The protease activity of B-casein was interpreted as being indicative of the protease in the leaf tissue. No protease activity was detected 6 h after infiltration of the bacteria into the leaf tissue. After 20 hr, slight protease activity was detected in samples infiltrated with the wild type strains only. At 48 and 72 h, both wild type strains and the mutant strain digested B-casein, but the mutant strain digested less than the wild type strains. B-casein was not digested by extract from the water-infused control, indicating that the zinnia leaf tissue does not contain proteases detectable by this method. This procedure should be useful in detecting and monitoring bacterially produced protease activity in plants.

A527

RESISTANCE TO RACE 2 OF XANTHOMONAS CAMPESTRIS PV. ORYZAE CONFERRED BY BACTERIAL BLIGHT RESISTANCE GENE Xa-10 IN RICE INVOLVES LIGNIFICATION OF HOST TISSUES. P.J. Reimers and J.E. Leach. Department of Plant Pathology, Manhattan, KS 66506. U.S.A.

Race-specific resistance (incompatibility) to bacterial blight (BB) of rice caused by Xanthomonas campestris pv. oryzae (Xco) is correlated with reduced bacterial numbers and shorter lesion lengths in incompatible leaves. When seedlings leaves of cultivars carrying the Xa-10 gene for BB resistance were infiltrated with suspensions of Xco (10^5 cfu/ml), a camouflage-blue color began forming in the inoculation site by 18-24 hours after inoculation (HAI) and reached a maximum by 48 HAI, with no water-soaking. Commutability resulted in uniform water-soaking. No response occurred after infiltration with water or UV-killed bacteria. Lignin-like materials accumulated throughout the inoculation site in the incompatible interaction, and reached a maximum level of 48-72 HAI. By 120 HAI, lignin-like components were detected only in the perimeter of the compatible interaction. The response, induced by races 2 and 5 of Xco on Xa-10-containing rice, resembled a hypersensitive response.

A528

CYTOTOLOGICAL AND BIOCHEMICAL CHANGES OF CELL WALL AS RELATED TO SYSTEMIC RESISTANCE TO BLUE MOLD (PERONOSPORA TABACINA) INDUCED BY TMV IN TOBACCO. X.S. Ye, S. Avdiushko, S.Q. Pan, U. Jarfors, S. Turun & I. Sac. Dept. of Plant Pathology, Univ. of Kentucky, Lexington, Ky 40546.

The effect of proteins in maize leaf leachates on the sensitivity of maize to BMT-toxin was determined by infiltrating leaves of Normal (N) and Texas male sterile (T) cytoplasm isolines (cv. U64A) with various dilutions of these proteins mixed with various dilutions of BMT-toxin. Infiltrated leaves were cut into 3 cm pieces, immersed in 20% for 20 min at 40°C, and then the rate of electrolyte leakage was measured. Toxicity of the T and N cytoplasm isolines showed increased electrolyte leakage in response to BMT-toxin. This was significantly reduced when T cytoplasm leaves were infiltrated with toxin solutions plus 2 μg/ml of more of proteins from N or T male sterile (cv. Bonton leaf) (Bonton) leaf extract (USA), a 66 kD protein, produced a similar response. Binding of maize proteins to toxin or to cell components in competition with toxin may be involved in the reduced activity seen when these polypeptides are mixed with toxin prior to its infiltration into T cytoplasm leaves.
Inoculation of lower leaves of tobacco Ky14, carrying the N gene, with TMV induced systemic resistance to P. tabacina and a concomitant systemic accumulation of cell wall hydroxylamine-rich glycoproteins by 12-16 hours increased significantly in uninfected plants in the presence of TMV induction with TMV, and more so after challenge with P. tabacina. During this period, HRGP levels in the controls remained unchanged. Four new salt-soluble proteins were detected in the cell wall preparation of the infected plants. These proteins are neither HRGP nor 6-1,3-glucanase or chitinase. One of these strong spots showed that blue mold development in the infected plants was severely restricted, infected cells were more plasmolyzed and 4 days after challenge some host cells and fungal hyphae became necrotic near the center of infection sites. Electron microscopy revealed that 4 days after challenge host cell walls in contact with fungal hyphae became more electron-opaque. Electron opaque materials were deposited against host cell walls at the host pathogen interface. Exostomal mosaic matrices were much wider, and fungal hyphae often lacked contents in the inoculated as compared to control plants.

A529
CHITINASE ISOMYZE PATTERN AND ITS COORDINATED INDUCTION WITH 6-1,3-GLUCANASE IN TOBACCO PLANTS IMMUNIZED BY PERONOSPOREA TABACINA AND TOBACCO MOSAIC VIRUS. S. Q. Fan, X. S. Ye, S. Tuzun and J. Ku, Department of Plant Pathology, University of Kentucky, Lexington, Ky 40550.

Stem injection of tobacco with sporangiospores of P. tabacina or leaf inoculation with TMV systemically protected plants against diseases caused by both pathogens and systemically increased chitinase and 6-1,3-glucanase activity. Among eight chitinase isozymes detected in tobacco, six did not change even after challenge in protected and control plants. Two were systemically increased in the plants protected by both methods of immunization and accumulated more rapidly in the protected than control plants after challenge with P. tabacina. One of these isozymes was not detected in control challenged leaves prior to 4 days after challenge. When symptoms appeared four days after challenge, two chitinase isozymes increased in the control plants. The increase of these two chitinase isozymes was coordinated with an increase of a 6-1,3-glucanase and both enzymes were associated with protection.

A530

The site of action of carbamate and organophosphate nematicides in plant-parasitic nematodes is the enzyme acetylcholinesterase. These pesticides block the hydrolytic action of the enzyme at the neuromuscular synapse, resulting in paralysis of the nematode musculature. However, nematicides are able to recover from the effects of carbamate and organophosphate nemacides rapidly, resulting in inconsistent field efficacy of applied compounds. The root-knot nematodes Meloidogyne arenaria and M. incognita produce an isozyme of acetylcholinesterase known to occur only in nematodes. This enzyme has an unusually high affinity for acetylcholine, but is very insensitive to carbamate and organophosphate nematicides. The enzyme purified from M. arenaria and M. incognita appears to be very similar to Class C acetylcholinesterase found in the free-living nematode Caenorhabditis elegans. It is known that this enzyme in plant-parasitic nematodes is responsible for the rapid recovery of the nematodes after exposure to carbamate and organophosphate nematicides.

A531
USE OF SLOW RELEASE FERTILIZERS FOR CONTROL OF SCROLETUM ROLFSII. G. H. Carullo and R. Rodriguez-Kabana. Department of Plant Pathology, Alabama Agricultural Experiment Station, Auburn University, Alabama 36849-5409.

Guaiadial thiochyanate, guayule sulfate, and thiourea were used in a microplot experiment at 0, 0.25, and 0.5 g/kg soil for 30 days with long-term replacement of fallow (0.5 g/kg formaldehyde). Guayule sulfate was the most effective compound in terms of the reduction of sclerotial production and improvement of soil microbiota. Guayule sulfate was tested in combination with other fungicides that were not effective in greenhouse conditions. The combination guayule sulfate + guandine thiochyanate was the most successful for controlling S. rolfsii. The treatment combination of 0.15 g guayule sulfate + 0.05 g guandine thiochyanate/kg soil was the lowest effective rate.

A532
ENHANCED SOYBEAN PLANT GROWTH AND NECTARINITY BY BRADYRhIZOBIUM IN THE PRESENCE OF STRAINS OF BACILLUS SUBTILIS. Z. Lu, Liu and J. B. Sinclair, Dept. of Plant Pathology, Univ. of Illinois at Urbana-Champaign, Urbana, IL 61801-4709.

Enhanced soybean plant growth and nodulation by Bradyrhizobium was obtained in nutrient culture in a controlled environmental chamber in the presence of Bacillus megaterium ATCC-55000 and M2144. Seedlings from seeds coated with Bradyrhizobium plus either 55000 or M2144 had significantly higher plant dry weight, nodule dry weight, nodule number, and nitrogenase activity than the control. None of the growth parameters was significantly increased above the control when the two strains were applied separately as a seed treatment at VI growth stage. Both strains used as seed treatments with Bradyrhizobium resulted in denser and more widely distributed nodules compared to the control. The mechanism involved in this enhancement appeared not to be the same as that of symbiotic interaction with Rhizobia solani, since M2144 was an antagonistic-deficient mutant. The nodulation enhancement was recorded in the field at VA to R1 growth stages but yield effects varied.

A533
ROLE OF 2,4-DICETYLTETRAFLUROROBORIC ACID IN DISEASE SUPPRESSION BY A STRAIN OF PSEUDOMONAS FLUORESCENS. C. Keel, C. Voisard, D. Haas, 5, and G. Dufay 5. Department of Plant Science/Phytopathology and of Microbiology, Swiss Federal Institute of Technology, 8092 Zürich, Switzerland.

Pseudomonas fluorescens strain CHA0, which is an effective biocontrol agent of soybean plant pathogens, produces several toxic metabolites, notably cyanide, acetylfluoroboric acid, and pyrolysin. By genetic manipulation of strain CHA0, cyanide was shown to be an important factor in the suppression of black root rot of tobacco caused by Thielaviopsis basicola. Strain CHA625, which was obtained after Tn5 mutagenesis, did not produce 2,4-diacetylfluoroboric acid and suppressed black root rot of tobacco and Ganassinymymes graminis var. tritici-induced take-all of wheat to a distinctly smaller extent than did wild-type CHA0. Under both phytobiotrophic and biotrophic conditions. A cosmid, P3E310, obtained from a genomic library of strain CHA0 mediated the restoration of the ability of strain CHA625 to produce this metabolite and partially restored its suppressive capacity. 2,4-Diacetylfluoroboric acid was shown to be produced by strains CHA0 and CHA625/pP3E310 but not by strain CHA625 in the rizosphere of wheat, grown under phytobiotrophic conditions. These results suggest that the production of 2,4-diacetylfluoroboric acid by strain CHA0 plays an important role in the suppression of soybean plant pathogens.

A534
MECHANISMS OF RESISTANCE TO ROOT-LESIOn NEMATODE (PRATYLENCUS PENETRANS) IN ALFAFA. J.A. Thies, D.H. Busskamp, D.K. Barnes, and R.D. Wilcoxson, USDA-ARS and Depts. of Plant Pathology and Agronomy & Plant Genetics, Univ. of Minn., St. Paul, MN 55108.

Resistance of alfalfa (Medicago sativa) to Pratylenchus penetrans has been reported, but mechanisms of resistance have not been determined. Rooted rames of 25 alfalfa clones inoculated with P. penetrans were grown for 6 weeks in free-choice and no-choice tests at 25C with a 16 hr photoperiod. An equal number of noninoculated rames served as controls. The clones varied for number of nematodes/plant, nematodes and eggs/g fresh plant weight, and for percent fresh plant weight (plant weight of inoculated clone/plant weight of noninoculated clone). Resistance mechanisms identified were antibiosis (characterized by low numbers of nematodes in roots) and tolerance (characterized by large plant weights and moderate to high numbers of nematodes in roots). Nematodes + eggs/g fresh plant weight and percent fresh plant weight of control were the most important criteria for selecting clones with highest degrees of antibiosis and tolerance, respectively.

A535
HEAT INACTIVATION OF GAEMANNOMYCES GRAMINIS VAR. TRITICI (GGT). W. W. Bockus and B. L. Norman, Department of Plant Pathology, Kansas State University, Manhattan 66506-5502.

Inoculum of GGT was exposed to 35, 40, or 45 C for various time periods to determine the inactivation point. Exposure occurred in moist (<0.1 MPa), autoclaved and nonautoclaved field soil in petri dishes. Natural inoculum (infected wheat crowns from the field) and artificial inoculum (colonized oat kernels) were compared. After exposure, inoculum was used to infest a assay to quantify the ability of GGT to cause rot and losses in fresh weight on wheat seedlings. Inactivation at 35 C required 10-15 exposures of 6 hr/day (depending upon the experiment); at 40 C, inoculum was inactivated after 3-5 exposures of 6 hr/day; and at 45 C, it was inactivated after a single exposure of 2.5-5 hr. No significant differences were detected between the response in autoclaved vs. nonsterile soil; however, natural inoculum was slightly more resistant to inactivation than artificial inoculum. Results of these experiments suggest that inoculum of GGT is sensitive to heat inactivation and may explain why it does not survive in bare soil for 8 wk during the summer in Kansas.

A536
CORRESPONDENCE OF RHIZOSPHERE COMPETENCE ON MAIZE TO CARBON UTILIZATION WITH SIX Fusarium SPECIES. Cynthia M. Gemb and Thor Kommedahl, Department of Plant Pathology, University of Minnesota, St. Paul, MN 55108.
Fusarium moniliforme, F. oxysporum, F. proliferatum, and F. solani extensively colonize the rhizosphere while F. equiseti and F. guepelii are not often encountered. Enzyme production for carbon utilization may reflect rhizosphere competence. All six species of Fusarium grew on various carbon substrates with Czapek-Dox salts: cellulose, cellulose-galactose, pectin, xylan, and xylose. Sporulation and mycelial dry weights were evaluated on cellulose, pectin, and dextrose. Fusarium moniliforme generally sporulated more than other species, especially on the dextrose and pectin substrates. Cellulose yielded significantly lower mycelial dry weights than pectin or dextrose. Mean mycelial dry weight produced on cellulose, pectin, and dextrose was greatest for F. moniliforme and generally least by F. solani. Rhizosphere colonization of these six Fusarium species appears not to be related to utilization of cellulose or pectin.

A537
EFFECT OF NaCl ON CARBOHYDRATES AND MALATE PRODUCTION IN ASPARAGUS ROOTS AND ON INFECTION BY Fusarium. W. H. Elmer, Dept. of Plant Pathology, and Ecology, The Connecticut Agricultural Experiment Station, Box 1160, New Haven, Connecticut 06504.

Asparagus plants, grown in sand culture in the greenhouse, received biweekly applications of 0.1, 0.3, 0.5, or 1.0 g of NaCl dissolved in 100 ml of Hoagland's solution. Half of the pots in each NaCl treatment were inoculated with 100 ml of H2O containing 10^6 conidia of F. oxysporum and 10^5 conidia of F. moniliforme. The other pots received 100 ml of sterile H2O and served as uninoculated controls. The percentage of roots with lesions (RL) and root colonization (RC) were measured in inoculated plants after 3 mo. Disease (RL, RC) did not increase as NaCl rates increased. Levels of carbohydrates significantly increased and malate significantly decreased in noninoculated roots treated with increasing rates of NaCl. Because the cause of NaCl did not affect the growth of these Fusaria in culture, it is postulated that NaCl suppresses disease by mechanisms associated with malate and carbohydrate metabolism.

A538
EFFECT OF IRRIGATION, FUMIGATION, AND SULFUR ON THE DEVELOPMENT OF STREPTOMYCYTES SOIL, ROOT, AND YIELD IN SWEETPOTATO. J. R. Ristaino, Department of Plant Pathology, North Carolina State University, Raleigh, NC 27605-7166.

Sweetpotato plants infested with Streptomyces ipomoeae were either not irrigated or drip irrigated during the season. Subsamples were seeded with sulfur (90 W 33131/B) and siblo subsamples were fumigated with Delone C-17 (10.5 gal/A) prior to transplanting. Soil pH was reduced from 5.8 to 5.2 in sulfur-treated plots. Fumigation increased yields by 50% and decreased disease on storage roots by 27%. Highest yields and lowest severity of disease on fibrous roots occurred in plots that were fumigated, irrigated and treated with sulfur. Fumigation increased the number of storage roots produced per plant, while fumigation and sulfur reduced the number of diseased storage roots produced per plant. Irrigation alone did not affect disease on storage roots but reduced disease on fibrous roots in combination with fumigation and sulfur. Management of soil root rot may not be as severe as in fibrous root media which allows rapid movement of zoosporangia through the soil.

A539
DETECTION OF PHYTOPHTHORA SPECIES IN CRANBERRY FIELD SOILS. M. J. Drillock and S. N. Jeffers, Department of Plant Pathology, University of Wisconsin, Madison, WI 53706.

Phytophthora species have been associated with a decline syndrome of cranberry in Wisconsin. A baiting bioassay was developed to detect Phytophthora spp. in cranberry field soils. Serially diluted soil samples were produced by mechanical production of cranberry stems (cv. Stevens) were used as baits. A 65-ml aliquot of soil, sieved through a 6-mm screen, was placed in a 150-ml, wax-coated paper cup. Three to four baits were placed in each cup so that the soil was below the water level. After 7 days, each stem was removed and the remaining soil was placed in a 1.0- to 1.5-cm layer of water over the soil surface. Baits were planted at constant temperatures for up to 2 wk. Baits were examined every 2-3 days, and those with discoloration were planted in commercial media corresponding to the genus Phytophthora. The number of baits colonized by Phytophthora spp. increased by 24 C. Phytophthora spp. recovered from field soils were morphologically similar to those previously isolated from cranberry plants.

A540
AN ISOLATE OF PHYTOPHTHORA ARECEA FROM FLORIDA PATHOGENIC TO Citrus. L. W. Timmer, S. E. Zilkka, and H. A. Sandler, University of Florida, Citrus Research and Education Center, Lake Alfred 33850.

A Phytophthora sp. recovered from soil in a citrus orchard near Ft. Pierce most closely fit the description of P. arecae (probably synonymous with P. palmivora). It produced papillate, caducous sporangia averaging 50 μm long and 34 μm wide with a pedicel 3 μm long. Asci were 30 μm long. Ascospores were 18 μm x 13 μm with A types of P. parasitica and P. palmivora. Oogonia, oospores, and chlamydospores averaged 30, 25, and 34 μm in diam., respectively. Optimum temperature for growth was 30°C with little or no growth at 15 or 37°C. The citrus isolate of P. arecae (P.a.-citrus) was as pathogenic as P. parasitica to fibrous roots of sweet orange, sour orange, and Swingle citrumelo but was not as pathogenic to citrus roots. The P.a.-citrus and the P.a.-palm isolates were pathogenic to grapefruit. This is the first report of a P. arecae pathogenic to citrus in the U.S.

A541
REDUCTION OF SEEDLING EMERGENCE DUE TO BIHZOSTOIA ZEA IN TWO TALL FESCUE VARIETIES. K. D. Quimlen and A. M. Gavin, University of Tennessee, Knoxville, TN 37901-1071.

Reduction of seedling emergence due to Hizostoria zeae (NC R2112J) was examined in two tall fescue varieties which differ in endophyte (Acremonium coenophialum) levels. Endophyte levels were determined to be 3% (Kentucky-31) and 3% (Forager). Seed (10 g/fl) were planted in flats of either Promix or Promix amended with B. zeae (NC R2112J). Number of seedlings in both varieties was determined; 3 cores/fl were counted. Five flats/treatment were used and the experiment was repeated three times. Seedlings/core in nonamended Promix was different (P<0.001) from seedlings/core in Hizostoria- amended Promix for both varieties in Forager and Kentucky-31. Seedling emergence in amended soil was 70% of emergence in nonamended soil, however for Forager, seedling emergence was reduced to 36% in amended soil. Whether differences are due to endophyte infection or genotype will be investigated.

A542
MOVEMENT OF PHYTOPHTHORA ZOOSPORES THROUGH COLUMNS OF CONTAINER MEDIA. D. M. Benson, Dept. of Plant Pathology, N.C. State Univ., Raleigh 27695.

Zoosporas of P. parasitica were added to 15-cm-high columns of saturated bark (PB), pine bark sand (PBS), and perlite sand (PSS; 1:1:1) media. The void volume (v) was collected and cultured on a PARP medium for colonies. Void volumes were 70, 40, and 10 ml for PB, PBS, and PSS, respectively. Additional void volume equivalents were added to each column and collected. For motile zoosporas, maximum counts (range 100-3200/v) were found in the 2nd, 2nd or 3rd, and 7th void volume for PBS, PB, and PSS, respectively. For nonmotile zoosporas, less than 20 zoosporas/v were recovered from any void volume or medium. Large pores with rapid drainage probably account for differences in rate of movement of motile zoosporas in pine bark media compared to PSS. Encysted zoospores apparently were trapped in small soil pores in all media. Phytophthora root rot may not be as severe in pine bark media which allows rapid movement of zoospores through the medium.

A543
RELATIONSHIP BETWEEN DEVELOPMENT OF PHYTOPHTHORA ROOT ROT AND YIELD IN COMMERCIAL FIELDS OF PROCESSING TOMATO. D. Neher and J. M. Dunivay, Dept. of Plant Pathology, University of California, Davis, CA 95616.

Six-meter-long rows of tomato variety 6203 were planted adjacent to five other varieties in several fields with a history of Phytophthora root rot. Above-ground symptoms of disease developed on a phenological stage in all varieties and the final disease incidence and severity ranged from zero to the maximum possible. The harvestable fruit decreased linearly with increased symptom severity in eight plots of 6203 in one field, and all other varieties and sites fit the same relationship within 95% confidence limits. Phytophthora parasitica was not detected by preplant soil dilution plating nor was Ph. infestans detected on artificially inoculated plants, but was detected by a baiting method. Final disease severity was correlated positively with soil clay content and cation exchange capacity and negatively with soil sand content.

A544
EFFECT OF SOIL MATHIC POTENTIAL ON INFECTION OF PEPPER BY COLONISERS OF PHYTOPHTHORA CAPITI. M. J. Nord and J. B. Ristaino, Dept. of Plant Pathology, North Carolina State University, Raleigh, NC 27695.

Pepper seedlings were grown in microwaved soil infested with 50 oospores/g dry soil in tension funnels. Soil was
maintained at 0–25, -50 or -100 °C for 5 or 10 days prior to a 24 or 24 hr saturation period. Above ground symptoms were recorded and final disease incidence was determined on a selective medium 10 days after saturation. A saturation period was necessary for infection. Longer incubation prior to saturation resulted in more rapid disease progress and greater disease incidence. Final disease incidence after the 24-hr saturation was 48, 45 and 58% with the 5-day incubation period, and 85, 100 and 9% with the 10-day incubation period at -25, -50 and -100 mb, respectively. With constant saturation, 20% of the plants became infected. Infection of leaf discs during the saturation periods indicated indirect germination of zoospores occurred in all treatments.

A545
A FLUORESCENT PSEUDOMONAS spp. ASSOCIATED WITH A NEW LEAF BLIGHT AND BULB ROT OF VIDALIA ONIONS IN GEORGIA. R. G. Alston, R. A. Ray, R. B. Fox, J. K. Dickey. Dept. of Plant Pathology and Horticulture, University of Georgia, Coastal Plain Experiment Station, Tifton, GA 31793.

A Foliar blight of onion (Allium cepa) resulting in a wet rot at the base of the leaves and of the bulb to occur in November with the bulb was observed for the first time in Georgia. A fluorescent-pigmented bacterium similar to Pseudomonas viridiflava was consistently recovered from all samples. The bacterium was not isolated for oxidase and arginine dihydrolase, and variable for HS in tobacco. It rotted potato and carrot slices, and degraded sodium polyphosphate gel at pH 8.5 and in CVP but not at pH 5.0. However it slowly utilized sucrose and weakly produced levan. Inoculation of greenhouse-grown onion plants with the bacterium resulted in the reproduction of the same symptoms observed in the field and in the recovery of a fluorescent bacterium with the same traits. A peccotylic Xanthomonas spp. was also recovered from many but not all field specimens.

A546
SURVIVAL OF COLLETOTRICHUM COCCODES IN NEW YORK. Helen B. Biddle. Department of Plant Pathology, New York State Agricultural Experiment Station, Cornell University, Geneva, NY 14456.

Sclerotia of C. coccodes and colonized tomato skin (fruit) tissue were enclosed in paper card (155 mm mesh) and placed at 0, 10 and 20 cm depths in soil on 11/15/88. The pouches were located at 49 sites in a 23 m strip in a 0.4 ha field and flagged. At regular intervals, a pouch was removed from each depth and sclerotia and tomato skin pieces were assessed for viability of C. coccodes. After 460 days, 96, 96% and 96% of the 100, 96% and 96% of the skin pieces of 100, and 92% of the skin pieces of 100, 96% and 96% of the sclerotia were viable in the 0, 10, and 20 cm depths, respectively. In a separate experiment, sclerotia of C. coccodes and colonized tomato stem and skin tissue were placed in two field soil (16% moisture by weight) and incubated at 7, 16, 25, and 31 C. After 47 days incubation, survival of C. coccodes in skin and stem tissues and as sclerotia was >50% at all temperatures.

A547
WHITE MOLD (SCLEROTINIA SCLEROTIORUM) IN FLORIDA CABBAGE DURING 1989-90. D. P. Weinreich, ARRC, Hastings, FL 32145.

Studies of a white mold epidemic on a 140 ha commercial cabbage farm were initiated in mid-October, 1989. Ascosporic inoculum was present the entire season. Apothecia were found either in cover crop or cabbage fields from 8 November to April. They were most prevalent in December. White mold developed during mid-November to April in different sets of potted bean plants following 4–7 day exposures to field inoculum. The first sign of ascospore infection in cabbage was observed in 1 December. Incidence increased slowly in December followed by a rapid increase after the hard freeze of 23–26 December. The percentage disease in 2 fields increased from 1.2 to 16.5% in 1 December, 3.7 to 37.3% in 2 December, 37.3 to 100% in 1 January, 100% in 2 January, 0 in 10 January, and 12 January, respectively. Similar increases in white mold following the freeze occurred throughout Florida. The disease increase in 11 of 13 fields was due to ascospores whereas infection in 2 was mostly from aerial spores. The mechanism of the increase in white mold is unknown but may result from freeze injury and/or infection of carpogenic and/or mycelial germination of sclerotia.

A548

A new race (4) of Pseudomonas farinosa f. sp. spinaciae was identified in California and Texas. The differentials used for race identification included Viciafaya (susceptible to races 1,2,3), Norea (resistant to races 1 and 2), Callifay (resistant to races 1 and 3) and Polka and St. Helens (resistant to races 1,2,3). All of the differentials were susceptible to race 4. All five isolates recovered in California were identified as race 4. Of the four isolates recovered from the Winter Garden area of Texas, one isolate was identified as race 4 and the other three isolates as race 3. Replicated growth chamber inoculation tests on 26 commercial cultivars and five Arkansas breeding lines were carried out using a single isolate of race 4 from California and a single isolate of race 3 from Washington. Of the 26 cultivars tested, 17 have reported resistance to races 1,2,3, seven have reported resistance to races 1 and 2 and two have reported resistance to races 1 and 3. Two of the Arkansas breeding lines have reported polygenic resistance to race 3. All cultivars and breeding lines tested to date were susceptible to race 4. Plant introductions of spinach are also being evaluated; those tested thus far have proven to be susceptible.

A549
A PROGRAM OF TESTING NEW AND STANDARD CRYSTAL CULTIVARS FOR RESISTANCE TO YELLOW DISEASE INCITED BY Fusarium oxysporum f. sp. apii. A. S. Greathood, Farm Advisor Emeritus, University of California Cooperative Extension, Salinas, California 93908

Celery yellows caused by Fusarium oxysporum f. sp. apii, has resulted in serious losses to celery growers in all the major production areas of California. The release of the cultivar UC 1 by the University of California has provided the industry with a highly resistant but horticulturally unsatisfactory variety. A program of field testing of commercial and experimental cultivars in the Salinas Valley of California was developed in 1987 and has continued through 1989. Reliable ratings of the resistance levels of a number of lines have been developed. Two new cultivars - Matador and Starlet - have proved to be highly resistant to yellows and to be satisfactory horticulturally. Other lines, not yet available for commercial use are showing merit.

A550
PESTS OF CAPER. CAPRARIS SPINOSA - SOME NEW RECORDS FOR CALIFORNIA. D. G. Kontaxis, Cooperative Extension, University of California, 1700 Oak Park Blvd., Bldg. A-2, Pleasant Hill, CA 94523.

Caper, Capparis spinosa, a member of the Capræales order, grows well in the Mediterranean area, where it is commercially cultivated. Caper is used as a food condiment (herb) and vegetable, and also as an ornamental. The U.S. imports about $20 million worth of caper every year from Spain, Morocco, Israel and other countries. Caper is practically unknown in the United States. Caper plants were planted in 1988 and 1989 to study crop adaptability and pest disorders. In California the fungi, Botrytis sp. and Pythium sp., insects, Pieris rapae and Brachyrhautus sulcatius, attacked caper plants. These are new records for this host and pests in California.

A551
INTERACTIONS BETWEEN PURPLE BLOTCH AND ORANGE THrips ON BULB YIELD. Marvin E. Miller, Texas Agricultural Experiment Station, 2415 E. Highway 83, Weslaco, TX 78599 and Jonathan Edelson, Oklahoma State University, P.O. Box 128, Lane, OK 74555.

Interactions between purple blotch (Alternaria porri) and onion thrips (Thrips tabaci) on bulb yield were determined for 9 cvs. Texas Grano 1015 (TCA 1015) and Ben Shemen (BS). Purple blotch severity levels were maintained by weekly treatments of either iprodione at 1.2 lg (kg) ha, azinazine at 1.2 kg (ai) ha, mancozeb at 2.69 kg (ai) ha or no fungicide on plot. Number of thrips were maintained by either applying pyrethrins to plots at 0.11 kg (ai) ha when populations reached 0-5, 5-10, 10-25 thrips per plant or no insecticide. Thrips (BS) populations were significantly (p<0.1) affected by purple blotch severity levels but thrips populations affected yields only on BS. Severity of purple blotch was significantly higher at the highest thrips population level. There were no significant interactions between purple blotch and thrips on yield.
A553

DEVELOPMENT OF BLACK ROOT ROT (Thielaviopsis basicola) AS A POST-HARVEST DISEASE ON FRESH MARKET CARROTS AND STRATEGIES FOR DISEASE CONTROL. Zairk K. Punja, Centre for Pest Management, Dept. of Biological Sciences, Simon Fraser University, Burnaby, British Columbia, Canada V5A 1S6.

Black root rot, caused by Thielaviopsis basicola (Berk. and Br.) Ferr. (= Chaetaria elegans Nag Raj and Kendrick) was a severe post-harvest disease on fresh market carrots grown in muh soils in the Fraser Valley of B.C. in 1989. Symptoms appeared at retail outlets as black lesions on the root; the pathogen was most destructive when carrots were stored in polyethylene bags at temperatures above 18 C. Chemical dips did not significantly increase the effectiveness of the treatment for preventing disease. Roots were inoculated with T basicola 48-72 hr prior to, and immediately after, each treatment and incubated at 19-21 C for 7-10 days. Among five salts, calcium propionate (1% aqueous solution for 60-90 sec) reduced infection to less than 5% of the control (40% of root colonization) if applied within 48 hr post inoculation or immediately preceding inoculation. Potassium carbonate, sodium bicarbonate, sodium formaldehyde, and NaOCl (each at 0.1% concentration) providing decreased levels of disease control, respectively.

A555

INHERITANCE OF TOMATO SPOTTED WILT VIRUS (TSWV) RESISTANCE IN TOMATO. J. J. Cho, J. C. Watterson, C. Wyatt, and D. M. Custer, University of Hawaii, Maui Research, P. O. Box 269, Rula, HI 96790, and Petoseed Co., Inc., Rt 4, Box 1255, Woodland, CA 95695.

Inheritance of TSWV resistance have been tested in inbred resistant tomato lines from an L. esculentum X L. esculentum cross. TSWV inheritance appears to be controlled by a single dominant gene which is simply inherited. Plants of the F1 generation from crosses between susceptible and resistant parents exhibited local lesions, but the virus failed to develop systemically infected, revealing that resistance was dominant. F2 populations also responded with local lesions, however, only approximately 25% of the plants became systemically infected, indicating that resistance was monogenic. Plants of the F1 x resistant parent backcross segregated in the ratio of one systemic resistant to one susceptible (1:1) giving further confirmation to inheritance of a single dominant gene for TSWV resistance.

A556


Aerial application of fosetyl-Al is currently the most widely used method of controlling lettuce downy mildew in Florida. Five lettuce cultivars, escarole, and endive were tested for sensitivity to simulated aerial fosetyl-Al applications in a replicated field trial. A commercially-available formulation and a pH-buffered formulation were applied to foliage alone and in combination with a copper fungicide and TechnoM AG, a micronutrient product. Significant phytotoxicity was observed across all leaf types when the nonbuffered formulation was tankmixed with copper fungicide. Nonbuffered fosetyl-Al alone resulted in slight phytotoxicity to all leaf types except escarole. Nonbuffered fosetyl-Al and TechnoM AG tankmix applications resulted in only slight phytotoxicity on romaine and bib lettuce types. Use of the buffered fosetyl-Al formulation reduced phytotoxicity to near nondetectable levels in all combinations across all leaf types.

A557

FIRST REPORT OF RESISTANCE OF HELMINTHOSTORIUM SOLANI TO THIABENDAZOLE IN THE UNITED STATES. C. L. Merida and R. Loria, Department of Plant Pathology, Cornell University, Ithaca, NY 14853.

Helminthosporium solani was isolated from tubers with silver scurf collected from five locations in New York State. Eight single-spore isolates were grown on V8 agar amended with 1, 3.2, 10, 31.2 or 100 mg thiabendazole (TBZ)/L, and radial growth was measured after 20 and 40 days at 21 C. Two of the eight isolates tested grew at concentrations up to 31.2 mg TBZ/L. The remaining isolates did not grow at concentrations greater than 1 mg TBZ/L. These results were repeatable. Fungicide dose response curves were constructed for each of the resistant isolates. The TBZ concentration that reduced growth of the resistant isolates by 50% (25 mg TBZ/L) was utilized to screen 50 isolates that had been obtained from diseased potato tubers. Twelve isolates from three locations within New York State were resistant to TBZ. Average growth rate of six resistant isolates was 1.2 times larger than that of two sensitive isolates on unamended V8 agar after 45 days incubation at 21 C.

A558

VERMICAL VIRUCIDUAL ACTIVITY: IMPLICATIONS FOR MANAGEMENT OF PATHOGENIC BIOLOGICAL WASTES ON LAND. L. S. Amaravadi, H. S. Biesi, R. F. Bozarth, Indiana State University, IN-47809.

The earthworm Pheretima fetida, known to contain bactericidal enzymes, was tested for virucidal activity using CPMV and TMV as models agents. Earthworms were fed cellulose saturated with a virus suspension and their excreted castings were analyzed by ELISA and local lesion assays. Our results indicated a considerable reduction in infectivity of both viruses. Virucidal activity was also observed when virus suspensions were incubated with the earthworm enzyme extract. The observed reductions in the infectivity of both viruses suggest that P. fetida may possess a virucidal enzyme system and, accordingly, may contribute to the inactivation of pathogenic viruses potentially associated with land application of sewage sludges and livestock manures.

A559


Pinus taeda L. seedlings from 2 families differing in ozone sensitivity were exposed to 0, 10, 40, and 110 percent of ambient levels for 3 weeks. Auxin production was enhanced by acid rain treatments and increased root development. Enhanced root systems were isolated and inoculated with mycorrhizal spores. The mycorrhizae were separated into groups based on surface morphology. No difference was found in the presence of mycorrhizal root systems and individuals mycorrhizae cm. amon treatments. Total numbers of morphotypes increased with increasing O2, but numbers of mycorrhizae tips cm did not vary among treatments.

A560

OCCURRENCE AND EXPRESSION OF SORGHUM YELLOW BANDING VIRUS IN SOUTH TEXAS. G. N. Odvody, R.W. Tole, and J. Remmers, Texas A&M Exp. Station, Corpus Christi, TX 78410. *Texas A&M University, Department of Plant Pathology and Microbiology, College Station, TX 77843.

By 1989 Sorghum Yellow Banding Virus (SYBV) was observed in 1 county nonadjacent to and 7 counties contiguous with the initial site of observation near Floresville in 1984. SYBV occurred naturally only on sorghum x sudan grass
hybrids except for one occurrence on grain sorghum tillers. No SYBV was observed on primary growth and initial or highest incidence was generally observed at field perimeters on basal rather than stem tillers. In an irrigated field, incidence of SYBV increased with each of two ratoon crops (1% and 10-30%). The slow recovery single ratoon crop of several dryland fields had a higher incidence of SYBV (10-25%) than the comparable irrigated ratoon crop. SYBV occurred in the third and subsequent ratoon crops of sorghum x sudan in greenhouse tests using soil from an SYBV-affected field but not another field soil. Aphids did not transmit the virus and vectors are yet unknown but results indicate a soilborne mechanism for SYBV.

A565
EFFECT OF CULTIVAR RESISTANCE AND INOCULUM POSITION ON ROOT INFECTION AND SYMPTOMS CAUSED BY PHYTOPHthora PARASITICA VAR. NICOTIANAE ON NICOTIANA TABACUM. K. T. Jones and D. G. Shew, Department of Plant Pathology, NCSU, Raleigh NC 27696-7616.

The effect of resistance of four cultivars of N. tabacum on above ground symptom (AGS) expression and root infection was quantified in the field at two sites for 2 yr. Seedlings were grown using standard cultural practices for 40 days. Inoculations were made by placing 25ml of naturally-infected soil followed by 100ml of water at 7.5 or 15 cm from the stem and 7.5 or 15 cm deep in the soil. The raised row was then covered with polyethylene to prevent flooding during rainfall. Susceptible cultivars had higher levels of AGS than more resistant cultivars. Inoculation close to the stem resulted in more rapid and greater development of AGS across all cultivars. Shallow inoculation of adventitious roots that arose from buried stems resulted in more rapid and greater development of AGS than deep inoculation of true roots. The more resistant cultivars had a lower percent infection and a lower percentage of the infections resulted in AGS.

A566

The movement of genetically modified bacteria from the site of application presents a risk to non-target organisms. To determine the extent of this movement, corn seeds were inoculated with a lac ZY modified rhizosphere inhabiting bacteria, Pseudomonas aureofaciens (L-11), and the stems, leaves, and gnutation drops were tested for 21 days. L-11 attained its maximum population size (1.1 X 109 CFU/gram) in stem 4 fresh wt on day 5 inoculation and then declined to its minimum at day 21 (1.9 X 103 CFU/g). However, the population of L-11 in the leaves declined from a maximum (1.9 X 103 CFU/g) on day 5 to be detectable in only 42% of the plants in subsequent samples. L-11 was detectable in the gnutation drops (5.5 X 103 CFU/ml) from the emergence of the corn shoot until day 6. The major source of inoculum for the foliar tissue was the contamination of the shoot as it moved through the soil and the subsequent ingress of the bacteria into the interior through stomata and hydathodes.

A567
SURVIVAL OF BINECULATE RHIZOCTONIA Fungi IN FIELDS SOIL AND BEAN STEMS UNDER FIELD CONDITIONS. M.A. Cubeta and E. Echandi, Dept. of Plant Pathology, North Carolina State University, Raleigh, NC 27695.

Survival of bineculate Rhizoctonia fungi (CG322 and J3N1, (AGA) in soil and on bean stems was studied. CG322 and J3N1 were buried at 15 and 30 cm deep in Clayton (CL) and Raleigh (RA) soil and sampled monthly for 1 yr. Populations of CG322 and J3N1 increased 73 and 40%, respectively, 1 month (mo) after burial at 1 cm in RA soil. Neither isolates were recovered 4 mo after burial at 30 cm in CL soil. Both isolates were recovered 2 and 7 mo after burial at 7 and 15 cm, respectively. In CL soil, CG322 was increased 29 and 54%, respectively, 1 mo after burial at 1 cm. After 9 mo in CL soil, neither isolate was detected at 1, 15, or 30 cm. In precolonized bean stems, CG322 and J3N1 decreased after burial at 1 cm but increased at 15 and 30 cm. In RA soil, both isolates initially increased after 1 mo in soil, but did not survive more than 9 mo in soil and 11 mo in bean stems.

A568
EFFECT OF SOIL pH ON GERMINABILITY AND VIRULENCE OF, AND LOSS OF ENDOGENOUS CARBON FROM, COCHLIOBOLUS SATIVUS CONIDIA. K. Hyakumachi1, N. Suzuki1, H. Ikeyama2, and J. L. Lockwood, 1Gifu University, Gifu 501-11, Japan and 2Department of Botany & Plant Pathology, Michigan State University, East Lansing, MI 48824.

Cochliobolus sativus conidia were exposed for 30 days to three different soils adjusted with H2SO4, (NH4)2SO4, KOH or K2CO3 to achieve a range of pH’s from 5-9. Conidial germination was not depressed on soils regardless of the pH, but germination occurred when soils were supplemented with glucose. A larger amount of glucose was required to obtain equal germination rates on soils of higher than of lower pH. The ability of conidia to germinate in soils solution without an exogenous energy source was rapidly lost during exposure to soils of higher pH. Virulence also declined more rapidly on soils of higher pH. Conidia lost more exogenous carbon during exposure of 1-Labelled conidia to soil at pH 9 than at pH 5. Thus, soils with a high pH were more fungistatic and imposed a greater energy stress on conidia than soils with a lower pH.

1028 PHYTOPATHOLOGY
A569
SUPPRESSIVENESS AND CONDUCTIVENESS OF SOIL TO RHIZOTOCIA SOLANI AFTER CONSECUTIVE CROPPING. M. E. de la Fuente and C. A. Martinson. Iowa State University, Dept. Plant Pathology, Ames, IA, 50011.
Soil was infested or not infested with R. solani AG-4, planted with radish (host), cucumber (host), wheat (nonhost), or not planted, and then replanted (or not) for six cycles. One unplanted treatment was reinfested with R. solani each cycle. Then all soils became highly suppressive to R. solani except the soil never cropped and not infested originally. Soil conductiveness was measured by the linear development of disease in radish planted radially around a R. solani pellet, and by weighing the soil aggregates formed by its hyphae radiating from the pellet after 8 days. The latter procedure was the most definitive. Adding R. solani inoculum to the soil each cycle with no cropping resulted in the least conducive soil; the most conducive one was never cropped nor infested with the pathogen. Infestation with R. solani resulted in less conduciveness than with no inoculation, and conduciveness was greater with wheat and cucumber cropping and less with radish cropping.

A570
GENOTYPIC REACTION OF WHEAT TO INFECTION BY PYTHIUM ARRHENOMENES, OR EXPOSURE TO ITS TOXIC METABOLITES. H. Nojiri, and L. L. Singleton. Dept. of Plant Pathology, Oklahoma State University, Stillwater, OK 74078.
Seven wheat genotypes, some of which were earlier reported to be resistant to P. arrhenomenes (PA), and the variety TAM-101 were inoculated by adding 2-day-old seedlings on the edge of a PA culture for 3 h. Infected seedlings were transferred into test tubes (18 mm dia.) with glass beads and 1 ml of sterile water, and incubated for 4 days at 25 C. For toxic metabolite study, non-inoculated seedlings were placed in test tubes with 1 ml of a 25-day-old culture filtrate of PA. In all genotypes, significant reduction in root, and shoot growth was obtained by infection, or exposure to toxic metabolites. However, TAM-101 had significantly longer root length, and greater fresh and dry root weights than all other genotypes except one. Root or shoot growth of TAM-101 was not different from most other genotypes after exposure to toxic metabolites.

A571
PECTIC ZYMOSOGRAM CHARACTERISATION GIVES MORE INFORMATION THAN ANASTOMOSIS GROUPING OF STAINS OF RHIZOTOCIA SOLANI WITHIN BARE PATCHES OF CEREALS IN WESTERN AUSTRALIA. G. C. Mac Nish and H. W. Sweetingham, Department of Agriculture, Esperance, 6450, Australia.
The Rhizoctonia spp. in a large rhizoctonia bare patch that stretched across four plots of alternating wheat and barley was studied. Wheat seedlings were grown in undisturbed soil cores removed from the patch over a 12 month period. K. spp. were isolated from the wheat roots and characterised using electrophoresis in pectin acrylamide gels. The zymogram patterns demonstrated that the patch was in fact a coalescing of two patches dominated by two strains (ZG1-1 or ZG2). These strains appeared not to mix, but maintained a distinct (but invisible to the naked eye) demarcation over the 12 month period. As both ZG1-1 and ZG2 belong to the same anastomosis group (AG8), the use of the latter method of identification would have failed to demonstrate the above phenomenon. Pathogenicity tests showed that the virulence of ZG1-1 and ZG2 is similar on wheat but differs on lupin.

A572
FUSARIUM ON SPRING WHEAT UNDER CONVENTIONAL AND REDUCED TILLAGE. B. Salas and R. W. Stack. Dept. Plant Pathology, North Dakota State University, Fargo, ND 58105.
The influence of conventional and reduced tillage on Fusarium infection of spring wheat was studied by quantitative isolation from roots and crowns of plants grown in field trials. Of twenty-two Fusarium species identified, just six accounted for over 90% of the 5500 cultures isolated in 1983 and 1989. F. equiseti was the most frequently recovered (33.4%) and was the only species which was found more often on plants grown under conventional tillage. The cereal root rot pathogens F.avenaceum, F. culmorum and F. graminearum together accounted for 21.9% of cultures. Each of these as well as F. acuminatum was isolated at twice the frequency from plants growing under reduced tillage as under those from under conventional tillage. Isolation of F. oxysporum, the second most common species, was not affected by tillage.

A573
Eggplants were grown in two fields that differed in initial inoculum densities of Verticillium dathialae. A 2 x 2 factorial design of black plastic (P) or bare ground with or without nitrogen sidedress (100 kg N/ha) at 65 days after planting (DAP) was studied for disease and yield response. Four replicated treatments were blocked twice in each field. Disease incidence, severity and yield were recorded during the season. At final harvest (DAP 95), plants were weighed, and stem and root colonization by V. dathialae was determined by placing tissue on selective media. The P + N, and P treatments increased yields, but did not affect incidence, severity or host colonization. Inasmuch as these treatments did not reduce symptom expression or host colonization, their ability to increase yield was apparently through increasing plant tolerance.

A574
COMPUTER ASSISTED STUDIES OF SOIL-BORNE FUNGI IN THE RHIZOSPHERE. H. T. Wilkinson and W. L. Pedersen. Dept. of Plant Pathology, Univ. of Illinois, Urbana, IL 61801.
A program designed to teach students about root pathogenesis was developed using wheat and the soil-borne pathogens, Geosminomyces graminis var. tritici and Magnaporthe poae. The study program integrates a literature review, a lecture, a wet laboratory experiment, analysis of data, and the generation of a rhizosphere model. Data generated in the lab experiment are also used to construct an existing data base, which is expanded by each group of experimenters. The computer software presents a menu driven program that the student can use to investigate the behavior of these pathogens in the rhizosphere. The student will select any combination of parameters listed and the computer calculates the maximum estimated distance from the root that a propagule can produce an infection. The program will also construct this rhizosphere cylinder in both two and three dimensions. The program is written in "C", a computer language, and operates in the Microsoft Windows environment.

A576
INFLUENCE OF TWO SOYBEAN CULTIVARS ON SOIL POPULATION DENSITIES OF MACROPHOMINA PHASEOLINA. S. R. Rondig and J. C. Rupe, University of Arkansas, Fayetteville, Arkansas 72701.
Soils were assayed for microsclerotia (ms) of Macrophomina phaseolina during the 1988 growing season. The cultivars, Davis and Lloyd, were planted and grown under the various irrigation regimes: no irrigation, full-season, until flowering, and after flowering. Ms soil densities decreased during the growing season with no differences between irrigation regime or cultivar. Although prevalent populations the following year were not influenced by irrigation, ms densities were significantly higher in plots planted to Davis (92 ms/3 g of soil) than those planted to Lloyd (60 ms/3 g of soil). Soybean cultivars did not influence ms soil densities during the growing season, but the cultivar Davis did contribute to a greater population of M phaseolina for the following growing season.

A577
GERMINATION OF PHYTOPHTHORA CAPSICII OOSPIRES IN VITRO. M. J. Lord and J. B. Kisitano, Department of Plant Pathology, North Carolina State University, Raleigh, NC 27695-7616.
Cospores were obtained by crossing opposite mating types of the heterothallic fungus *Phytophthora capsici* on clarified V8 juice agar and incubating in the dark at 24°C for approximately 60 days. Cospores were collected by centrifugation and treated with enzyme to remove cytoplasm. A droplet of cospore suspension was placed on sterile distilled water (SDW), root extract or soil extract in petri plates. Germination was microscopically examined directly in the petri plates. Cospores germinated between 16°C and 72 h, with maximal germination at 24°C. Germination of cospores produced in the dark was 18%, whereas germination of cospores produced in the light was 24%. Germination after 12 days in SDW, soil extract and root extract was 24%, 36 and 39%, respectively. Cospores incubated in soil extract produced germ tubes with apomaria, while those incubated in root extract germinated to form long germ tubes with small or no apomaria.

**A578**

THE INFLUENCE OF SOIL pH ON THE ECOLOGY OF *P. aphanidermatum* AND *P. ultimum*. Frank N. Martin and C.R. Sencer, Plant Pathology Department, University of Florida, Gainesville, Fla. 32611.

The influence of soil pH on the ecology of *P. aphanidermatum* and *P. ultimum* was investigated in autoclaved and natural field soils using a model system which evaluated the saprophytic activity of the isolates. Soil pH was adjusted from 3.5 to 8.5 and allowed to equilibrate for 10 days prior to use. All tests were conducted with soil maintained at 0.1 bars. The saprophytic activity of *P. aphanidermatum* was minimal at pH 3.5, increased to maximum at pH 5.5, and decreased slightly thereafter. Results were similar for *P. ultimum* with the exception that there was a significant decrease in saprophytic activity as the soil pH was increased from pH 7.5 to pH 8.5. Both species behaved similarly in autoclaved and natural field soils, indicating that the influence of soil pH was a direct effect on the fungus and not an indirect effect mediated by other soil microflora.

**A579**


Two software shells were used to demonstrate how an inductive reasoning expert system and a neural network could be used to build a knowledge base and to identify logic in pest risk assessments (PRAs) that are defined here as the scientific estimation of the likelihood of introduction and magnitude of the effects of establishment of an exotic pest. An example from USDA-APHIS-PPO was used to demonstrate how PRAs could be performed for certain insects. Both systems were formulated by formulating questions that mimicked the general assessment logic as much as possible. An expert familiar with the data was involved in the development of the knowledge base. The neural network was the preferred tool of the expert, but may not be directly applicable for a regulatory agency due to the "lack of transparency" of the computer program. Applications in PRA for plant pathogens are suggested.

**A580**

SPATIAL DISTRIBUTIONS OF *Pseudomonas syringae* STRAINS ON POTATO LEAVES. L. L. Kingsley and D. E. Lindow, Department of Plant Pathology, University of California, Berkeley, CA 94720.

The spatial relationships of bacteria coexisting on leaves are not well described. We investigated the spatial patterns of *Pseudomonas syringae* strains inoculated singly or in pairs onto potato leaves. Plants were maintained under alternating wet and dry conditions and leaf sampling was taken 24-72 h after inoculation. Entire leaves were sectioned into 0.09 cm² quadrats and population sizes of individual bacterial strains were counted for each quadrat. Population sizes on single quadrats ranged from 0 to 1500 individuals. Bacteria were aggregated on the leaf surface (variance/mean >> 1). Quadrats located along the central vein tended to have the largest population sizes. Spatial patterns were not consistent in the spatial distribution of strains when inoculated onto leaves singly or in pairs. More sophisticated approaches to describing the spatial patterns of bacteria on leaves in relation to specific microsites will be suggested.

**A581**

EVALUATION OF THE IOWA POT TEST FOR FOLIAR FUNGICIDES ON SOYBEANS. Douglas J. Jardine, Department of Plant Pathology, Kansas State University, Manhattan, KS 66506-5502.

The Iowa pot test to determine the need for foliar fungicide sprays in improving seed quality was tested in Kansas. Pods were harvested and evaluated for presence of *Pseudomonas* at corn growth stage R6. In 1987 and 1988, percent pod infection was 50, 14, and 50, respectively. Accordingly, spraying would have been required in 1987 and 1988. After harvest, seeds were evaluated for the presence of *Pseudomonas*. In 1987 and 1988, there were no significant differences in the level of pathogens present on seeds from the untreated or benomyl sprayed plants. In 1989, pathogen populations in seeds from benomyl sprayed plants, but Alternaria and Fusarium significantly increased. Fungicide treatment did not improve germination in 1987 and 1989 when a fungicide treatment was called for. Germination in 1988 was significantly increased in 1988 when no spray was predicted. The model did not reliably predict the need for foliar fungicides in Kansas.

**A585**

ASCORC PRODUCTION BY *PYRYPHORA TRITICI-REPENTIS* IN WHEAT STRAW UNDER INTERMITTENT WETTING. W. Zhang, and W. Pfender, Dept. Plant Pathology, Kansas State Univ., Manhattan, KS 66506.
The effect of wetting duration on ascospore production by *Puccinia graminis* (Pth) in wheat straw was studied using two types of straw: sterilized, then re-colonized with *Pth* and field straw (field-grown, naturally-infested with *Pth* and saprophytic microorganisms). Both types of straw were exposed to three regimes of alternating moisture and desiccation, in which the repeated wetting period was 5, 10, or 36 hr, respectively. Ascocarps developed in all 3 wetting treatments. However, in the 36-hr treatment, ascocarps formed earlier than in the 5-hr treatment (Table 1). 5-hr wetting cycle was earlier for small and large ascocarps, respectively; the 10-hr treatment was intermediate. Ascocarp production in field straw was more variable than that in axenic straw, and differences between 36-hr and 5-hr treatments were accentuated. Ascocarp formation was correlated with wetting-cycle duration. The relationship of these data to field observations of straw wetness duration will be discussed.

**A586**

**Influence of surface corn residues on disease gradients of gray leaf spot of corn.** L. M. de Nazareno, P. E. Lippins and L. V. Madden, Dept. of Plant Pathology, The Ohio State University and Ohio Agricultural Res. and Dev. Center, Wooster, OH 44691.

Spread of corn gray leaf spot (GLS), caused by *Cercospora zeae-maydis* Teh. and Dan., was studied at Dresden and Columbus, Ohio. Dresden had a history of GLS epidemics and Columbus had no previous record of GLS. Square blocks (900 m²) of two hybrids (Pioneer Brand 3352 and LHI 29 x LH51) were planted on May 18, 1979. Corn residues were removed from the surface of a field that had GLS the previous growing season and spread on the surface of a 9.3 m² area in the center of each block when plants were at fifth leaf stage. Disease spread was assessed weekly from appearance of first lesions within and across rows from the edge of the lesion. At Dresden, a steep disease gradient was observed extending from the inoculum source out 3 m, with more pronounced spread down than across rows. However, at Dresden, no definite gradient was detected, presumably due to the presence of other sources of inoculum in addition to the inoculated corn residue placed in the center of the blocks.

**A587**

**Effect of surface topography and rain intensity on rain splash dispersal of *Colletotrichum acutatum* of strawberry.** X. Yang, L. V. Madden, L. L. Wilson, and M. A. Ellis, Dept. of Plant Pathology, Ohio State Univ., Wooster, OH 44691.

Effects of ground cover (straw, soil, and plastic), leaf area index (0, 2.7, and 4.9) of a strawberry row canopy, and rain intensity (15 and 30 mm/hr) on rain splash dispersal of *Colletotrichum acutatum* were studied, using a rain simulator. Dispersal was measured by collecting droplets containing conidia in gravity samplers positioned at various distances from the source at selected times. Ground cover had a major effect, as measured by plastic. The following order of dispersal was observed: the finest cover, plastic the most, and soil intermediate. Differences in covers were due to differences in steepness of gradients (straw/stripe the steepest), not release rate from the source. Colleotrichum increased with rain intensity; this was due to the release rate, as measured by the intercept parameter of a gradient model, not gradient steeperness. Leaf area index was inversely related to colony number.

**A588**


Ascospores of *A. anomala*, the cause of eastern filbert blight, were trapped in a hazelnut orchard from November 1980 to June 1981. In 1981, rain collectors were placed under cankers in ten trees. Collector reservoirs were changed weekly and spore discharge per canker per week was calculated by counting spores within subsamples of the rain water. Precipitation was monitored on an hourly interval. Samples from November accumulated for 70% of the total number of spores trapped. This month had 177 hr of precipitation. Ascospores were trapped in each week with rain from December to April. Few spores were trapped in May after a cumulative 660 hr of rain for the sampling period. In a growth chamber, spore discharge from cankers ceased after 720 hr of intermittent mist at 35 C. Additional growth chamber and field data were collected in 1989-1990.

**A589**

**Effect of endophyte infection of perennial ryegrass on growth under drought stress.** M. L. Gleason, N. E. Christians, and M. Agnew, Departments of Plant Pathology and Horticulture, Iowa State University, Ames, IA 50011.

Reports that infection by a fungal endophyte, *Acremonium lolii*, improves tolerance of perennial ryegrass to drought stress were evaluated in greenhouse experiments. Endophyte was eliminated by growing tillers in sand amended with 200 ppm benomyl. Sanecolonic plants with and without endophyte were grown in field soil amended with 5 g/l 14-14-14 fertilizer. Pots were weighed daily and watered to saturation when pot weights corresponded to soil water potentials of -0.4, -2.0, or -15 bars. After 7 wk in one trial, shoot and root dry weights of endophyte-free plants were significantly greater than for endophyte-infected plants at all watering treatments. In two other trials, endophyte infection had no significant effect on growth at any watering treatment.

**A590**

**The effect of temperature on ascospore release, germination and infection by uncincula necator on grape.** C. S. Thompson, W. D. Guibler, and D. Pogue. Department of Plant Pathology, University of California, Davis, CA 95616.

Mature cleistothecia of *Uncinula necator* (Sowh.) Burks were collected from a commercial chardonnay vineyard (*Vitis vinifera* L.) in Monterey Co., California. The number of ascospores released was determined on water agar at seven temperatures from 5°C to 30°C. Ascospore release, germination and infection were determined at five temperatures from 10 to 30°C on Caragana grape leaves. The greatest number of spores were released at 15 and 20°C. Of the ascospores that were released at each temperature, a significantly greater percentage germinated at 15 and 20°C. Infection occurred throughout the temperature range tested (10-30°C) but was greatly reduced at 30°C.

**A591**

**EPIMODEL - A COMPUTER PROGRAM TO TEACH PRINCIPLES OF MODELLING PATHOGEN POPULATION GROWTH AND ANALYSIS.** P. W. Nutter, Jr. and O. Worawilkit. Department of Plant Pathology, University of Georgia, Athens, GA 30602.

EPIMODEL was developed for the purpose of teaching students principles of pathogen population growth modeling. Example data sets are available within the program to demonstrate the application of several growth models (monomode, exponential, logistic, gompertz). EPIMODEL can also be used to help students select the most appropriate growth model for temporal disease assessment data sets. The program is menu-driven and allows easy entry of actual data sets for study. The program will produce graphs of the transformed data vs time, dy/dt (rate) vs time, the transformed data vs time, and the residuals vs time. Regression statistics and parameters are also displayed for each growth model and hard copies of all data and graphs can be produced.

**A592**

**Time-sequenced inoculation indicates a susceptibility rhythm in soybean blight disease.** B. W. Kennedy and R. Denny, Department of Plant Pathology, University of Minnesota, MN 55108.

Glycine max L. cv. balsam were grown in controlled environments at constant 25°C, 85 ± 5 percent RH and a 12:12 h light (L) dark (D) regime in which 12 h darkness followed 12 h of light. One primary leaf off of a subset of 7-day-old seedlings was inoculated every four h for 20 h with buffered extracts of fluid from soybean plants infected with tobacco streak virus. Leaf movements were recorded by measuring leaf angles every four h for 96 h and data were subjected to curve-fitting procedures to detect relationships between leaf movement and symptom development (epinasty of growing tips and inoculated leaves). Leaf angles from plants during the first 10 cycle following inoculation (day 1) were subtracted from those in the second (day 2) and the second from the third. Amplitude of leaf angle differences from day 1 to day 2 and from day 2 to day 3, were statistically analyzed by t-tests; there were no differences in cadian timing of the change but differences within the first day after inoculation predicted symptoms to come.

**A593**

**Effective vesicular-arbuscular (VA) mycorrhizal fungi for Pueraria phaseoloides grow in a high-aluminum acid soil.** H. T. Bartolome and C. C. Schenck. Plant Pathology Department, University of Florida, Gainesville, FL 32611.

Thirteen isolates from the International Culture Collection of VA Mycorrhizal Fungi (INVM) were screened for effectiveness in improving the growth and nodulation of *Pueraria phaseoloides*.
A594
CHARACTERIZATION OF MYCORRHIZOPLANT-ASSOCIATED STREPTOMYCETE EFFECTS ON ECTOMYCORRHIZAL FUNGI. J.M. Patechow, S.T. Bagley, and J.N. Brunn. Michigan Technological University, Houghton, MI 49931
Up to 25 different streptomycete morphotypes were isolated by enrichment technique from the mycorrhizosphere of red pine seedlings during monthly sampling over a 3 year period. Morphotypes were screened for effects on three red pine ectomycorrhizal fungi, Laccaria bicolor, L. laccata, and Thelephora terrestris. Six morphotypes showed the desired pattern of promoting growth of Laccaria spp. but inhibiting T. terrestris. The effects of growth medium (MMN, nutrient, and yeast extract), temperature (25, 30, and 35°C), pH (5.5, 6.0, 6.5, 7.0, 7.5), and shaking vs. static incubation on biomass and fungal effects were determined. Fungal growth effects were measured by incorporating sterile spiked (100 mg/l) into MMN agar. Optimal conditions for streptomycete biomass and fungal effects are compound production for all six morphotypes were obtained with MMN broth at pH 7.0 and 30°C with static incubation. Peak effects were seen with streptomycin filter. Studies are continuing with the two morphotypes causing the greatest responses to determine the type of compound(s) produced.

A595
COLONIZATION OF RESISTANT AND SUSCEPTIBLE CORK ROOT LETTUCE CULTIVARS BY GLOMUS INTRARADICIS, I.E. Datnoff, R.T. Nagata, Univ. of FL-ERE, Belle Glade and T.E. Wood, NPI, Salt Lake City, UT
Cork root caused by Rhizomaras suberificans is a disease of lettuce (Lactuca sativa L.) grown in south Florida. Vascular-arbuscular mycorrhizae (VAM) could be used as a biocontrol agent and affect cork root development. VAM colonization of resistant (R) and susceptible (S) lettuce cultivars to cork root were investigated. 'Shamnee', S, and 'South Bay', R, crisp-head lettuce cultivars were inoculated with Nutri-Link, a VAM inoculant containing spores of G. intraradices. Seeds were planted in a potting mix and amended with 500 spores per cell. Plants were fertilized with a 20-20-20 liquid formulation. After 28 days, plants were observed for colonization and effects on dry weight. Generally no differences were noted for percent colonization between cultivars or dry weight between colonized or non-colonized plants.

A596
RELATEDNESS STUDIES ON PISOLITHUS TINCTORIUS FROM NORTH AMERICA, ASIA, AND EUROPE. J.B. Sprenger, R.A. Taber, and R.F. Pettit, Department of Plant Pathology and Microbiology, Texas A&M University, College Station, Texas 77843-2132.
Isolates of the ectomycorrhizal gasteromycete Pisolithus tinctorius from 15 different geographical regions throughout the world were used in this study. The fungi were grown on synthetic medium at 22°C for 21 days. Their buffalo proteins were electrophoretically compared by microprocessor-controlled NATIVE-PAGE, SDS-PAGE, IEF-PAGE, and two-dimensional electrophoresis. Twenty five proteins were resolved from the gasteropodium of the fungus under the presence of SDS (MW range 14,100-72,400 daltons) and 40 proteins using isoelectrofocusing (pl range 3.60-7.45). Isolates from Texas and Georgia had identical SDS and IEF electrophoretic patterns. The SDS and IEF patterns of the Philippine isolate were different than U.S. or European isolates; SDS gels revealed the Philippine isolate lacked 2 major and 2 minor polypeptides, and IEF revealed it lacked 14 components found in the U.S. isolates. Other isolates also showed differences in banding patterns. There were also differences among the isolates in activities and banding patterns of certain isoamines. General native protein, polypeptide, and isozyme patterns of all isolates investigated were similar, with slight differences in these patterns allowed to distinguish certain isolates.

A597
Virus-like Particles in Plasmopara halstedii, Sunflower Downy Mildew. T.J. Gulya, T.P. Freeman, and D.E. Mayhew. USDA Northern Crop Science Lab; NDSU Electronic Microscope Lab, Fargo, North Dakota 58105; and Analysis & Identification Lab, CDSA, Sacramento, California 95814.
Virus-like particles (VLP) were observed in high-titer in hypaea, haustoria, and zoosporangia of Plasmopara halstedii race 2. The particles were incapsulated and measured 26 nm when stained with phosphotungstic acid in leaf discs or 24.3 nm in sectioned material. Four species of double stranded RNA with approximate molecular weights of 3.08, 1.28, 1.76 and 0.36 were found in VLP by gel filtration on Sephadex G-50. P. halstedii. No ds-RNA was found in healthy sunflower tissue. This is the first report of VLPs in Plasmopara spp. All isolates of race 2 examined contained VLPs. Attempts at mechanical transmission using triturated zoosporangia failed to yield any symptoms on any indicator hosts.

A598
Three stable, single component viruses, southern bean mosaic (SBMV), bean mild mosaic (BMMV), and blackgram motte (BMV) were transmitted with different efficiencies by Mexican bean beetles using Phaseolus vulgaris 'Black Valentine' bean as the acquisition and test host (SBMV = 95%, BMMV = 41%, BMV = 21%). When equal concentrations of purified viruses were inoculated by beetles or by gross wounding, SBMV > BMMV > BMV. Translocation in cut stems and indirect immunofluorescence analysis of the three purified viruses in gross-wounded primary leaves demonstrated rapid movement in vascular tissue. Comparisons of viral infectivities using mechanical inoculation demonstrated that Black Valentine bean is less susceptible to BMV. Furthermore, BMV and BMMV concentrations are lower than SBMV in infected bean. Thus, reduced transmission rates result from lower host susceptibility and virus titer in the acquisition host.

A599
BIOCHEMICAL ALTERATIONS ASSOCIATED WITH BEAN POD MOTTLE VIRUS (BPMV) PATHOGENESIS OF PHASEOLUS VULGARIS (BEAN) CV. PINTO. P. L. Pughan, A. Novacky, and O. P. Sehgal. 108 Waters Hall, Dept. of Plant Pathology, University of Missouri, Columbia, Mo. 65211.
Active oxygen production, lipid peroxidation, electrolyte leakage, cell death, and necrosis are characteristics of a typical hypersensitive reaction (HR). BPMV does not induce necrotic lesions on Pinto, but an active host resistance mechanism that limits viral spread exists. Lesions caused by BPMV are in the form of discolored areas of the minor vascular bundles in the infection area. Superoxide production has been demonstrated during BPMV pathogenesis of Pinto, but lipid peroxidation has not been detected. The percentage of dead cells is higher in BPMV-infected tissues than in the controls. In a typical viral induced HR, however, all cells within a necrotic lesion are dead. Physiological experiments demonstrate that the electrical potential across the plasmalemma is reduced upon BPMV infection. The reaction of Pinto to BPMV infection does not completely parallel events involved in HR; however, some similarities exist.

A600
RELATIONSHIPS AMONG SEROTYPES OF COKEEA SEVERE MOSAIC VIRUS AS DETERMINED BY SIGNATURE ANALYSIS. B. Di, J. H. Hill, and R. A. Van Denusen, Dept. of Plant Pathology, Iowa State University, Ames, IA 50011.
Isolates of coepea severe mosaic virus (CSMV) can be grouped into nine serotypes (I to IX) by immunodiffusion tests using polyclonal antibodies (Lin et al., 1981, 1984). Several common and serotype-specific antigenic determinants have been identified. In this study, the nine serotypes were compared using "signature analysis". Radioimmunoassays, using seven monoclonal antibodies (MAbs) that recognize CSMV serotype-specific or cross reactive epitopes, were used to construct antigenic signatures of each of the nine serotypes. A statistical (iterative least-squares) method was used to align unknown CSMV antigen concentrations from different virus preparations to allow comparison of binding profiles from different assays. The technique could identify antigenic divergence among different serotypes of the virus.

A601
FRAMESHIFT IN TRANSLATION OF BYDV RNA. B. Di, W. A. Miller, Dept. of Plant Pathology, Iowa State University, Ames, IA 50011.
Based on the genome organization of BYDV, the polymerase gene (60K ORF) was proposed to be translated via a frameshift event
in the overlapping region of the 39K and 60K ORFs. In vitro translation of BDV (PAV serotype) genomic RNA gives products consistent with the translational frameshifting, a major event in this virus. The frameshifting event has been demonstrated as a tandemly integrated monocistronic RNA with an internal ribosome entry site.

Distinctive aggregates of potyviral cylindrical inclusion protein (CI) molecules are found in the cytoplasm of infected cells. There have been many reports of the development of cylindrical inclusions in cells of infected leaf tissue, but none to our knowledge of their development in isolated protoplasts. Tobacco mesophyll protoplasts were inoculated with tobacco vein mosaic virus (TVM) and monitored at various times p.i. Cylindrical inclusions in the form of bundles and pinwheels were observed in protoplast sections as early as 15 h p.i. When sections were subjected to immunogold labeling using anti-CI serum and gold-conjugated Protein A, cylindrical inclusions were detected as early as 10 h p.i. Cylindrical inclusions were either associated with the plasma membrane or observed in the cytoplasm (with no apparent membrane association). There was an increase in the number of cylindrical inclusions over time (15-30 h p.i.). The rate of increase in the number of cytoplasm-associated cylindrical inclusions appeared to be greater than that observed for cylindrical inclusions associated with the plasma membrane.

Host tobacco protoplasts were infected with sonchus yellow net virus (SYNV), a plant pathogen, and analysed for viral protein and RNA synthesis. Transcription of SYNV mRNA was detected within 2 h post-inoculation (PI), reached maximal levels by 24 h and declined to undetectable levels by 60 h. Expression of the coat protein was downregulated. Viral RNA was evident between 24 and 36 h, but decreased appreciably by 60 h PI. Synthesis of the major viral structural proteins was detected by western analyses within 24 h PI and these proteins achieved maximal synthesis by 72 h PI. Cylindrical inclusions accumulation with a switch from a transcriptional to replicative phase parallels some animal rhabdovirus systems. Among various glycosylation inhibitors applied to protoplasts, only tunicamycin effectively inhibited viral protein synthesis, resulting in synthesis of a G protein about 10% smaller than in untreated protoplasts. Two specific cleavage products of the nucleocapsid (N) protein with molecular weights of about 22 kDa and 37 kDa and 37,000 appeared in cells by 60 h PI, but in the absence of tunicamycin, the cleavage products were present by 38 h PI. It may be possible that this specific cleavage of the N protein, activated in cells stressed by viral accumulation and/or tunicamycin treatment, accounts for the loss of virions and nucleocapsids during the chronic stage of SYNV plant infections.

Bean yellow mosaic virus (BYMV)-specific, BYMV subgroup-specific and potyvirus group cross-reactive monoclonal antibodies (McAbs) were evaluated with respect to their ability to detect and differentiate potyviruses. Different extraction buffers, antigen preparation regimes, solid phase coating conditions and ELISA protocols were used to evaluate the McAbs. All McAbs detect viral antigens in antigen-coated plate (ACP) ELISAs. Virus-specific McAbs could also detect virus in triple-antibody-sandwich assays. All of the tested potyviruses were more readily detected in ACP-ELISA compared to at least one McAb (PSY1) with substrate incubation times ranging from 1 to 7 h; however, some low virus titer-containing plant extracts required overnight substrate incubation for reliable detection. BYMV isolates from gladiolus, irises, orchids, and pea, as well as most of the other distinct potyviruses tested, could be differentiated with the panel of McAbs. Individual or specific mixtures of McAbs were capable of identifying certain virus clusters or related subgroups.

Monoclonal antibodies reacted with the homologous antigen trapped in rabbit anti-CyMV serum coated ELISA plates; twenty-two of the 45 antibodies reacted with CyMV on antigen coated ELISA plates. In IEM on monoclonal antibody-coated grids the delimitation of the virions produced on ELISA plates was about 1/5000 while the end point of crude sap from infected orchids was about 1/1000. Sequences within the 3' untranslated region of the same monoclonal antibodies was about 1/4000 in ELISA and 1/3200 by dot-blot assay on nitrocellulose membranes. An indirect immunological method was developed to detect CyMV antigens in direct tissue blots on nitrocellulose membranes. CyMV was detected in 30 of 155 plants in tissue blots on nitrocellulose membranes. Two and four samples of the 30 that were positive in tissue blots showed Ag5 values lower than 0.05 and 0.2, respectively, when tested at 1/20 sap dilutions.

Spleen cells of BALB/c mice injected with SDS-PAGE purified cylindrical inclusion protein (CIP) from an isolate of papaya ringspot virus type W (PRSV-W) were fused with SP2/0 myeloma cells. Two resulting monoclonal antibodies (MCAs) reacted with the CIP band (MW = 68-70k) when crude sap from PRSV-infected tissue was analyzed by Western blotting. MCA CI-1 reacted with 15 PRSV-W isolates and with an isolate of papaya ringspot virus type-P (PRSV-P). MCA CI-2 reacted with 12 of 15 PRSV-W isolates, PRSV-P and the Tigner isolate of PRSV. Neither MCA reacted with the Moroccan isolate of watermelon mosaic virus (WMV-M), watermelon mosaic virus-2 (WMV-2), zucchini yellow mosaic virus (ZYMV) or healthy pumpkin. Polyclonal rabbit antiserum (PCA) to the CIP reacted with all isolates of PRSV tested, and cross-reacted with WMV-M, WMV-2 and ZYMV. The differential reactions of the two MCAs and the PCA indicate that the CIP has both specific and common epitopes which could be useful in the classification and diagnosis of potyviruses.

Bean yellow mosaic virus (BYMV)-specific, BYMV subgroup-specific and potyvirus group cross-reactive monoclonal antibodies (McAbs) were evaluated with respect to their ability to detect and differentiate potyviruses. Different extraction buffers, antigen preparation regimes, solid phase coating conditions and ELISA protocols were used to evaluate the McAbs. All McAbs detect viral antigens in antigen-coated plate (ACP) ELISAs. Virus-specific McAbs could also detect virus in triple-antibody-sandwich assays. All of the tested potyviruses were more readily detected in ACP-ELISA compared to at least one McAb (PSY1) with substrate incubation times ranging from 1 to 7 h; however, some low virus titer-containing plant extracts required overnight substrate incubation for reliable detection. BYMV isolates from gladiolus, irises, orchids, and pea, as well as most of the other distinct potyviruses tested, could be differentiated with the panel of McAbs. Individual or specific mixtures of McAbs were capable of identifying certain virus clusters or related subgroups.

Although cultivars (cvv) of hard red winter wheat (Triticum aestivum L.) with resistance to WSMV have been developed, the mechanism(s) of resistance is not understood. Larsen, et al. (Plant Disease 69:857) proposed that resistance to WSMV is expressed as resistance to Polymyxa graminis carrying WSMV or a close relative. All cvv tested were mechanically inoculated on nitrocellulose and were mechanically inoculated on nitrocellulose and were mechanically inoculated on nitrocellulose and were mechanically inoculated on nitrocellulose. WSMV was found in the shoots of cvv as cvv and cvv resistant cvv when mechanically inoculated, but only in the roots when cvv were inoculated with root washings. An inhibition of virus movement is suggested as the mechanism of resistance.

The virus associated with citrus ringspot (CRSV) was further characterized. Nucleic acids associated with the short and long filamentous particles were isolated and appeared to be single-stranded RNA. Nucleic acid preparations from crude extracts or partially purified preparations were not infectious. A sedimentable double-stranded RNA was associated with CRSV infections. An antiserum to purified CRSV was used to detect virus particles and coat protein in nitrocellulose from agarose gels and by serologically specific electron microscopy. The antiserum was also used to detect the 48 kilodaton protein associated with CRSV in western blots.

A610
ULTRASTRUCTURE OF MAIZE LEAVES SINGLEY OR DOUBLY INFECTED WITH MAIZE CHLOROPLASTIC MOBILE (MCMV) AND MAIZE DWARF MOSAIC (MDMV) VIRUSES. E. D. Ammar and D. T. Gordon, Dep. of Entomology, Fac. Agric., Cairo Univ., Egypt; Dep’t of Plant Pathology, Ohio State Univ., Wooster, Ohio 44691

Maize seedlings mechanically inoculated with MDMV (strain B), MCMV, or both were processed for electron microscopy 2 or 3 wk post-inoculation. Particles and inclusions of MDMV were abundant in leaves of singly or doubly infected plants at both intervals; those of MCMV were abundant only at 3 wk in singly infected plants, and at both intervals in doubly infected ones. Previously undescribed ‘complex’ inclusions containing apparently deformed, enlarged or coalesced mitochondria, large aggregates of fibrous structures, MCMV and MDMV particles and inclusions, were abundantly found in doubly infected plants. Complex inclusions, without MDMV particles or its inclusions, were occasionally found in MCMV singly infected plants. The significance of these ultrastructural alterations in the development of the corn lethal necrosis disease is discussed.

A611
DETECTION OF CITRUS TRISTEZA VIRUS (CTV) WITH A MIXTURE OF MONOCLONAL ANTIBODIES. M. Cambra, J.V.I.A., Moncada, Spain; S. M. Garnsey, T. A. Permar, C. T. Henderson, USDA, ARS, Orlando, FL; D. Gump, University of California, Riverside; and C. Vela, Ingenasa, Madrid, Spain.

Although several epistries of CTV are well conserved, none of the existing monoclonal antibodies (Mabs) react with all CTV isolates. Eighty-four isolates of CTV from 18 countries (selected for maximum serological and biological diversity) were tested with a mixture of two Mabs (IgG2b2 specific to different epistries and against polyclonal antisera in four variations of enzymed linked ELISA). The Mab mixture did react to all isolates tested and proved a suitable substitute for polyclonal antisera in double antibody sandwich ELISA. The best results were obtained with a biotin-streptavidin protocol using unlabeled and biotinylated Mab mixture to trap and detect CTV antigens.

A612
CROSS PROTECTION WITH TOBACCO MOSAIC VIRUS IN ABRIDOPHOTO THALIANA. L. A. Urban, J. L. Sherwood, and K. M. Melcher, Department of Plant Pathology, North Carolina State University, Raleigh, NC 27695; Department of Plant Pathology, and Department of Biochemistry, Oklahoma State University, Stillwater, OK 74078-9947.

To test the role of tobacco mosaic virus (TMV) coat protein in cross protection in A. thaliana, three-week-old plants were initially inoculated with 50 µg/ml of TMV isolated from petunia (TMV-P, Virology 119:150). Plants were challenged with 10 µg/ml of TCV from the same strain (TCV-C; 7 days later). After 10 days, the presence of coat protein of each strain was determined in the initially inoculated leaf, the leaf that was challenged inoculated, and a leaf above the challenge inoculated leaf. TMV-P was detected throughout the plants. TCV-C was rarely detected out of the leaf onto which it was inoculated. Similar results were obtained when vireions of TCV-C were used as the challenge inoculum. These results suggest that A. thaliana infection by TMV may be the primary mechanism of cross protection.

A613
THE EFFECT OF DAY LENGTH ON ALFALFA MOSAIC VIRUS MULTIPLICATION AND RNA STABILITY. S. Flissinger, C. W. Garrett, and A. Kluge, Department of Plant Pathology and Physiology, Clemson University, Clemson, SC 29634-0377

Alfalfa mosaic virus (AMV) strain 425, 106, and Soy were purified from Nicotiana clevelandii, 7, 10, and 14 d after inoculation. AMV-106 virion yields were highest 7 d after inoculation while AMV-425 yields were highest at 10 d and AMV-Soy at 14 d. Yields of AMV-425 were greater from plants growing in an 18/6 photoperiod than in a 12/12 photoperiod, but not for AMV-106 and AMV-Soy. Different amounts of RNA 1, 2, 3, and 4 were purified from virions of each strain at the different harvest dates and photoperiods. In general, high virus yields resulted in intact strands of all four RNAs. Amount of RNA-4 per mg virion did not change with time of inoculation or photoperiod but RNA 1, 2, and 3 occurred in lower amounts relative to RNA 4 14 d after inoculation. Photoperiods also affected the ratios of RNA 1, 2, or 3 to RNA-4. RNA degradation, in vivo or in vitro, could explain some of these differences.

A614
PROPERTIES OF THE MENTHA STRAIN OF LYCINIS RINGSPO T VIRUS. L. Beczeri (1), R.I. Hamilton (2), and D.M. Rochon (2), (1) Plant Protection Institute, Hungarian Academy of Sciences, Budapest H-1525, Hungary, and (2) Agriculture Canada Research Station, 6660 N.W. Marine Drive, Vancouver, B.C., Canada V6H 1Z2.

A hordeivirus, isolated from horsemint (Mentha longifolia Huds.) in Hungary, was designated as the mentha strain of lychnis ringspot virus (LRSV-M) on the basis of serological relationship with the type strain (LRSV-T), reciprocal nucleic acid hybridization, and similar properties of their ss (genomic) and ds RNAs. Four smaller ssRNAs in addition to the three genomic RNAs were encapsidated by LRSV-M coat protein. No hybridization was detected under high stringency conditions between randomly primed cDNA of LRSV-M and other hordeiviruses (barley stripe mosaic [BSMV] and potato semitectant [PSLV] viruses). Reciprocal hybridization experiments using cDNAs to BSMV and PSLV confirmed that the hordeiviruses are a group of morphologically similar but genetically distinct viruses.

A615

High molecular weight double-stranded RNA (dsRNA) was detected in five isolates of Alternaria solani, the causal agent of early blight of potato. The five isolates were obtained from potato leaves collected in Pennsylvania, Maine, and New York. dsRNA was extracted with phenol and purified by cellulose chromatography from cultures grown in liquid media. Each isolate showed a characteristic rDNA electrophoretic pattern in agarose gel consisting of 1 to 4 bands ranging in molecular weight from 3,2 to 18,5 x 10^6 d. Some bands were common among isolates. No virus-like particles or abnormal cytological structures were observed in any of two to six hyphal tips of each isolate analyzed by transmission electron microscopy.

A617
SIMULATION OF ULTRAVIOLET ABSORPTION SPECTRA OF VIRUSES AND PROTEINS. L.C. Lane, Dept. of Plant Pathology, Univ. of Nebraska, Lincoln, NE, 68583-0722.

Ultraviolet absorption spectroscopy is a convenient method of characterizing viruses and proteins. To a first approximation the UV absorption spectrum of a protein is the sum of spectra for tryptophan, tyrosine, phenylalanine and disulfide bonds. Spectra of individual proteins can be simulated by entering amino acid spectra into a computer spreadsheet and combining them in ratios dictated by the protein amino acid content.
Virus spectra can be simulated by adding, in addition, spectra for RNA and light scattering. Tryptophan/tyrosine ratios strongly influence protein spectra. Tryptophan/tyrosine ratios of unknowns can be estimated by comparing actual spectra to computer generated spectra. Comparing UV spectra of viruses to computer generated spectra is a useful criterion of purity. Generating spectra by computer is a useful way for students to learn spectroscopy principles.

A622

SPECIFIC ENDORIBONUCLEASE CLEAVAGE OF TMV GENOMIC RNA BY AN ENGINEERED RIBOZYME. B.V. Edington, A.D. Choudhary, R.A. Davison, and R.S. Nelsen. The Rockefeller Foundation, New York, NY 10021 and The Rockefeller University, F.G. Box 2180, Armondo, OK 73402.

Ribozymes are RNA molecules which possess an enzymatically self-cleaving or self-splicing activity. Properties of this self-cleaving activity may be used in the design of endoribonucleases, with the potential of altering gene expression. Such a ribozyme has been constructed and targeted to cut the plus strand of TMV at nucleotide 2467, which will bisect the coding region of the TMV RNA-dependent RNA polymerase. The ribozyme itself is flanked by 40 nucleotides which are complementary to sequences surrounding the cleavage site. This RNA enzyme has been used in in vitro assays to cleave purified positive strand TMV RNA. Experiments are now in progress to assay the ability of this ribozyme to cleave TMV genomic RNA in tobacco protoplasts, and thus prevent viral replication.

A623

CONSTRUCTION OF CDNA PROBES FOR THE DETECTION OF TOMATO RINGSPOT VIRUS. J. R. Guo and T. A. Chen. Department of Plant Pathology, Rutgers University, New Brunswick, NJ 08903.

Complementary DNA of the New Jersey isolate of tomato ringspot virus was synthesized using reverse transcriptase with random primers. The double stranded DNA was cloned into pUC18 and transformed into E. coli DH5a cells. 116 recombinant plasmids were selected by colony hybridization using the double stranded CDNA as a probe. Cleared inserts ranged from 0.3 to 2.6 kb. Comparison of restriction enzyme maps and southern hybridization patterns of 8 clones indicated that they represented about 50% of the RNA genome. 5 clones were 3P-labeled by nick translation and used in dot blot hybridization. The dot blot assay allowed the detection of as little as 20 pg of RNA from purified virions as well as virus in a 1:512 dilution of crude sap from infected tobacco plants. The cloned DNA probes hybridized with all 8 isolates tested. The probes will be used to detect virus infection of fruit trees and to investigate the biology of the nematodes which transmit the virus.

A624

RESISTANCE IN TRANSGENIC POTATO EXPRESSING THE POTATO LEAFROLL LUTOVIRUS COAT PROTEIN GENE. L.M. Kavchuk (1), B.R. Martin (1), and J. McPherson (2). (1) Agriculture Canada Research Station, Vancouver, B.C. V6T 1X2 and (2) Department of Plant Science, University of British Columbia, Vancouver, B.C. V6T 1X2.

Three constructs of the potato leafroll luteovirus (PLRV) coat protein gene were inserted into the commercial potato cultivar Russet Burbank via an Agrobacterium tumefaciens mediated gene transfer. One construct possessed 12 nucleotides of the untranslated leader sequence 5' to the coat protein AUG and the other construct, which was also inserted in the reverse orientation to produce negative sense RNA, had 112 nucleotides from this leader sequence. Introduced as chimeric genes under the control of the duplicated CaMV 35S promoter, transcription levels were very high but coat protein levels were less than 0.01% of total leaf protein. Results show that significant levels of sustained resistance are obtained with each construct.

A625


Two strains of cucumber mosaic virus (Fny- and M-CMV) differ greatly with regard to aphid-transmissibility, symptom expression, and the ability to infect the squash cultivar ‘Black Beauty’. With respect to these differential phenotypes, transcripts of cDNA clones of the Fny-CMV genomic RNAs 1, 2, and 3 produce infections and progeny virus typical of Fny-CMV, whereas replacing the Fny-CMV RNA 3 transcript with an M-CMV RNA 3 transcript results in infections and progeny virus typical of M-CMV. Reciprocal recombinants between the two CMV RNA 3 clones and the resulting infections on tobacco and squash implicate the coat protein gene as the determinant of these differential phenotypes. A comparison of the
nucleotide sequences of the two strains revealed 14 nucleotide differences in the coat protein genes, resulting in 8 predicted amino acid differences. Recombinants within the coat protein gene are being used to further delimit the amino acids involved in determining these phenotypes.

A626

The group 2 isolate of barley yellow dwarf virus (BYDV), NY-RPV, can be distinguished from group 1 BYDV isolates by serological relationships, cytopathological ultrastructure of infected cells, and dsRNA profiles obtained from infected tissues. To investigate the genomic basis for these differences, cDNA libraries were constructed from NY-RPV viral RNA in both plasmid and bacteriophage vectors. From these libraries, overlapping clones representing the NY-RPV genome were identified by restriction analysis and by hybridization, and subsequently sequenced. The genome of NY-RPV is 3.6 kb in length, within which six major (+) strand open reading frames (ORFs) are identified. Based on the sequence and the organization of the genome, NY-RPV is clearly different from group 1 BYDV isolates. Furthermore, the genome of the NY-RPV isolate of BYDV more closely resembles that of two other luteoviruses, best western yellow virus and potato leafroll virus, than those of group 1 BYDV isolates.

A627

The NY-MAV-PS1 and P-PAV isolates of barley yellow dwarf virus (BYDV) are serologically related, but are not identical. Both BYDV isolates are transmitted by the aphid Sitobion avenae, but P-PAV is also transmitted by Rhopalosiphum padi. To evaluate the genomic basis for these and other differences, cDNA libraries were constructed from the RNA of each BYDV isolate in both plasmid and bacteriophage vectors. From these libraries, overlapping clones representing the genome of each viral isolate were identified by restriction analysis and by hybridization, and subsequently sequenced. Each genome is 5.2 kb in length with six identified (+) strand open reading frames (ORFs). The greatest diversity between the NY-MAV-PS1 and P-PAV sequences was found in ORF5 located at the 3' end of the respective genomes, indicating that this region of the genome may be involved in the properties which differentiate BYDV-NY-MAV-PS1 and BYDV-P-PAV, taken to incorporate the non-representative nucleotides at the 3' terminus of the clones as well as incorporate a convenient SmaI restriction site. Following SmaI restriction and T7 transcription in the presence of m'GpppG, infectious RNA-1 and -2 run-off transcripts were made. Incubation of the RNA-1 and RNA-2 in vitro transcripts resulted in systemic infection and typical symptom formation on Nicotiana clevelandii.

A630

Wheat streak mosaic virus (WSMV), a putative member of the potyvirus group, has a single-stranded RNA genome of approximately 8.5 kb. To date, the capsid protein cistron has been mapped proximal to the 3' terminus. A 1.0 kb cDNA clone not 3' co-terminal with clones containing capsid protein coding sequence was synthesized by oligo-dT priming. The nucleic acid sequence of this clone has been determined, and its amino acid sequence deduced. The amino acid sequence shares homology with a cucumovirus, potato leafroll, and potato leafroll mosaic virus, and plum pox, and tomato vein motting viruses and potato virus Y within the N terminus of the cylindrical inclusion protein. The consensus sequence for nucleotide binding, GXXGXXGKS, which is highly conserved among the four sequenced potyviruses, is present at the 5' terminus of the WSMV clone. These data further support the inclusion of WSMV within the potyvirus group.

A631
MOLECULAR ANALYSIS OF TOBACCO VEIN MITTLING VIRUS (TVMV) PATHOGENICITY BY INFECTIONAL TRANSCRIPTS OF CHIMERIC(119,350),(940,500)

A632
HETEROLOGOUS ENCAPSULATION IN MIXED INFECTIONS AMONG THREE ISOLATES OF BARLEY YELLOW DWARF VIRUS. E. Wen and R. M. Lister. Dept. Botany and Plant Pathology, Purdue Univ., W. Lafayette, IN 47907.

Immunohybridization (J. gen. Virol. 71:211) and ELISA were used to study heterologous encapsidation between paired isolates of barley yellow dwarf virus (BYDV) in mixedly infected oat plants. One-way heterologous encapsidation was detected between NY-RPV and NY-MAV-PS1 isolates, and between NY-RPV and P-PAV isolates. Apart from homologous encapsidation, some of the RNAs of either P-PAV or NY-MAV-PS1 were heterologously encapsidated in the protein capsids of NY-RPV to form "hybrid" virions, but there was no evidence of such encapsidation of the RNAs of NY-RPV in the protein capsids of either P-PAV or NY-MAV-PS1. Two-way heterologous encapsidation was detected between P-PAV and NY-MAV-PS1, i.e. some of the viral RNAs of P-PAV or NY-MAV-PS1 were detected in virions trapped with NY-MAV-PS1-specific or P-PAV-specific antibodies, respectively. Further analysis, including two-site ELISA, to determine whether the heterologous encapsidation involves transcription, phenotypic mixing, or both, is in progress.

A629
Red clover necrotic mosaic virus infectious transcripts synthesized in vitro from full-length cDNA clones. Z. Xiong. and S. A. Lommel, Dept. of Plant Pathology, North Carolina State University, Raleigh, N.C. 27695-7616.

The red clover necrotic mosaic virus (RCNMV) genome is split among two non-homologous ssRNAs of 3.9 kb (RNA-1) and 1.5 kb (RNA-2). Near to full length cDNA clones were generated to both RNAs. The clones were determined to be short of full length by several nucleotides at both termini. Oligo-directed mutagenesis was employed to incorporate the missing 5' terminal nucleotides as well as fuse the pBS(+)

T7 promoter to both clones. Authentic viral RNA-1 and -2 begin with a m'GpppA. The T7 promoter extends one nucleotide into the transcript sequence, preferring that the transcript begins with a guanosine. Consequently, an additional non-viral guanosine residue was engineered to the 5' end of the viral sequence. The same approach was

1036 PHYTOPATHOLOGY

La France disease of Agaricus bisporus is associated with a conserved electrophoretic pattern of nine dsRNAs. In addition, several virus-like particles, including a 19 x 50 nm RNA bacilliform virus (MBV), have been detected in diseased tissues. We investigated the relationship of MBV RNA and dsRNAs. Upon electrophoresis in denaturing 1.5% agarose gels, the genome of MBV migrated as a single 4.4 kb RNA molecule. A 1.4 kb CDNA to MBV RNA was synthesized by oligo (dT)-primed reverse transcription, cloned, and radiolabeled by random priming. Northern analysis showed that the cDNA probe hybridized to the genomic RNA isolated from purified virus, but not to a comparable fraction from healthy basidiocarps. Similarly, the probe detected MBV RNA in diseased tissue but not in healthy basidiocarps. No sequence homology existed between the cDNA and either the dsRNAs associated with La France disease or those present in healthy tissues. The results suggest that MBV is distinct from the putative dsRNA viruses.


Copea mottle virus (CMv) is a non-enveloped plant virus reported only from Nigeria. It has one (+)-ssRNA as a genome (mol. wt. 1.4 x 10^6 (4 Kd)) and one capped protein (mol. wt. 40 K). cDNAs were generated using random primers and cloned into phagemid vector PT/7/THI 18U. The insert was isolated from 500 bp to 2100 bp. A CMV capsid gene sequence was obtained from cDNA library CMv16-12 from CMv. The CMv capsid gene is composed of 1104 nt and the codons represent 367 amino acids. The amino acid sequence deduced from the nucleotide sequence of capsid gene was compared to those of Carmoviruses. The S-domain showed about 30% sequence homology eventhough Western blot and Northern blot analysis showed no cross-reaction among CMv and Carmoviruses.

CONSTRUCTION AND ANALYSIS OF A VIRAL VECTOR FOR RAPID ANALYSIS OF GENE EXPRESSION IN WHOLE PLANTS. R. Cassidy and R. Nelson, The Noble Foundation, P.O. Box 2180, Ardmore, OK 73402.

Elements presumed to be necessary for replication and translation from an attenuated strain of tobacco mosaic virus (TMV) were isolated from cDNA clones or chemically synthesized and combined to create an expression vector. This vector has characteristics of both defective interfering particles and satellite viruses. It contains DNA homologous to the TMV origin of assembly sequence and the 3' untranslated region. A series of restriction sites inserted in the cDNA locus allow the insertion of foreign genes. When coinfected with an attenuated strain of TMV these elements will allow for replication and encapsidation of the vector RNA and gene expression. Expression of foreign genes will allow the observation of effects in differentiated tissues and at the same time avoid the long delays in analysis when producing transgenic plants. The expression and systemic movement of a reporter gene, β-glucuronidase, will be presented.


We have characterized the upstream sequences needed for functioning of the polyadenylation signals from cauliflower mosaic (CaMV) virus and a pea ribulose-1,5-bisphosphate carboxylase small subunit gene. Sequences between 55 and 181 bases upstream from the CaMV poly(A) site are sufficient for efficient polyadenylation at this site, as is an AAUAAA sequence located 16-21 bases upstream from this site. An element located between +137 and +137 bases upstream from the poly(A) addition site is a pea ribulose-1,5-bisphosphate carboxylase small subunit gene is needed for functioning of these sites, indicating that upstream sequence elements far from polyadenylation sites are a common feature in plant genes. Our studies indicate that upstream sequences other than AAUAAA are important for efficient mRNA 3' end formation in these two genes. We suggest that multiple elements are involved in mRNA 3' end formation in plants. These components of the plant polyadenylation apparatus appear to interact with their respective sequence elements and with each other to result in efficient mRNA 3' end formation.


Crown gall tumors induced by octopine-type Agrobacterium tumefaciens strains produce four mannose-containing opines. One of these, called agrocinopine (AGR), is a second, called mannopine (MOP). We now show that extracts from tumors producing agropine contain an activity that cyclizes MOP to AGR and that this activity is the product of Tg gene 26. Strains harboring octopine-type Ti plasmids also contain a catabolic activity that lactonizes MOP to AGR. This cyclase is encoded by a Ti plasmid gene linked to the Ti DNA. A fragment encoding the catabolic MOO cyclase was subcloned from the Ti plasmid and defined by deletions. The region was sequenced and an open reading frame encoding a predicted 45.3 kd protein was identified. In a coupled transcription-translation system the subclone yielded a single protein with a molecular size estimated at 45 kd. The derived amino acid sequence of the encoded protein from Tg gene 26 and the catabolic cyclase gene show 53% identity and 79% homology including conserved changes.

DEVELOPMENT OF A DNA PROBE TO DETECT COPPER-RESISTANCE GENES IN XANTHOMASMA CAPRESCIA PV. VESICATORIA. S. Garde and C. Bender. Dept. of Plant Pathology, Oklahoma State Univ., Stillwater, OK 74078.

We recently demonstrated that Cu^2+ resistance in X. caprescia pv. vesicatoria strain XVD was encoded by a large 110 kb replicative plasmid designated pXV10A (Appl. & Environ. Microbiol. 56:170-175). A cosmid library of pXV10A DNA was constructed in plasmid pACYC184. A 44 Kd cosmid clone, designated pCuD, conferred Cu^2+ to a copper-sensitive strain of Xv when it was transformed by electroporation. Subcloning and transposon mutagenesis experiments were performed to further characterize the specific location and size of the Cu^2+ determinant on pCuD. Several probes constructed from DNA fragments internal to the Cu^2+ region were hybridized to Cu^2+ and Cu^2+ Xv strains as well as other pathogenic and aphthophylic bacteria to assess their specificity for Cu^2+ in Xv.

GENETIC EVIDENCE FOR A TRANS-ACTING FACTOR OF ERWINIA CAROTOVORA SUBSP. CAROTOVORA (EC) THAT STIMULATES THE PRODUCTION OF EXTRACELLULAR DEGRADATIVE ENZYMES. Y. Furuta, and K. Chatterjee, Department of Plant Pathology, University of Missouri, Columbia, Missouri 65211.

Ec strain 71 produces extracellular enzymes such as pectate lyase (Pel), polygalacturonase (Feh), cellulase (Cel), and protease (Prot). Using Tn5, TnphoA, and Tn0-1acZ, we isolated pleiotropic mutants deficient in these enzymatic activities. The phenotypes did not result from insertions in genes specifying enzyme (out) or adenylyl cyclase, or cAMP receptor protein. We isolated a cosmid, pAKC264 that restored extracellular enzyme production to Tn5 in A1.7.4.8.3. In addition, pAKC264 restored their original phenotype to a strain carrying Tn-phoA, pAKC264 or its subclone, pAKC262, stimulated production of Pel, Feh, Cel and Prot in Ec71 by ca. 3-fold. pAKC262 also stimulated the production of Feh, Pel and Cel in Ec14. Ec strain carrying Tn-phoA, pAKC264 or its subclone, pAKC264 also allowed the insertion of the same was designated as spg for the activation of extracellular protein production.


A. tumefaciens bivar 3 causes both crown gall and root necrosis of grape. Activity-stained isoelectric focusing gels of culture supernatants show the production of a single polygalacturonase (PG) by bivar 3 strains from different geographical origins but reveal no pectolytic activity from bivar 1 and 2. The pl optimum and pI of the PG are around 4.5. The enzyme is produced in Kado 532 medium, but not minimal medium, is largely extracellular, and is produced independently of auxin. A PG of a 3 with a pl of 4.5 was recovered from lesions in grape seedlings infected with A. tumefaciens bivar 3 strain C489, but not from healthy seedlings. Several PG-deficient Tn mutants of strain C489 have been isolated and will facilitate further characterization of the role of the PG in Agrobacterium pathogenesis.

BROAD-HOST-RANGE COSMID AND PLASMID VECTORS FOR USE IN PSEUDOONEMAS. D. N. Bauer and A. Colliner. Department of Plant Pathology, Cornell University, Ithaca, NY 14853.
A new cosmid vector, pCPP34, was constructed from the broad-host-range, IncF vector, pMP92 (Spink et al. 1987, Plant Mol. Biol. 9:27-39). The cosmid is small (ca. 8.9 kb) and contains two cos sites flanking a unique blunt-end restriction site, thus simplifying construction of libraries. When packaged, 1.5 kb of the vector is lost, allowing inserts of 30-45 kb to be cloned. The vector contains a BamHI cloning site flanked by T3 and T7 promoters and MboI sites for rapid mapping of inserts. The vector was mobilized from Escherichia coli into Pseudomonas syringae pv. syringae where it was similar in stability to plAFR5. The cosmid has been used to construct libraries of P. syringae pv. syringae and P. syringae pv. lachrymans. Several additional derivative phages of pMP92 were constructed which contain the laco region from pUC19 or pUC128 and have been useful in subcloning fragments of P. syringae DNA due to their small size (ca. 7.2 kb) and ability to stably maintain inserts.

A643

SIV-DIRECTED MUTAGENESIS OF CORONATINE SYNTHESIS GENES IN PSEUDOMONAS SYRINGAE PATHOVAR GLYCINEA, ATROPURPUREA, AND MORSERPONR. C. L. Bender and S. A. Young, Dept. of Plant Pathology, Oklahoma St. Univ., Stillwater, OK 74078.

In P. syringae pv. tomato P0T23.2, plasmid pP0T23A (101 kb) is involved in synthesis of the pytoxocime coronatine. The characterization of Tn5 insertions and deletions in pP0T23A suggest that a 30 kb region of this plasmid is necessary for coronatine production. Coronatine biosynthesis genes in extracellular polysaccharide (EPS) production by AW1-83 is drastically reduced when cultured alone, but is reversibly induced to normal levels when AW1-83 is grown on a split-plate adjacent to the wild-type strain AW1. The inducer produced by AW1 is volatile, but is not ethylene. A volatile compound from the isoprenoid metabolism of AW1 is a volatile inducer for AW1 and isolates the P. solanacearum wild-type gene responsible for the inducible phenotype of AW1-83 is in progress.

A644

VOLATILE COMPOUNDS INDUCE EXTRACELLULAR POLYSACCHARIDE PRODUCTION BY A PSEUDOMONAS SOLANACEARUM MUTANT. S. G. Clough and T. M. Donny, Dept. of Plant Pathology, University of Georgia, Athens, GA 30602.

Mutant AW1-83, derived from Pseudomonas solanacearum strain AW1 by insertion of an IS65 element, has a pleiotropic phenotype similar to that of a spontaneous phenotypic conversion mutant. Extracellular polysaccharide (EPS) production by AW1-83 is drastically reduced when cultured alone, and is reversibly induced to normal levels when AW1-83 is grown on a split-plate adjacent to the wild-type strain AW1. The inducer produced by AW1 is volatile, but is not ethylene. A volatile compound from the isoprenoid metabolism of AW1 is a volatile inducer for AW1 and isolates the P. solanacearum wild-type gene responsible for the inducible phenotype of AW1-83 are in progress.

A645


A syrb::lacZ gene fusion, obtained by Tn3HoIo1 mutagenesis, was marker exchanged into strain BS3-R of Pseudomonas syringae pv. syringae. The resultant strain, BS3AR132, expressed high β-galactosidase activity when specific plant signal molecules were present in the culture medium. Signals that transcriptionally activated syrb::lacZ were extracted from cherry leaves and purified by C-18 reverse-phase HPLC. A reverse-phase sequence for the nucleotide sequence of the predicted protein product from this fragment is homologous to members of the superfamily of prokaryotic response regulator proteins. The proteins Hrp5 from Pseudomonas syringae pv. phaseolicola, HrpM from Pseudomonas syringae pv. glycinea, and Hrp4 from Pseudomonas syringae pv. syringae, and HrpQ from Pseudomonas syringae pv. syringae are significantly similar to the predicted E. amylovora Hrp protein.

A646


A pectinase gene from a plasmid-carrying strain (SJ074) of Pseudomonas viridiflava was cloned and expressed in E. coli using pBR322 as a vector. All four resulted pectolytic transformants produced only one type of pectate lyase with P/L 9-7 and no endopolygalacturonase. The smallest insert (9-10 kb) from one of the four clones was restricted with various endonucleases and a restriction map of the cloned fragment was constructed. One of the SphI subfragments (1.6-3.7 kb) encoded the pectate lyase gene (pel). Further investigations are being made to determine if the pel is plasmid-coded and if pel homologs exist in other strains of P. viridiflava.

A647


P. viridiflava is a postharvest pathogen, which causes soft rot of vegetables by producing a single pectate lyase (PL: P/L 9.7). We have previously shown that insertion of transposons into genes controlling synthesis of cellulases and pectolytic enzymes results in the loss of pathogenicity. Here we describe another pathogenicity-related mutant, which produces PL normally in cultures but fails to induce soft-rot symptoms on plants. When inoculated onto pepper fruit, this mutant causes browning and necrosis of tissues. The mutation is pleiotropic and reversible at relatively high rates. A genomic library of the parent strain has been constructed in plAFR5 and will be used to identify genes responsible for induction of this hypersensitive-type response in host plants.

A648

CHARACTERIZATION AND ISOLATION OF GENES CODING FOR TOXIN PRODUCTION IN BACILLUS THURINGIENSIS. M. C. Kosinski, D. A. Kuepfei, and G. A. Carner, Department of Plant Pathology and Physiology, Clemson University, Clemson, SC 29634-0377.

B. thuringiensis is a gram-positive bacterium which produces a proteolytic crystal during sporulation. When ingested by lepidopteran larvae, the crystal becomes an endotoxin deadly to lepidopterans but not to other organisms. Bioassays to determine the LD 50 of four strains of Bacillus thuringiensis (HD-1, HD-73, HD-187 and HD-263) against Heliothis virescens showed that strain HD-263 was two to seven times more effective against H. virescens than the benchmark strain HD-1. Chromosomal and plasmid DNA from HD-263 were extracted and purified; DNAs were separated on 0.7% agarose gels, transferred to nitrocellulose paper by the Southern blot procedure, and probed with a 32P-labelled, truncated B. thuringiensis clone from strain B. thuringiensis subspecies berliner 1715. The delta endotoxin gene in strain HD-263 was identified, located, and cloned to allow study of its elevated level of insecticidal activity.

A649

A PATHOGENICITY GENE FROM EWINIA AMYLOVORA ENCODES A PREDICTED PROTEIN PRODUCT HOMOLOGOUS TO A FAMILY OF PROCRARYOTIC RESPONSE REGULATORS. B. S. Snath, J. M. Howson, and S. V. Beer. Department of Plant Pathology, Cornell University, Ithaca, NY 14853.

A cluster of hrg genes from Ewinia amylovora spanning ca. 40 kb was cloned previously. The naturally occurring Hrp strain PSE (from E. Billing) has been complemented by a 2.7 kb BamHI-HindIII fragment from the middle of the hrg cluster. The nucleotide sequence of the predicted protein product from this fragment is homologous to members of the superfamily of prokaryotic response regulator proteins. The proteins Hrp5 from Pseudomonas syringae pv. phaseolicola, and HrpM from Pseudomonas syringae pv. syringae, and HrpQ from Pseudomonas syringae pv. syringae are significantly similar to the predicted E. amylovora Hrp protein.

A650


1038 PHYTOPATHOLOGY
The entire hrp gene cluster of *E. amylovora* was cloned previously as a 46 kb insert in cosmid pG340. Subclones from the cosmid were used to screen a *Porphyromonas gingivalis* W83 library. Hybridization between certain subclones and genomic DNA of *E. carotovora*, *E. chrysanthemi*, *E. glycines*, *E. maltovora*, *E. nigrifaciens*, *E. rubrifaciens*, *E. salicytica*, and *E. stewartii* was observed. Hybridization between certain subclones also occurred with pE5044, a cosmid containing genes of *E. stewartii* involved in water-soaking. With higher stringency (47°-57°C) washes, hybridization between certain subclones and *E. amylovora* was observed. Certain subclones hybridized with pHR1, a cosmid containing the hrp cluster of *Pseudomonas syringae*, and with genomic DNA of *P. syringae* at low stringency. These results indicate that the genome of *E. amylovora* shares DNA homology with other phytopathogenic bacteria, including some that do not elicit the HR.

**A651**


We have developed biologic methods for transforming *B. megatennium* strain 7A17 with plasmid pUB110. This is the first report of biologic transformation of a prokaryote. Cells were spread on solid LB medium plus 50 μg/ml methionine and sorbitol (0.175 M), bombarded with DNA-coated (0.8 μg DNA per bombardment) tungsten particles (0.1-μm 1.0) and then overlayed with LB containing 50 μg/ml kanamycin. Transformation efficiency was highest when the cell density was 7.5 x 10^9 cells/ml and high efficiency transformation of transformants at 1.25 M sorbitol was 5.5 times greater than at 0.75 M. Transformation was confirmed by restriction and gel electrophoresis of plasmid DNA. An improved particle accelerator design dramatically increased transformation efficiency. Hundreds (>500) of transformants per petri dish were produced as compared to <10 with the old particle accelerator. The effects of bacterial strain, pre- and post-bombardment treatment of cells, and DNA size are being tested as well as transformation of other species. Particle bombardment may prove to be a universal process for transformation of prokaryotes as well as eukaryotes.

**A652**


A cosmid (pCPP430) containing the entire hrp gene cluster of *E. amylovora* was identified previously. Strains of *E. coli* harboring pCPP430 elicited the hypersensitive response (HR) in tobacco and other plants. Two other cosmids pCPP440 and pCPP450, which do not complement hrp mutants at the left end of the cluster, also bestow on strains of *E. coli* the ability to elicit the HR. A 12.5 kb EcoRI fragment from the left end of pCPP430 was mutagenized with a transposon and the insertions marker-exchanged into the *E. coli* genome. Mutant *E. coli* transconjugants containing pCPP440 or pCPP450 failed to elicit the HR. However, the HR was restored (in trans) by a 2.9 kb HindIII subclone from the left end of pCPP430. These results indicate that some genes of *E. coli* can functionally complement the left-hand region of the hrp cluster of *E. amylovora*.

**A653**

**APPARENT RESISTANCE TO SCLEROTINIA STEM ROT IN OILSEED BRASSICAS,** D. W. Phillips, P. L. Raymer, and D. L. Auld. University of Georgia, Georgia Experiment Station, Griffin, GA, and University of Idaho, Moscow, Idaho.

Oilseed Brassicas from the USDA World collection were planted in a field naturally infested with *Sclerotinia sclerotiorum* at Calhoun, GA. Plants began dying in late March and over 57% of the plants were killed by *Sclerotinia* stem rot. Fifty of 380 lines tested had all plants killed and 5 lines had no plants with symptoms. There was a correlation (R = 0.53, P < 0.0001) between date of initial flowering and percent mortality. Late flowering lines were killed by winter frosts because of cold, ascospore discharge, and high moisture and conditions coincide. Lines flowering in early March were the most heavily damaged, with 50% of the lines with mortalities above 75% and 85% of the lines with mortalities above 50% or higher. Several lines which flowered during that period had fewer than 25% plants damaged by stem rot and are being evaluated as sources of genes for resistance to *Sclerotinia* stem rot.

**A654**

**DENSITY OF SCLEROTIA OF RHIZOCTONIA SOLANI AND INCIDENCE OF SHEATH BLIGHT IN MISSISSIPPI RICE FIELDS,** J. P. Damiane, M. V. Patel, and W. J. Moore. Mississippi State University, Stoneville, MS 38876.

Sixty-seven fields representing various rotation sequences of crops with soybean were sampled over 2 yr for pre-plant levels of *R. solani* and incidence of sheath blight in 'Lemont' rice. Sclerotial densities were positively correlated with percent diseased tillers (PDT; r = 0.54-0.63) and percent disease flood (PDF; r = 0.64-0.67). Adjustment of sclerotial density for viability did not increase the degree of correlation with PDT or PDF. In the 3-yr period prior to sampling, years cropped to rice was generally correlated with increased sclerotial density and viability and incidence of sheath blight. Years cropped to soybean were converse to those of rice. Determination of preplant sclerotial density was not a sufficiently accurate method for predicting development of sheath blight. Rotation of soybean in an area where occurrence of soybean aerial blight is rare also did not appear to increase inoculum of *R. solani* or incidence of sheath blight in rice.

**A655**


Disease infestation by cyst nematode (SCN), stem canker (SSC), bacterial blight (BBB), combination of these diseases, and their effect on the agronomic performance of soybean cultivated at a site studied in the field from 1985-1988. The mean of three-year study for the various treatments: disease infestation of each of SCN, SSC, BBB, combination infestations of all three, and no disease infestation. Mean differences were noted in the number of days to flowering, podding, and protein content of the seeds when compared with the control. Significant negative correlations were observed between disease rating-protein content of the leaves, and highly significant negative correlations were observed between size of nodules, disease rating, and incidence of disease ranking. Incidence of plants with 5 or more podding plants were significantly correlated with the size of nodules, protein content of the leaves, and disease rating-protein content of the leaves, and disease infestation-protein content of the leaves. Significant positive correlations were observed between the size of nodules-protein content of the leaves, the number of pods-plant height, and protein content of the leaves. Highly significant correlations were observed between plant height, protein content of the leaves, disease rating-disease infiltration, and incidence of the leaves-leaf blight. High-yielding plants were associated with increased protein content of the leaves, disease rating-disease infiltration, and incidence of the leaves-leaf blight. No significant effects were observed on soybean cv Bragg affected with combination of diseases.

**A656**

**TIME-TEMPERATURE RELATIONSHIPS IN THE DEVELOPMENT OF SEEDLING DISEASES IN WATER-SEENED RICE,** R. A. Thompson, R. W. Schneider, and S. S. Quesenberry. Department of Plant Pathology & Crop Physiology and Entomology, Louisiana State University Agricultural Center, Baton Rouge, LA 70803.

Seeding disease, caused by *Pithum spp.*, is a major cause of stand failure in water-seeded rice. The disorder has been associated with cool weather shortly after planting and particularly soil temps and emergence data collected from sequential plantings in field soil, either untreated or treated with metalaxyl, were used to construct correlation matrices in order to assess these relationships between relative percent emergence (RPE) and mean, min, and max temps, number of hrs within 2.8 C temp range, and number of hrs below certain temps for up to 8 days after planting. There were significant inverse linear relationships (r) between RPE and mean temps. Hrs below 21.1 (-0.886), 23.9 (-0.925), and 26.7 C (-0.886) during the first 4 days after planting. Cumulative hrs within 2.8 C temp range were not significantly correlated with RPE.

**A657**

**EFFECT OF TEMPERATURE ON THE RATE OF INFECTION OF SOYBEAN SERVINGNOS BY PHOMOPSIS LONGICOLLA,** J.C. Rupel, University of Arkansas, Fayetteville, 72701.

The infection rate of 2-wk-old soybean seedlings (cv Forrest) by *Phomopsis longicolla* was determined at temperatures ranging from 15 to 35°C. The percentage of plant segments infected with *Phomopsis* Longicolla was decreased to a potential degree. The rate of infection and rates of infection were determined by linear regression for each temperature. All regression equations were significant (P<0.0001) and the coefficients of determination varied from 0.76 to 0.96. The optimum temperature for infection was 30 C followed by 25, 30, 35, 40 C in that order. The results were incorporated into a previously published model relating field infection of soybean seedlings to environmental conditions. The linear relationship between conditions rate data and the fit of the model of a coefficient of determination (r^2) of 0.73 to 0.79.

**A658**


*Phomopsis leptostromiformis* (teleomorph Diaportha woodiae) forms latent infections on stems of narrow-leaved lupins (*Lupinus angustifolius*) that normally develop into lesions on senescing plants. The fungus produces a hemiparasitic colonization of stems that causes lupinosis in grazing animals.
Percent prematurely ripened plants was significantly correlated with both external and internal symptoms as well as negatively correlated with seed yields. One hundred seed weights were negatively correlated with external but not internal symptoms. These results support the hypothesis that PM is a factor in premature ripening of sunflower, but also suggest that other factors may be involved and that losses to PM are slight.

A663

effect of calcium sulfate on pod rot of peanut incubated by pythium myriotylum. T. E. Clemente and A. Filonow, Departments of Plant Pathology, North Carolina State University, Raleigh, N. C. 27695 and Oklahoma State University, Stillwater, Ok 74078.

Calcium sulfate was evaluated for reducing pod rot of peanut in the greenhouse and in field microplots. Sand/vermiculite mix in the greenhouse or fumigated soil in microplots were non-infested or infested with 10 or 50 propagules of P. myriotylum/g. At early bloom CaSO4 was applied at 0, 560 and 1,120 kg/ha to the cv. Early Bunch in the greenhouse or at 0, 1,120 and 2,240 kg/ha to the cvs. Florunner and Spano in microplots. At harvest, P. myriotylum was consistently associated with pod rot. Calcium sulfate was generally ineffective (P=0.05) in reducing pod rot at 10 or 50 propagules of P. myriotylum/g. There were no significant (P=0.05) linear correlations between calcium contents of hulls and pod rot severity. Florunner generally had more pod rot and less calcium in hulls than Spano. Calcium sulfate rates had no effect (P=0.05) on the final populations of P. myriotylum in infested mix or soil.

A660

Colonization of soybean pods and seeds by Cercospora kikuchii at different reproductive stages. F. A. Fernández and J. B. Sinclair. Dept. of Plant Pathology, University of Illinois, 1102 S. Goodwin Avenue, Urbana, Il 61801-4709.

In two separate field experiments, individual plants of soybean cvs. Corsoy 79 and Williams 82 were spray-inoculated with a conidial suspension (3.2x10^6) of an isolate of Cercospora kikuchii (CK) at growth stages R2, R3, R4 and R6. Pods and seeds from inoculated and uninoculated plants were harvested at maturity and staged separately. On pods, the earlier the inoculation, the larger the area of pod colonization in both cvs., but the extent of colonization was greater on Williams 82 than on Corsoy 79. At later inoculations, there was an increase in the occurrence of Phomopsis spp. with a concomitant decrease in CK on pods but not on seeds. The incidence of purple seed stain was negligible in both cvs., but CK was recovered from asymptomatic seeds. There was no relationship between pod infection and percentage recovery of CK from seeds.

A661

Characterization of resistance in sunflower to Macrophomina phaseolina, the cause of charcoal rot. L. Ahmad, K. Burney, and P. S. Dyar. Crop Diseases Research Institute, PARC, P.O.Box 1031, Islamabad, Pakistan and *The Botany School, Downing Street, Cambridge, CB2 3EA, U.K.

Four sunflower hybrids, NK-212, Cargill-204, SF-100 and Hysun-30 were tested for disease resistance against 26 isolates of Macrophomina phaseolina collected from growing areas in Pakistan. Stem inoculations were made using a toothpick method and spread of charcoal rot was measured from the point of inoculation. Disease reaction was scored on a 0 to 5 scale where 0=no rot and 5=plant dead. Resistance was characterized using an analysis of variance (Vanderplank, 1984). A highly significant main effect for both varieties and isolates was detected. This indicated a highly significant difference in horizontal resistance between varieties and in aggressiveness between the isolates. However, the interaction varieties x isolates was insignificant, indicating no evidence of vertical resistance.

A662


The effects of injection of hybrid sunflower stems with a conidial suspension of Phoma macdonaldii (PM) 2 wk prior to anthesis, at anthesis, and 2 wk post-anthesis were evaluated over 3 yrs at Brookings, SD. Inoculation with PM consistently produced greater external symptoms and internal stem decay when compared to uninoculated and sterile water infected control treatments. Premature ripened plants was greater in PM inoculated than the uninoculated control plots. Inoculation with PM had no significant effect on either seed yield or oil content, but 100 seed weight was significantly reduced by inoculation with PM 2 wk post-anthesis.
A667

RESISTANCE TO BENZIMIDAZOLE FUNGICIDES IN PSEUDOCRISPORELLA HERPOTRICHIOIDES IN WASHINGTON AND OREGON. T. D. Murray*, R. W. Smiley*, and W. Uddin*, Department of Plant Pathology, Washington State University, Pullman, WA 99164, and Columbia Basin ARC, Oregon State University, Pendleton, OR 97801.

Mycoelia of *P. herpotrichioides* were isolated on water agar + 100 µg/ml of fubinamid from 62 wheat samples with symptoms of eye spot. Pure cultures were maintained on potato dextrose agar (PDA), then transferred to FDA amended with Gabeym, Thidibenzol, Thiophanate-methyl, Propiconazol, Flusilazole, or prochloraz at concentrations (µg/l) of 3, 3, 4.5, 10.6, 5.1, and 5.1 µg/ml, respectively. Of 275 total isolates collected, 108 isolates from 9 fields, 3 in Oregon and 6 in Washington, were resistant to the benzimidazole fungicides. None of the isolates were able to grow on PDA amended with the demethylation inhibitors. Cropping practices in fields with resistant isolates ranged from 10-30 continuous wheat under irrigation to 2-3 yr rotations of wheat in dryland production; all fields with resistant isolates had received 5-8 applications of a benzimidazole fungicide for control of eye-spoor since 1977.

A668


Common root rot, caused by *Cochliobolus sativus*, is widespread problem on spring wheat and barley. Crop rotation and partially resistant cultivars have been the main controls but some newer fungicides have potential to control root rot when applied as seed treatments. Five seed treatment fungicides were compared in replicated trials planted on land known to have high inoculum levels of *C. sativus*. Treatment with nuarmil and imazalin reduced root rot disease rating (DR) but yield responses were inconsistent. Triadimenoil also reduced root rot and BARLEY gave an 8% average yield increase in four years of trials. The subchron internode index was a poor predictor of yield response and its suitability as a disease measurement in evaluation of seed treatments for control of common root rot is questioned.

A669

EFFECT OF TIME OF FUNGICIDE APPLICATION ON SHEATH BLIGHT CONTROL IN RICE. B.E. Groth, Rice Research Station, La. Agri. Exp. Stn., L.S.U. Agricultural Center, P.O. Box 1449, Crowley, LA 70327-1449.

Experimental plots of the rice cultivar Leont were inoculated with Rhizoctonia solani (Ag-lA). Propiconazol, benomy and iprodione fungicides were applied with a CO2 backpack sprayer delivering 95 1/3a at the panicle 2 mm, hooping, and/or % percent heading stages of growth at 8:00, 10:00, 12:00, 14:00, 16:00, and 18:00 hours at labeled rates. Plots were rated for disease development at maturity and harvested. Fungicides significantly reduced increased yields. Time of fungicide application did not affect sheath blight control but yields were significantly reduced compared to the unsprayed control at certain timings. The only weather factor that appeared to affect fungicide performance was rainfall before or after spraying reduced yield increases due to fungicide application.

A671

FAILURE OF ALTERNATIVE FUNGICIDE REGIME TO DELAY DICARBOXIMIDE RESISTANCE IN *BOTRYTIS CINEREA*. B.J. Vail and G.W. Moorman, Dept. Plant Pathology, Penn State University, Univ. Park, PA 16802.

Strains of *Botrytis cinerea* resistant to the dicarboximide vinclozolin were detected in 80% of greenhouses surveyed in Pennsylvania, indicating the need for alternative fungicide regimes for effective disease control. Fungicide regimes were evaluated using a leaf disc assay which quantified disease incidence (df discs infected) and percent visually resistant control. One hundred percent resistance to vinclozolin was detected after 1 application of vinclozolin, with a concurrent loss of disease control. Disease control with the non-systemics, chlorothalonil and copper hydroxide, was 72% and 50%, respectively. Neither of these fungicides selected for vinclozolin resistance. Rotations, or full or half-strength mixtures of vinclozolin, with either non-systemic failed to delay the development of resistance. In all cases, disease control was characteristic of the non-systemic companion fungicide. In the rotations, the level of vinclozolin resistance did not decrease when the non-systemic was applied, suggesting that vinclozolin resistant populations were stable. Stability of the resistant strain in the absence of vinclozolin may be partially due to the similarity between the two strains of three for five fitness parameters evaluated.

A672

TEMPORAL DYNAMICS OF CHLOROTHALONIL RESIDUES ON PEA-NUT FOLIAGE. V.J. Elliott and H.W. Spurr Jr. USDA-ARS-SAA Crops Research Lab, Oxford, NC and Department of Plant Pathology, North Carolina State University, Raleigh.

Field studies were conducted to determine the persistence of chlorothalonil residues on the foliage of peanut (*Arachis hypogaea*) cv. Floringle. Bravo 720 (formulated at 7200/l Fumigant Protection Co. Mentor, OH) was applied at a rate of 1.5 pints per acre in 40 gal of water per acre using a hand drawn plot sprayer operated with D3-25 nozzles at 40 psi. Upper canopy leaves were sampled at increasing times after application by taking 10.1cm disks from each plot. Five replicate samples were taken at each disk. Leaf disks were washed in toluene to remove residues and chlorothalonil levels were quantified using electron capture gas chromatography. An exponential decay model reasonably described the decline in chlorothalonil residues over time. The half-life varied between 13 and 17 days. Variations in half-life values was correlated to rainfall rates during each experiment.

A673


The effects of simultaneous infection by BYDV-PAV and *Puccinia coronata* f. sp. *avenae* (P.c.a.) on the yield and yield components of two spring oat varieties were examined in 1989. The objective of this field study was to develop an empirical crop loss model. Two varieties, Noble (susc. P.c.a., intol. BYDV) and Ogle (susc. P.c.a., tol. BYDV), were used in the study. All plots were inoculated with BYDV-PAV by application of viruliferous aphids (*Rhopalusphilus padi*). P.c.a. treatments were a combination of two disease levels, and three fungicide levels (Othane N-45). The experiment was repeated at two locations. Disease severity, yield, # florets and seeds per head, # oil, # protein and seed coat integrity were determined. Crop rot severity was assessed on several dates. BYDV-PAV incidence was determined using ELISA. The results of a second croploss study of P.c.a. infection alone also will be reported.

A674


Tomato (cv. Rutgers) seedlings infected with Meloidogyne incognita and *N. javanica* (RKN) were transplanted at a high density to raised beds in the trial site. Tomato foliage was removed 3 m, later the plot area was planted to RS-2 susceptible bean cva, which were removed at the time of trial establishment. A border of *Crotalaria spectabilis* was established on three sides of the infestation, and furrow irrigation was supplied on the open side. The RKN-susceptible cv. Calina was planted in the infested beds and uninfested beds outside the *Crotalaria* border at 15 seeds/m. Each treatment plot was 4 rows, 4 m-long,
and replicated 6X. Dry seed weight was 147 and 398 kg/ba for the RKN-infested and uninfested plots, respectively, indicating a significant 632 yield reduction (kg/ha) due to RKN. Similar results obtained when the trial was repeated using the bean cv. Calima and CIAT bean line PVA 916.

A675
NOCLEUS NUMBER IN FUSARIUM CONIDIA. L. J. Stepanske and J. E. Partridge. Dept. of Plant Pathology, University of Nebraska, Lincoln, NE 68083-0722.

Using fluorescence microscopy and the DNA-binding stain 4',6'-diamidino-2-phenylindole (DAPI), the number of nuclei per conidium was compared in cultures grown on liquid and solid (agar) media. A minimum of ten isolates each of nine species (S. rolfsii, F. subglutinans, F. proliferatum, F. anthophilum, F. dimani, F. nymagani, F. napiforme, F. beemiforme, and F. oxysporum) were examined. The number of conidia per isolate in both liquid and solid medium was assessed in each replicate. Harvested conidia were fixed in Carnoy's solution, rinsed in phosphate buffer, stained for 5 minutes in DAPI, rinsed, suspended and examined. The range of conidia with multiple nuclei was from <1% to >5%. Conidia with multiple nuclei from solid media did not exceed 4% whereas their conidial equivalents from liquid were normally above 4%. The number of nuclei/conidium was more isolated than species dependent.

A676

M. tassiana (de Not) Johans. (MT), with a Gladiosporella herbarum (Pers.) Link ex Gray anamorph, was detected in overmowed apple scab infected leaves from Utah. Many morphological characteristics are similar to V. inaequalis (VI), and when both fungi were present it was tedious to determine the maturity of the VI ascospores. We present the following comparisons of both fungi to minimize confusion. MT and VI pseudohyphae are similar size, but in MT they lack setae and pseudopharynges. Ascii of MT are saccate versus cylindrical for VI, and are about one-third as numerous. Ascospores of both fungi have two to four unequal size cells, but in MT they are 20-22u long and hyaline, instead of 14u long and colored as in VI. Mature MT ascospores can germinate within the pseudothecium, grow through the ostiole, and produce conidiophores bearing the anamorph. No disease developed when mature apples were inoculated with mycelium and conidia of C. herbarum.

A677

An association between dogwood anthracnose severity and acid rain has been suggested. Also, tolerance to extreme acidity for germinating Discella conidia has been described. Complete liquid medium (CM) was amended with HCl to pH 2.5, 3.5, 4.5, 5.5, and 6.2. Growth of Discella isolates from GA, MA, NY, and TN was monitored over this pH range for a 14-day period. Growth of the GA isolate (GA-1) was limited at pH 2.5 with mg hydroxide (CaCO3) weight per milliliter. Growth of GA-1 increased as pH increased, to a maximum of 51mg at pH 6.2. Based upon this preliminary study, Discella isolates do not appear unusually tolerant of increased hydrogen ion concentrations in vitro.

A678

Morphology of sclerotial development was studied in Rhizoctonia solani Kuhn among anastomosis groups (AG) 2, 3, 4, 5, 6, 7, 8, 9 and AG-BI (Bridgeport). Sclerotial isolates from purified cultures belonging to the various anastomosis groups growing on PDA and were processed for scanning electron microscopy. The development of sclerota in AGs 2, 3, 4, 5, 6, 7, 8 and 9 was from a lateral "trunk" hypha and the sclerotia were made up of monilioid cells developing from the trunk hypha. This form of sclerotial development was referred to as the lateral type. In AG-7 and AG-BI, sclerotia developed as interwoven loose hyphae and monilioid cells within the loose hyphae. Monilioid cells were observed in all the AGs studied and dense material binding the monilioid cells was also detected.

A679
PRODUCTION OF A SELF-INHIBITOR BY COLLETOTRICHIUM GRAMINICOLA: SIGNIFICANCE TO SURVIVAL. B. Leir and R.L. Niswander, Department of Botany and Plant Pathology, Purdue University, West Lafayette, IN 47907.

The conidial mucus of Colletotrichum graminicola contained a low molecular weight material that inhibited conidial germination. The inhibitory material was water soluble and extractable into aqueous solvents by partitioning mucus against organic solvents. Production of the inhibitor depended in part on the strain. Conidia germination required 3 days maturation after their formation in order to germinate. Depending on the substrate, conidia either formed germ tubes or appressoria, and this was influenced by conidium concentration. At high concentrations of conidia germination was inhibited completely. As concentrations were reduced, germination occurred first by appressorium formation and then by germ tube formation. Appressorium formation and/or germ tube formation was completely prevented by a partially purified inhibitor preparation. The results indicate that a self-inhibitor is present in conidial mucus and leaches from conidia, suggesting that the inhibitor prevents conidia from germinating under adverse conditions.

A680
IMPROVED MEDIA FOR TESTING THE USTILAGO HORDEI MATING REACTION. Alfredo U. Martinez-Espinoza, Michael L. Bjarko, and John E. Sherwood, Dept. of Plant Pathology, Montana State University, Bozeman 59717.

Mating of U. hordei sporioid, which is controlled by a single locus with two alleles, results in the formation of dikaryotic mycelium which is pathogenic on barley. Ex planta mycelium formation can be used to determine the mating-type of unknowns and for population analysis and study of mutants. Several media and growth conditions were analyzed to optimize dikaryotic mycelium formation and to determine the incubation time before mycelium was observable. Mycelial growth was consistent at 20°C or 25°C, but generally stable at 6°C or 16°C. The nuclear condition of yeast and mycelial cells was confirmed by fluorescence microscopy.

A681 Withdrawn

A682
GENETIC DIVERSITY WITHIN POPULATIONS OF FUSARIUM SECTION LISOGLA FROM CORN AND SORGHUM IN KANSAS. C. Chaisrisopok and J. F. Leslje. Dept. of Plant Pathology, Throckmorton Hall, Kansas State University, Manhattan, Kansas 66506-5502.

We examined 359 isolates belonging to Fusarium section Liseola recovered from separate plants at 32 sites in Kansas for mating-type and vegetative compatibility group (VCG) phenotypes. Members of all six known mating populations (A-F) were identified in this set of isolates. From corn tissue, 4 isolates were analyzed from each of 13 sites and at least 60 isolates were analyzed from each of two sites. Among isolates recovered from corn, members of the A and D populations accounted for 50-70% of the total population although there was some variation from site to site. From sorghum tissue, 4 isolates were analyzed from each of 15 sites and at least 60 isolates were analyzed from each of two sites. Among isolates recovered from sorghum, members of the D and F populations were the most common. More than 50% of the isolates from the sites with 60 isolates per site belonged to the F mating population. In the total population, the distribution of the mating type alleles was not skewed, although some local perturbations were found. Thus, the possibility for sexual genetic exchange under field conditions exists. Based on preliminary data, the relative number of VCGs appears to be quite large.

A683
CRYOGENIC PRESERVATION OF RHIZOCTONIA CULTURES. B. O. Nelson and I. Kural, Dept. of Plant Pathology, North Dakota State Univ., Fargo, ND 58105.

Mycelium of Rhizoctonia solani and binucleate Rhizoctonia spp. on potato dextrose agar (PDA) can be maintained for extended periods in cryogenic storage at -80°C. Blocks of PDA (100x45mm) with hyphae from 6-12 day old cultures were placed in sterile, polypropylene cryogenic vials (12.5 x 41 mm) with 1 ml of 10% sterile glycerol. Vials were then cooled at 4°C for 24 h followed by 24 h at -80°C and stored in an ultrafreezer. To initiate cultures from cryogenic storage, vials were immersed in water at 35°C until the ice had dissipated, then the PDA blocks plus mycelium were immediately
transferred to fresh PDA. Growth was observed within 24-48 hr at 22 C. Eleven anastomosis groups of R. solani plus 13 anastomosis groups of binecule Rhizoctonia spp. were successfully maintained from 4-24 months in cryogenic storage. Rhizoctonia solani also survived in infected soybean stems stored at -50 E.

A684
VEGETATIVE COMPATIBILITY GROUP DIVERSITY WITHIN POPULATIONS OF FUSARIUM MONILIFERUM ISOLATED FROM CORN SEED. R. Farrokh-Nelad and J. F. Leslie. Dept. of Plant Pathology, Throckmorton Hall, Kansas State University, Manhattan, Kansas 66506-5502.

Fusarium moniliforme was isolated from corn seed from two cultivars both grown at four locations in the North Central United States. From the eight seed lots 123 isolates of F. moniliforme were recovered. Six mutations were generated in each isolate and each resulted in a hetero-typed reaction to determine compatible and incompatible interactions. Within a seed lot 45% and 80% of the isolates belonged to a unique vegetative compatibility group (VCG), with an average of 60%. At two sites, isolates belonging to a common VCG were recovered from each of the two cultivars. Each of the 74 VCGs identified, only three were present at more than just one site. Of these three, one was found at three sites and the other two at two sites each. Isolates belonging to the most frequent VCG were present at a frequency of 47% within their seed lot, but only 6% within the population as a whole. These data suggest that populations of F. moniliforme are very localized and are genetically diverse, and that seed movement could provide a mechanism to explain the variability observed in commercial fields.

A685
APPRESSORIUM DEVELOPMENT IN UROMYCIDS: VESICLE AND MICROBULBULE DISTRIBUTION. Y.H. Kwak and H. C. Hock, Department of Plant Pathology, Cornell University, New York State Agricultural Experiment Station, Geneva, N.Y. 14456.

Uromyces appendiculatus uredospores germinated in appressorium development in response to soluble factors (e.g. artificial ridges on plastic substrates, host stomata) by switching from polarized to non-polarized (vesicle) growth. Distribution of apical vesicles and microbulbules during appressorium development were determined for germinations grown on polycarbonate substrates bearing inductive ridges that trigger appressorium initiation. Germinations were examined through 12 min time course following contact with the ridge. Serial longitudinal sections through non-differentiating germinations revealed that most apical vesicles were located within 2 μm of the substratum, and 4 μm distal to the apex. The majority of cytoblastic microbulbules were nearest the substratum and were oriented in longitudinal profiles parallel to the direction of germination growth. During early appressorium development (4 min after ridge contact) the vesicles became redistributed at sites nearest the ridge in actively swelling regions of the appressorium. Vesicles in the actively swelling region were likely associated with apical vesicles, whereas the microbulbules in other regions of the developing appressorium exhibited longitudinal profiles, especially over the ridge. These changes in vesicle and microbulbule distribution indicate that signal reception for appressorium initiation occurred within 4 min of ridge contact. Because the vesicles were always located in the region of apical growth or swelling, they were likely involved in the expansion of the germination apex. The microbulbules may be involved in vesicle transport to the new regions of growth.

A686
SEQUENCE HOMOLOGY BETWEEN NEUROSPORA CRASSA MATING TYPE GENES AND DNA FROM OTHER ASCOMYCETES. C.R. Cramer, D.O. TeBeest and F.W. Spiegel, Departments of Plant Pathology and Botany and Microbiology, University of Arkansas, Fayetteville, AR 72701.

Heterothallism in ascomycetes increases the likelihood of outcrossing and has been reported in numerous taxa. Whether this theoretically advantageous mode of reproduction has evolved once or has been the historical product of independent origin from thallic ancestors is unclear. Starting with the assumption that the commonly found, one locus, two allele mating type system (α/α or α/α) is homologous for many ascomycetes, the recently isolated mating type genes from Neurospora crassa (Glass, N.L. et al. Science, 1988, 241: 570) were used to probe Southern blots containing genomic DNA from a wide taxonomic range of heterothallic ascomycetes. Some of these ascomycetes show homology with the Neurospora mating type genes. These results may be useful for understanding the evolution of sexual systems in ascomycetes and for determining the phylogeny of the group.

A687
SUDDEN DEATH SYNDROME: SCANNING ELECTRON AND LIGHT MICROSCOPIC OBSERVATIONS OF SOYBEAN ROOTS INFECTED WITH FUSARIUM SOLANI FORM A. K. S. McLean, G. W. Lawrence, and K. W. Roy. Dept. of Plant Pathology and Weed Science, Mississippi State University, Mississippi State, MS 39762.

Soybean roots inoculated in vitro with Fusarium solani form A (FS-A) were examined by scanning electron (SEM) and light microscopy (LM) to observe pathogenesis. Tissue was collected from tap roots at 4-hour intervals and prepared by standard fixation procedures. Callus on root surfaces germinated with one or two germ tubes within 4 hours. Eight hours after inoculation hyphae and germ tubes were observed to have entered roots by direct penetration. After 24 hours, hyphae were observed growing intercellularly within epidermal and cortical tissues. Epidermal and cortical cells in the infected region began to collapse 48 hours after inoculation. Intercellular hyphae were found within the cortical tissue after cell degradation occurred. Chlamydospores of FS-A were observed within the cortex.

A688
REACTI ON OF TALL FESCUE CULTIVARS INOCULATED WITH STEM RUST. R. K. Wells and K. L. Barker, USDA ARS, NIFSPR, Corvallis, Oregon 97331-7102.

Twenty cultivars of tall fescue were inoculated with uredospores of stem rust. Five-week-old seedlings (2-3 leaves on 2-3 tillers) were inoculated and scored 14 da later (3 replications). F. graminearum (Pustule types 0 & 1 = resistant; 2, 3, or 4 = susceptible) were used to calculate % resistant plants and a Disease Severity Index of 1-5 (DSI). Resistant plants as 5-week-old seedlings ranged from 4-10% for K-31, Flamin 1, and Mesa (DSI range 3.7-4.14). Resistant plants as 10-week-old seedlings ranged from 0 to 6% for CIMMWR 1 and CI-307 (DSI 4.61-4.99) to 26% for Arid and KY-31 (DSI 3.71-3.76). A highly significant (P<0.01) difference in DSI among cultivars occurred, but all cultivars were considered susceptible to stem rust; 39 stem-resistant plants were saved as a source of germplasm.

A689
 FUNGI ISOLATED FROM WHITE LUPINS IN MINNESOTA. R. A. Kalis, E. L. Stewart, and R. A. Meronuck, Department of Plant Pathology, University of Minnesota, St. Paul, MN 55108.

Lupinus albus (white lupin) is currently being grown on a limited acreage in Minnesota as an alternative crop for a source of animal protein and for human consumption. Potential fungal pathogens were isolated from tissue and seed material during production in 1988. Three regions of the state were studied: Faribault, CI-307, Mankato, Maximize, Red Rock EF, and Shortstop (DSI ranged 4.72-4.98) for K-31, Flamin 1, and Mesa (DSI range 3.76-4.14). Resistant plants as 10-week-old seedlings ranged from 0 to 6% for CIMMWR 1 and CI-307 (DSI 4.61-4.99) to 26% for Arid and KY-31 (DSI 3.71-3.76). A highly significant (P<0.01) difference in DSI among cultivars occurred, but all cultivars were considered susceptible to stem rust; 39 stem-resistant plants were saved as a source of germplasm.

A690
 OCCURRENCE OF CLAVICLUPUS PURPUREA AND HYPERPARASITES ON BERMUDA GRASS (CYNOGLOSSUM DACTYLOIDES) AND OLD WORLD BLUESTEM (BROMETHOCHLOA SPR.) K. E. Conway and C. M. Tafasefro, Depts. of Plant Path. and Agronomy, Oklahoma State Univ., Stillwater, OK 74078.

Production of range, pasture and turf grass seed in the Great Plains has increased as marginal lands are being removed from wheat production. In Oklahoma there are approximately 121,500 ha of seed production and may reach 405,000 ha. Heavy haydow production by C. purpurea (CP) has occurred in Oklahoma and Texas on old world bluestem (OWB) each year since 1986. An OWB seed sample from Houston, TX contained 13 to 22% sclerotial contamination. Honeydow occurred each year on bermudagrass (BG) but only at Stillwater. This is the first report of CP on BG. Sclerotia were easier to observe in OWB because seed is larger than BG. Sclerotia were observed growing in OWB and BG within OWB but not from BG. Sclerotia occurred on OWB and BG in both early summer and fall seed production, but was more prevalent in the fall. Two hyperparasitic fungi were recorded on the hondeny stage of CP. Fassianou heterospermum was common on both and Cerebellaria androgynopsis was on OWB.

A691
 INFECTION PERIOD AND SITE OF INFECTION OF ANISOCYMA ANOMALA ON CORVUS AVELLANA. J.K. Stone, K. B. Johnson, and J.W. Pechelt. Dept. of Botany and Plant Pathology, Oregon State University, Corvallis, OR 97331.

Between 1 Feb - 30 April, 1988 and 2 Dec - 26 April, 1989. 13 sets of thirty 2-yr old hazelnut trees were serially exposed to natural inoculum of A. anomala with a diseased orchard. Individual sets of trees were exposed for 1 wk, replaced with a new set, and then inoculated in an isolated area until symptoms developed (14-16 mo). Only trees exposed after bud break developed symptoms. In 4 hours between 14 March and 4 April, and 32% of trees exposed after April 4 became diseased. In a related study, 3 sets of 10
trees were serially inoculated with an ascospore suspension applied to opening buds and young shoots at weekly intervals between 4 - 25 April, 1988. Of trees inoculated, 80% developed symptoms. The natural infection period for S. anomala appears to coincide with bud burst and early shoot elongation.

A692

PRODUCTION OF HUILACOHE, USTILAGO MAYSIS. J. K. Pataky
Dept. of Plant Pathology, Univ. of Illinois, Urbana, IL 61801.

Common smut, caused by Ustilago maydis, is a devastating disease of sweet corn (Zea mays); but in Mexico, smut galls on ears are an edible delicacy known as huilacoches. Recently, huilacoche has been marketed as "maize mushroom" in the U.S. Buyers in New York City paid growers $0.50/ear for sweet corn with large ear galls. Huilacoche is harvested about 1 wk prior to sweet corn for fresh market. To commercially produce huilacoche, ear galls must be induced consistently. Sweet corn hybrids with greater than 40% incidence of ear galls from natural infection were identified and crossed to create a population from which new varieties with high incidence of ear galls were selected. In 1989, stalk infection and leaf whorl spray inoculation procedures induced only 42 and 0.4% incidence of ear galls, respectively, and 46% incidence of stalk or leaf galls. Plant growth stage affected the host tissues on which galls formed. For example, naturally-infected plants of 'Candy Bar' planted 17 May had 41% ear and stalk galls, respectively, compared with 0% and 24% when planted 29 May.

A693

ISOLATION AND PATHOGENICITY OF BORTYCI CINEREA ASSOCIATED WITH CLAVODE ROT OF PRICKLY PEAR (OPUNTIA SPECIES). J. O. Kuit, Horticulture Research Lab., College of Agriculture, Texas A&M University, Kingsville, Texas 78363.

A field survey for pathogenic mycoflora of naturally infected stems (cladodes) of economically important prickly pear germplasm at Texas A&M University farm was made over two years. Seven species of fungi were frequently isolated. Botrytis cinerea accounted for 25% of the isolates; species of Colletotrichum and Glomeraea accounted for 16%, Aspergillus 15%, Phytophthora 11% and Macrophomina and Sclerotium 9%. This is the first record of B. cinerea as a pathogen of prickly pear. Pathogenicity of the B. cinerea isolate was tested on 20 accessions of 8 Opuntia species under greenhouse conditions. Fifteen accessions were found to be susceptible or highly susceptible while 10 accessions were resistant or highly resistant. Symptoms of pathogenicity of B. cinerea on the Opuntia species include soft rot and discoloration of affected tissues.

A694

PHYTOXICITY OF CULTURE FILTRATES FROM FUSARIUM SOLANI ISOLATED FROM SOYBEAN. S. M. Lim, H. S. Song, and L. E. Gray, USDA-ARS, Department of Plant Pathology, Univ. of Illinois, Urbana, IL 61801.

Culture filtrates from isolates of Fusarium solani pathogenic to soybeans were phytotoxic to soybean callus, cotyledons, and germinating seeds. Culture filtrates of the fungus grown in a synthetic culture medium for 3 weeks at 24°C caused browning in soybean, maize, tobacco, carrot and cotton callus indicating that the toxin produced by F. solani is not a host-specific phytotoxin. Drops of the culture filtrates on wounded soybean cotyledons produced chlorosis 4 days after inoculation at 24°C. The root growth of germinating seeds of soybean and maize was inhibited by the culture filtrates. Variation in responses of soybean callus and cotyledons to culture filtrates was observed among fungal isolates from soybean cultivars. Ethyl acetate extracts of the culture filtrate were not phytotoxic and the extracts were negative to known T-2 toxins indicating that T-2 toxin may not be involved in the development of soybean sudden death syndrome.

A695

PARTIAL CHARACTERIZATION OF A HOST-SPECIFIC PHYTOXIN FROM CULTURE FILTRATES OF SEPTORIA GYLCINES. H. S. Song, S. M. Lim, and J. M. Clark, Jr., Department of Plant Pathology and USDA-ARS, University of Illinois, IL 61801.

A host-specific phytotoxin isolated from culture filtrates of Septoria glycines is an autoclave-stable, anionic, water-soluble, high molecular weight substance(s) causing typical disease symptoms of brown spot on cotyledons and seedlings of soybean. The toxin was purified by sequential use of CM-cellulose treatment, DEAE-cellulose chromatography, dialysis, gel filtration, and 5% charcoal treatment. The molecular weight of the phytotoxin is approximately 20,000. Drying the toxin under flash-evaporation at 46°C in vacuo destroys more than 99% of the toxin activity. Partial acid hydrolysis with 1 N HCl at 90°C for 3 hr does not abolish the toxicity. Toxin activity is destroyed by periodate oxidation. Incubation of toxin with D-mannosidase, D-galactosidase, and D-glucosidase also reduces activity. These results indicate that the toxin contains polysaccharide, a glycosyl component is essential for the activity, and mannose, galactose, and glucose may be an essential portion of the toxin.

A696

PRODUCTION OF PERITHEICA IN NECTRIA HAEMATOCOCCA MPV, PATHOGENIC ON PEA. P.S. Brey, Botany School, Cambridge University, Downing Site, Cambridge, CB2 3EA, England.

The influence of light intensity on the production of perithecia in Nectria haematococca MPV, a broad host range pathogen and the perfect state of Fusarium solani f.sp. pisi, was investigated. Four isolates were grown under different regimes of light and dark, both before and after crossing. Cool white fluorescent tubes were used as a light source of varying intensity 20-150 μmol m⁻²s⁻¹. Cultures were incubated at 21°C. A marked variation in perithecia production with post-spermatization light intensity was found in three isolates. Maximum numbers of perithecia were formed at light levels 20-40 μmol m⁻²s⁻¹. In addition, incubation in the dark prior to crossing significantly increased perithecia production in these three isolates.

A697

EFFECT OF SUPPLEMENTAL CALCIUM ON DECAY OF APPLE CAUSED BY GLOMERELLA CINGULATA. W. S. Conway, Hort. Crops Quality Lab., Beltsville, MD 20703, C. E. Sams, Univ. of Tennessee, Knoxville, TN 37996, and J. A. Abbott, Instr. and Sensing Lab, Beltsville, MD 20703.

Apples were pressure infiltrated at harvest with solutions of CaCl₂ to determine the effect of increased tissue calcium content has on decay caused by Glomerella cingulata. The fruit were inoculated with a conidial suspension of G. cingulata following 6 months storage at 0°C. Analysis of fruit tissue calcium indicated that a 4x solution of CaCl₂ pressure infiltrated into the fruit, resulted in a fruit calcium concentration of approximately 1000 μg/g dry weight. Decay caused by G. cingulata was reduced by approximately 60%. In previous work, similar calcium content which were inoculated with Penicillium expansum had only about 30% less decay than nontreated fruit. Increased tissue calcium may differentially inhibit tissue maceration by pestic enzyme activity of postharvest decay fungi.

A699

INJECTION OF SOYBEAN ROOTS WITH SCLEROTIO FORMING ISOLATES OF COLLETOTRICUM TRUNCATUM. Mahmood Khoo, R. E. Wagner, and J. B. Sinclair, Dept. of Plant Pathology, University of Illinois at Urbana-Champaign, Urbana, IL 61801-4709.

The pathogenicity of three sclerotia-forming isolates and one non-sclerotia-forming isolate of Colletotrichum truncatum (CT), cause of soybean foliar anthracnose, were compared on the top-root of soybean plants cv. Coronoy using aeronic culture at 25°C. Plants were inoculated by dipping roots in a conidial suspension (2.2x10⁶) or by attaching to the roots a piece of agar-infiltrated foam colonized by the fungus. One wk after inoculation, a 5-cm section of top-root was removed and placed on moist filter paper in a sterile culture plate. After 1.0 wk at 25°C, the aver number of acervuli of CT ranged from 25-50/root. Seedling root systems of each isolate produces more acervuli than the non-sclerotia-forming isolate. Acervuli number was the highest on roots inoculated using the foam colonized with CT. This is the first report of CT causing disease on soybean roots.
A700
SELECTIVE MEDIUM FOR NITRATE NONUTILIZING MUTANTS OF FUSARIA OXYSPORUM. K. F. Toth and M. L. Lacy. Dept. of Botany & Plant Pathology, Michigan State University, East Lansing, MI 48824.

An agar medium was developed which slows the growth of F. oxysporum f. sp. apii race 2 wild-type (WT) strains but not chloride-tolerant nitrate-nonutilizing mutants (nits) of those strains. The medium (PC*+) consisted of 500 ml potato-carrot broth (filtrate from 20 g each of carrots and potatoes autoclaved for 30 min in 500 ml Hg), 500 ml H2O, 40 g KCl, 0.8 g PO4, 0.25 g chloramphenicol, and 15 g agar. PC*+ was steamed for 1 hr, dispensed, and was tested by scraping a sterile needle across colonies growing on other media, stub-inoculating plates of PC*+ 5 times, and measuring colony size after 5 days. The mean colony diameters from nits were 1.3-5.9 X larger than colonies from WT strains. Colonies grown from suspensions of conidia and chlamydospores of nits had diameters of 0.2-3 cm after 5 days on PC*+. The colony diameters from WT strains were all <0.2 cm. PC*+ is being tested for its ability to enumerate populations of nits added to soils.

A704
VIRULENCE AND RACE FREQUENCIES OF PIUCCINIA CORONATA IN CANADA DURING 1974-1989. L. Chong and J.A. Kolmer. Agriculture Canada, 195 Dafoe Road, Winnipeg, R3T 2M9

The changes in virulence and race frequencies of Puccinia coronata f. sp.avenae in Canada since 1974 were examined. To 1986, wheat and prairie rust populations were dominated by avirulent and simple races. Since 1987, races with virulence to Pc39 predominated in the east. During 1974-1989 in the east, virulence frequency to Pc35 fluctuated at levels 22-53%, to Pc56 at levels 9-42%, and to Pc40, Pc46, and Pc50 at levels 1-24%. During the same period in the prairie region, virulence frequency to Pc35 fluctuated at 20-51%, to Pc40 at levels 24-60%, while virulence to Pc46 increased from 8% to 24%. Gene Pc39 has been used in the east since 1983. Virulence to this gene was first detected in 1985 and increased to 87% by 1988. Cultivars with Pc38 and Pc39 have been grown in the prairie region since 1982. Virulence to this gene combination was first detected in 1987 and increased to 22% by 1989.

A702
SENSITIVITY OF TELIOSPORES OF TILLETIA TRITICI AND T. CONTROVERSA TO METHYL BROMIDE. J. L. Smilnick, P. L. Hartsell, B. J. Benson, R. J. Coates, J. D. McKinney, and J. C. Tehets. USDA, ARS, PNA, HCR, 2011 South Peach Avenue, Fresno, CA 93727

Teliospores and spores of Tilletia tritici (Tt) and T. controversa (Tc) were mixed with wheat seeds (cvs. Daws and Itana) and fumigated 24 hr at 24-27C with methyl bromide (MB). The wheat moisture content (MC) was 11% or 15% before use. After fumigation, the teliospores were incubated at 20C 1 wk or 5C 8 wk for Tt and Tc, respectively. Tt was about twice as resistant to MB as Tc. Spores did not protect teliospores from MB. Results from both wheat cvs. were similar. MC greatly influenced MB activity. On wheat of 11% MC, ED95 (± 95% CI) MB doses (g/cu m) to prevent germination were 161 (127.238) and 320 (279,473) for Tt and Tc, respectively, while on wheat of 15% MC, ED95 (± 95% CI) MB doses were 25 (20,30) and 58 (46,111), respectively. Since high MB doses and high wheat MC were needed for efficacy, the practicality of MB to decontaminate wheat containing wheat is questionable for plant quarantine purposes. Repeated fumigations, vacuum fumigations, or other fumigants may hold promise.

A706
A CHARACTERIZATION OF FUSARIA ASSOCIATED WITH CORN, COTTON, AND SORGHUM IN SOUTH TEXAS. A.S. Ring and G.N. Odvody, Texas A&M Exp. Station, Route 2, Box 589, Corpus Christi, TX 78410

Numbers and species of Fusaria from corn, cotton, and sorghum leaves, roots, inflorescences, stems, and rhizosphere soil grown in a farming systems crop rotation experiment, Corpus Christi, Texas, were determined during crop development. Mean numbers of Fusaria were from highest to lowest on corn tassels; corn and sorghum leaves, roots, and soil; cotton seed; and cotton tissues and soil. Numbers of Fusaria increased with crop maturation on corn and sorghum, decreased in soil, and remained low on cotton tissues. Fusarium species isolated were: F. solani, F. equiseti, F. semitectum, Fusarium spp. section Liseola, F. oxysporum, and F. chlamydosporum. Predominant Fusaria were F. solani and F. equiseti below-ground, and Fusarium spp. section Liseola and F. semitectum above-ground. Corn and sorghum tissues contained large numbers of Fusarium spp. section Liseola spp., whereas cotton did not contain large numbers of any Fusaria. Overall relative percentages of Fusarium species did not change during maturation.
A708
TRANFORMATION AND COTRANSFORMATION OF THE TAKE-ALL FUNGUS, GAEANNOMENOCES GRAMINIS, TO PHLOEMYCIN RESISTANCE. Alice L. Peterson and Joan M. Hensen, Deps. of Plant Pathology and Microbiology, Montana State University, Bozeman 59717.

Gaeannomencyes graminis var. graminis and G. g. tritici (the take-all fungus) were transformed to phloemycin resistance by an improved transformation procedure with pAN6-1, a plasmid encoding the bgl gene of Streptomyces griseus. G. graminis var. graminis transformed with pAN6-1 and pP13, a plasmid encoding resistance to benomyl. Vector DNA apparently was integrated into the fungal genome in all transformants analyzed, at different sites in the genome and with varying copy numbers. The selected phenotypes (Phleob or BenR) were stable through mitosis in most transformants. Integrated plasmid DNA was stable through meiosis in all transformants tested.

A709

Two hundred North Carolina Design I progenies were produced by crossing 50 male plants with four female plants each in the South Dakota Plant Pathology Synthetic (SDPPS). Progenies were evaluated over two years at two locations for ind strength (RST) and thickness (RT), nodal plate thickness (NT), internode length (IL), ear height (EHT), stalk lodging (SL), stalk cross-sectional area (SA), Diopida stalk rot reaction (DSR) and grain yield (YLD). Significant additive genetic variance was present in SDPPS, but at all traits except RST. Dominance genotypic variance was not important for any of the traits measured including YLD. SL was significantly negatively genetically correlated with RST and RT, but was not correlated with DSR or any of the other stalk traits. Selection for SL per se appears to be the best means of improving SDPPS for lodging resistance.

A710

In Canada the eastern and prairie populations of Puccinia recondita had similar identities and frequencies of Unified Numeration (UN) races during 1931-1937 when susceptible cultivars were grown in both regions. The release of wheat cultivars with genes Lr14a and Lr3 in the prairie region in 1937 and 1943 was followed by the first directional virulence shift in the prairie leaf rust population. The continuing use of resistant cultivars in the prairie region has exerted a continuous selection pressure on the rust population in the prairie leaf rust population. Susceptible cultivars continued to be grown in the eastern region, resulting in a different succession of UN races. The current distinct regional populations most likely originated from a common introduced population of P. recondita and evolved through differences in the use of resistant cultivars.

A711
VERIFICATION OF GENES HYPOTHEZIZED FOR RESISTANCE TO LEAF RUST IN WHEAT CULTIVARS OF SOUTH DAKOTA. S.S.A. Rizvi, C.W. Buchenau, and F.A. Hollick, Plant Science department, South Dakota State University, Brookings, SD 57007.

Various spring and winter wheat cultivars with gene(s) previously hypothesized for resistance to leaf rust, were crossed to each of Leaf rust (Lr) near isogenic line for low reaction to Puccinia recondita tritici. Failure of F2 plants from such crosses to segregate verified presence of hypothesized genes and proved the validity of analytic method. Verified genes were: A99AR, Apex 83, Challenger: Lr 1B; Butte, Norwegian, Wheaton, Oslo, Norak and Olof: Lr 10; Erik, Marshall and Len: Lr 1A, Lr 1B, Lr 10; Butte#6: Lr 10, Lr 26; Guard and Shield: Lr 2A, Lr 3, Lr 10; Bennet, Brule, Lancer, Rita and Rose: Lr 3; Dawn, Nell: Lr 3, Lr 10; Centura and Sage: Lr 3, Lr 24; and finally Sioux: Lr 3, Lr 24, Lr 26.

A712

Scanning electron photomicrographs of densely pubescent wheat leaves inoculated with urediinospores of Puccinia recondita f. sp. tritici provided evidence that infection was influenced by leaf hairs. Spores were frequently trapped by hairs, 'sticking' them above the leaf surface. Contact with leaf hairs disrupted normal germ tube growth along the leaf surface often resulting in failure to find a stoma and infect. Subsequent light-microscope observations of inoculated seedlings provided further evidence of erratic growth patterns by leaf rust germ tubes and reductions in the number of germinating spores which initiate infection. Over 90% of germ tubes observed on cv. Hunter's glabrous leaves exhibited normal growth whereas 40% were normal on Combo's densely pubescent leaves. Slight reductions in infection in each disease cycle can significantly slow epidemics of leaf rust. Therefore leaf pubescence may provide an important level of protection.

A713
COMPARISON OF MYCOphaereaella SPECIES IN WHEAT STUBBLE. R. B. Madarigas and D. G. Gilmourh. Department of Plant Pathology, University of California, Davis, CA 95616.

Two species of Mycophaearella occur on wheat stubble in California. They produce morphological similar hyaline didymoascopores but differ in their biology and are connected to different anamorphs. The ascospores of one species measured 18-20 μm and on water agar germinate by germ tubes which develop into typical fruiting structures of Cladosporium herbarum, a cause of the Black Point disease of wheat. Its teleomorph is M. tassiana. In the second species the ascospores measure 13-16 μm; they produce only blastospores which are indistinguishable from blastospores formed by gynioospores of Septoria tritici the causal agent of Septoria leaf Blotch of wheat.

A714
EFFECTS OF TRICHODERMA HARZIANUM ON REPRODUCTION OF HELIOIOGYCN SPECIES ON TRIFOLIUM REPENS. G. L. Windham and H. T. Windham, USDA-ARS, Mississippi State, MS, 39762; and Dept. of Entomology and Plant Pathology, Univ. of Tennessee, Knoxville, TN 37901.

The effect of Trichoderma harzianum (TH) isolate T-12 on the reproduction of Helioioygena inconspicua (MI) and M. arenaria (MA) on Trifolium repens cultivar Regal (nematode susceptible) and germplasm SC-1 (nematode tolerant) was determined in greenhouse studies. Apeat-white bran infested with TH was added to soil prior to transplanting clover seedlings into 10-cm pots. MI or MA were added at transplanting at a rate of 5000 eggs per pot. MI reproduction was significantly (P = 0.05) lower on Regal and SC-1 grown in TH treated soils. MI reproduction was reduced by 38% on Regal and 58% on SC-1. TH had less of an effect on MA, with reproduction reduced by 8% on Regal and 29% on SC-1.

A715
DISTRIBUTION AND INCIDENCE OF HETERODERA GLYCINES IN IOWA SOYBEAN FIELDS. L. E. Sweets and N. R. Baker. Iowa State University, Ames, IA 50011.

A study to monitor the distribution of Heterodera glycines (SCN) with production fields and the changes in population levels of SCN with various crop rotations within those fields was initiated in 1988. A grid pattern of sampling sites was laid out through each field using permanent markers so that the same site could be sampled annually. Soil samples have been taken from all sampling sites in all fields each spring prior to or immediately after planting. A 100 cm³ subsample of each sample was processed for eggs of H. glycines using a mechanical separation technique. SCN infection levels were widely distributed in all of the fields sampled. Levels (based on SCN egg counts) within an individual field varied greatly (ex. 100 eggs/100 cm³ soil to 11,100 eggs/100 cm³ soil). One year of SCN susceptible soybeans led to a significant increase in SCN levels within monitored fields.

A716
BEHAVIOR OF CRICONEMELLA XENOPLAX ON ROOTS IN MONOCENIC CULTURE. S. W. Westcott, III, Department of Plant Pathology and Physiology, Clemson University, Clemson, SC 29634.

Criconemella xenoplax (Raski) Luc and Raski was perpetuated in monoxenic culture on root explants of Trifolium incarnatum L. 'Udine' by repeated subculturing. The medium contained Gamborg's B-5 salts (Exp. Cell Res. 50:151-158, 1968.). Second stage juveniles (nematodes) were frequently accumulated in these cultures (4% of the nematode population was observed feeding at any one time. On roots from cuttings of Prunus besseyi L. H. Bailey
the proportion of J-2 in the population was lower (35%) and more nematodes fed at once (13%) than on P. incanum. *Criconemella xenoplax* could not be perpetuated by subculture on seedling roots of *Prunus persica* L. "Nemaguard" or *Dianthus carpophyllus* Ait. *Jassidella l.* "Double Greenwood", on root explants of *Zea mays* L. "Golden Sweet Bantam", *Lycopersicon esculentum* Miller "Rutger's", or *Cucumis sativus* L. "National Pickling". Adults fed and subsequently laid eggs, but the J-2 accumulated in cultures: up to 80% of the population were J-2 on roots of *L. esculentum* and *D. carophyllus* roots after 7 wk. Few eggs were laid and juveniles did not develop on the other plants.

**A717**

**EFFECTS OF YELLOW NUTSEDGE (CYPHERUS ESCULENTUS) AND PURPLE NUTSEDGE (C. ROTUNDUS) ON MELIODIOGENE INCOGINTA POPULATIONS IN CHILE PEPPERS (CAPSICUM ANNUUM).** J. H. Thomas and J. Schroeder, Department of Entomology, Plant Pathology and Weed Science, New Mexico State University, Las Cruces, NM 88003.

Root-knot nematodes (*M. incognita* host race 3) and both yellow and purple nutsedge are severe and frequently concomitant pests of chili peppers in Texas. The effects of different pest combinations on plant growth parameters and nematode populations were investigated in a factorially designed greenhouse experiment. Nematode numbers (injective J2) were 50% less in pots containing chili than in those without plants. Both C. esculentum and C. rotundus were hosts of *M. incognita*, but egg production per gram of root tissue was less than 3% of that observed on chili roots. Such information on the effects of root competition between two weed species on population dynamics of plant-parasitic nematodes is needed in the development of representative pest management models.

**A718**

**CORN–COTTON ROTATIONS FOR THE MANAGEMENT OF THE RENIFORM NEMATODE.** G. W. Lawrence, G. L. Windham*, K. S. McLean, W. E. Batson, Jr., and J. C. Borbon, Dept. of Plant Pathology and Weed Science and USDA-ARS, Mississippi State University, Mississippi, MS 39762.

Corn (*Zea mays*) and aldicarb were evaluated for the management of *B. enchecnecus* weeds, but corn rotation for the management of *Heterodera glycines*. Corn (Pioneer Brand 3165) and cotton (Beltpaine 20) were planted in a field previously planted to cotton and naturally infested with a high population of *H. reniformis*. Each crop was planted alone and with aldicarb (Temik 13C) at 1.18 kg a.i./ha. *H. reniformis* population densities at harvest were significantly larger in plots where cotton was planted without aldicarb, with a reproductive factor (RF) of 2.3. The observed populations of *H. reniformis* recovered from the corn plots with an average (RF) of 0.07. Corn cultivar Pioneer Brand 3165 does not appear to be a host to *H. reniformis* and may be a useful rotation crop in cotton production systems.

**A719**

**EFFECT OF PROLONGED EXPOSURES TO ROOT LEACHATES FROM RESISTANT AND SUSCEPTIBLE SOLANUM MICHIGANENSE EMBERS ON POPULATION DENSITIES OF HETERODERA GYCLINES.** E. J. Sikora and G. R. Noel. USDA-ARS, Dept. of Plant Pathology, Univ. of Illinois, Urbana, IL 61801.

Egg hatch and emergence were determined for second-stage juveniles (J2) of *Heterodera glycines* races 3 and 4 exposed to soybean root leachate of cv. Fayette (resistant to J. glycines) and cv. Asgrow 2575, Asgrow 3127, and Williams 82 (susceptible to H. glycines) in a 2-d period. Leachate was removed from plants at 4-d intervals beginning 7 d after planting using double-distilled water. Twenty cysts from field-grown soybeans were incubated in 2 ml of leachate at 25 C. At 4-d intervals, emerged J2 were counted and removed and leachate from 4-d older plants was added. Leachate obtained from Asgrow 2575 stimulated more hatch (88 and 72%) and emergence (66 and 65%) of race 3 and 4, respectively, than leachate from the other cultivars. Leachate obtained from Fayette stimulated less hatch (25 and 28%) and emergence (5 and 8%) of race 3 and 4, respectively. Hatch and emergence were greatest during the initial 12 days of the experiment.

**A720**

**NEMATODE ASSOCIATIONS WITH OAK DECLINE.** G. W. Lawrence, V. Almada, J. S. McLean, T. T. Kottke, T. E. Goosen, L. W. Kottke, and J. D. Solomon, Dept. of Plant Pathology and Weed Science, Mississippi State University, Miss., MS 39762 and *Southern Hardwoods Lab., USDA, Stoneville, MS.*

Nematodes were extracted from soil taken from oak decline and control sites within the Mississippi and Tennessee-Tombigbee River basins. Soil was collected from trees with decline and trees with no evidence of decline. Nematode communities were separated into trophic groups based on characteristics of the stomodeum. A total nematode population density of 526 and 651 nematodes/500 cm² of soil were recovered from decline and healthy trees, respectively. Fifty-nine species of nematodes were recovered in highest frequency (55%), followed by fungivores (26%), bacterivores (14%) and predators (4%). Fifteen species of stytel bearing nematodes were recovered. Nematodes included *Tylenchus (17 species),* in highest frequency, *Xenocriconemella sp. (24%), Criconemella sp. (9%), Meloidogyne sp. (11%), Helicotylenchus sp. (7%),* and *Dolichodorus sp. (2%).*

**A721**

**APPLICATION OF CLONED DNA FRAGMENTS TO DIFFERENTIATE AND DETECT PERONOSCLEROSA SPECIES.** C. L. Yao, C. W. Magill, R. A. Frederiksen, and M. R. Bondo. Dept. of Plant Pathology and Microbiology, Texas A&M University, College Station, TX 77843 and USDA-ARS, Frederic, MD, 21701.

*P. sorgii,* *P. sorghii* (Thai isolate), *P. maydis,* *P. philippinensis* and *P. sacchari* were readily distinguished by the EcorI RFLP patterns produced when six radioactively labeled probes were hybridized to Southern blots containing DNA from the respective fungi. The patterns were the same whether DNA was extracted directly from the fungus or from infected leaves. No intraspecific RFLPs were detected in limited tests. The probes were selected from a *P. sorghii* pathotype 3 DNA library in pUC 19. Sorghum downy mildew and maize downy mildew inoculum in sorghum seeds and maize seeds collected from plants infected with *P. sorghii* and *P. maydis* respectively, were detected by probe pMly 12 which contains 1.5 and 1.3 kb DNA fragments. This probe did not hybridize to DNA extracted from 10 of the common fungi of sorghum and maize, or to DNA isolated from plant tissue infected with other downy mildews, *Sclerospora graminicola* or *Sclerophthora macrospora.*

**A722**

**THE IDENTIFICATION AND CHARACTERIZATION OF A RACE 3-SPECIFIC REGION FROM PSEUDOMONAS SOLANACEARUM.** Douglas Cook and Luis Sequeira, University of Wisconsin-Madison, Madison WI 53706.

Race 3 strains have the unique ability to cause wilting of potatoes at low temperatures and represent the most homogeneous group within *Pseudomonas solanacearum*. Members of race 3 have remarkably similar RFLP patterns and are thought to have evolved in geographical isolation in the Andean region of South America, although strains have been disseminated worldwide via infected potato tubers. By means of subtractive hybridization, we have identified a 2 Kb cosmid clone that has homology with all race 3 strains tested, but not with other members of the species. Overlapping cosmid clones with homology to the original race-specific cosmid were obtained from a race 3 gene library; subsequent analysis of these clones has established that the race specific region is comprised of two clusters containing at least 26 Kb of DNA.

**A723**


Himoderni azalea growing in pine barksand (31) in a container nursery were inoculated with *P. cinnamomi* c. 5. 1. 3. 9. 30, or 90 colonial oat grains/plant. One wk after introduction of *P. cinnamomi* and then at 2-wk intervals for the next 13 wk, root samples were collected from each plant. Each sample was divided into two sub-samples with one cultured on PPF medium and the other assayed by ELISA in a multiwell kit-E (Agri-Diagnosis Assco, Cinnamomum PDA). Prosecticism was detected after 1 wk from 10% of the plants sampled by both culture and ELISA methods. After 3 wk, detection was 45% and 35% for culture and ELISA, respectively. Detection by ELISA reached 50% by 7 wk, and 90% by 13 wk. The number of culture-ELISA positives was consistent beginning at 3 wk and thereafter. No correlation was found between inoculum rate and absorbance. Cross-reaction was not apparent. Comparable results were found in a subsequent experiment in the greenhouse with a rapid assay kit. ELISA was a reliable method for detection of *P. cinnamomi*.

**A724**

**INDOLEACETIC ACID ENHANCEMENT OF C4 METHIONINE UPTAKE BY TOMATO CELL CULTURES AIDS ASSAY OF PATHOGEN EXTRACTS.** Clarence MadhooSingh, London Research Centre, Agriculture Canada, 1400 Western Road, London, Ontario, Canada, NGL 2V4.
One micromolar indoleacetic acid (IAA) produced a six-fold increase in 14C methionine uptake by washed tomato cells in suspension. This system allowed a practical differentiation of pathogen (Fusarium oxysporum f. sp spirogrodii) and non-pathogen (F. oxysporum) metabolites used for trials based on 14C methionine uptake. This IAA effect facilitates the development of a rapid assay system for Fusarium wilt pathogens of tomatoes.

A725
NON-ANTIBODIES TO A SALINE EXTRACTABLE ANTIGEN ASSOCIATED WITH CONIDIA OF PYRICULARIA OXYSPORUM, RACE 1849. J. Q. Xia, L. N. Raymond, F. N. Lee and H. A. Scott. Hybridoma Laboratory and Dept. of Plant Pathology, University of Arkansas, Fayetteville, AR 72701.

Two mouse monoclonal antibody-producing hybridomas, 285A1 and 2DIF1, were developed using germinated conidia of Pyricularia oxysporum Race 1849 as the immunogen. Monoclonal antibodies produced by both hybridomas reacted in an enzyme immunoassay and an immunofluorescent assay with a reaction (IFA) with a phosphate-buffered saline extractable substance associated with F. oxysporum conidia, and normal and infected rice leaf tissue, and rice seed. IFA with ascites produced by hybrid 285A1 also resulted in identification of the same or a cross-reactive epitope on conidia of Aspergillus sp., Penicillium sp., and five isolates of Pyricularia grisea. No reactivity with Alternaria sp., Curvularia sp., or Fusarium sp. conidia was observed. IFA indicated that the epitope is localized on the surface of both F. oxysporum conidia and conidiospores but not on germ tubes.

A726
USE OF IMMUNOGOLD LABELLING WITH SCANNING ELECTRON MICROSCOPY TO DETECT BACTERIA ON LEAF SURFACES. C. L. Davis, R. H. Bransky and D. S. Wood. University of Florida, CREC, Lake Alfred 33850.

Scanning electron microscopy coupled with backscattered electron detection of immunogold labelling was used to detect colloidal gold-labelled specific immunoglobulins (IgG) attached to epiphytic bacteria. Greenhouse-grown Citrus paradisi Macf, cv. 'Duncan' plants were spray inoculated with 0.5 X 10^7 cfu/ml of strains of Xanthomonas citri pv. campestris pv. citri and Xanthomonas citri pv. campestris pv. campestris. Leaf disc samples were collected at 1, 2, 3, 4, 5, and 7 days post-inoculation and fixed in glutaraldehyde/ruthe- nium red. Afterwards the discs were incubated on gold-labelled IgG, washed, dehydrated, and carbon coated, and observed with a fluorescent stereoscope. No specific IgG attachment was detected on the other bacteria when labelled with homologous gold-labelled IgG. This method was used in the scanning electron microscope with BSE imaging. Reverse polarity was used to observe individual gold particles attached to the bacteria.

A727

The sensitivity of a Phytophthora specific ELISA immunoassay (Kit E, Agri-Diagnostics, Cinnaminson, NJ) was tested on 17 species of Phytophthora including 18 isolates each of P. cinnamonii and P. cactorum collected throughout the world. Isolates were grown in liquid (GYP) media for 7-10 days at 19°C. A 20 mg sample of aspirated mycelia was ground in sterile sand and 2 ml extract solution, boiled and diluted such that 5 ug fresh wt mycelia was tested. Absorbance of the assay at 405 nm was compared to 'extract solution' control ground in sand. All Phytophthora isolates produced a positive reaction with the immunoassay. The lowest absorbance value of the positive control to other species was obtained from P. cinnamonii and P. megasperma (subgroup Apple/Cherry). Variation in absorbance was high among isolates of P. cinnamonii but low among P. cactorum. The 'Kit E' immunoassay reacted to a wide range of Phytophthora species found worldwide, however, the sensitivity of the assay was variable among species and isolates.

A728
COMPARISON OF TOMATO RINGSPOT VIRUS DETECTION BY IMMUNOLOGICAL AND NUCLEIC ACID HYBRIDIZATION ASSAYS. E. V. Podleski', R. A. Owens', and A. Haddi'. USGA, ARS, Microbiology and Plant Pathology Lab., Beltsville, MD and USDA, ARS, National Plant Germplasm and Quarantine Lab., Glenn Dale, MD.

Tomato ringspot virus (ToMSV) detection by nucleic acid hybridization assays and indirect enzyme linked immunosorbent assay (ELISA) were compared to determine their relative sensitivities. Total nucleic acids extracted from infected plants were bound to nitrocellulose membranes and probed with radiolabeled complementary RNA or DNA probes. The 100bp nucleotide cDNA riboprobe detected amounts of ToMSV RNA equivalent to picogram quantities of intact virions. Synthetic cDNA oligoriboprobes, 15-28 nucleotides long and 1-ELISA of equivalent dilutions of crude sap from ToMSV-infected plants were 100 and 1980-fold less sensitive, respectively.

A729
ELISA MICROTRITTER PLATE UNIFORMITY STUDY. E. M. Buske, S. G. Carmack, A. D. Hewings, and F. L. Kohl. Dept. of Plant Pathology and USDA/ARS, University of Illinois, Urbana, IL 61801.

To characterize the ability to detect treatment differences, the variability between and within 12, Immulon 1 "U" plates was determined using an indirect monocular enzyme-linked immunosorbent assay for the barberry yellow dwarf virus (BYDV-PV-IL) using virulent and avirulent sample of Citlaln 64 tissue infected with BYDV-PV-IL was prepared and partially clarified by centrifugation to assure uniformity. All reagents used in the assay were prepared in bulk and applied to all 12 plates. The single sample was placed in all wells and the plates were read 60 min. after the substrate was added. Plate absorbances ranged from 0.955 to 1.249 OD. The coefficients of variation for each plate ranged from 8.44% to 16.90%. Incomplete blocks were imposed on each plate to determine if variability within plates could be controlled through experimental design. The results did not indicate consistent control. Patterns of variability within plates and results of tests to evaluate the assumption that absorbances are normally distributed with a common variance will be discussed.

A730
GAERTNERIOMYCES SP. AS A POTENTIAL BIOLOGICAL CONTROL AGENT OF SYSTEMIC DOWNY MILDEW INFECTION OF SORGHUM BICOLOR (MOENCH). I. S. Kucur, G. N. Odvoy, and R. A. Frechen. Department of Plant Pathology and Microbiology, Texas A&M University, College Station, Texas 77843.

Incorporation of Gaertneriomyces sp., a chytrid, in microplants containing soil with Penicillium or Sordaria spores reduced systemic downy mildew infection in Sorghum bicolor plants. Reductions in systemic infection were dependant on the amount of chytrid added. Microplants containing 13,100 cm^3 of silty clay loam soil at pH 8.1 were treated with 250 ml of chytrid (high) and 125 ml chytrid (low) at a concentration of 100 thall/ml. Systemic infection was reduced by 42% in low concentration treated plots and by up to 58% in high concentration treatments. Spraying had no influence on levels of disease.

A731
THE ROLE OF PYLOUTERIN AND FLUORESCENT SIDEROPHORE PRODUCTION BY PSEUDOMONAS FLUORESCENS P53 IN BIOCONTROL OF PYTHIUM DAMPING-OFF OF COTTON. J. Kraus and J. E. Loper, USDA, ARS, HCRIL, Corvallis OR 97330.

Pseudomonas Fluorescens P53, a biocontrol agent of Pythium damping-off of cotton, produces many anti-fungal compounds in culture, including pylouterin (2R,3S)-3-hydroxy-1,2,3,4-tetrahydroisocoumarin, and a fluorescent siderophore (Flu), ammonia, and cyanide. Thirteen Poyo' and 14 Flu' mutants, with single Tns insertions in 8 and 7 distinct EcGFI fragments, respectively, were obtained. Single Pyle and Flu' derivatives were antagonistic against P. ultimum on 253 medium in the presence of FeCl2, while Py' derivatives were not. PFS, Flu', and Py' strains were indistinguishable with respect to antagonism on nutrient agar supplemented with 2% glucose. PFS, two Py' mutants, and two Flu' mutants established similar rhizosphere population sizes and were indistinguishable statistically with respect to biocontrol of cotton damping-off in a Wizard sandy loam soil at approximately 5 ppg. Results suggest that biocontrol of Pythium damping-off is not mediated by pylouterin or fluorescent siderophore production of P53 in this soil.

A732
PRODUCTION OF AMMONIA BY PSEUDOMONAS CAPACIA INTERFERENCE WITH SEED GERMINATION AND ROOT ELONGATION. M. Bajish, K. E. Conway and N. A. Delgado, Department of Plant Pathology, Oklahoma State University, Stillwater, OK 74078 and Universidad Nacional de Puebla, Puebla, Peru.

P. capacia used as a biological seed treatment on vinca and sage to control soilborne diseases interferes with germination and root elongation. High concentrations of bacteria on seeds (10^9 to 10^10) inhibited more than 90% of the germination concentrations. Germination was affected in split-half petri dishes in the presence of P. capacia. Because no direct contact occurred this indicated that a volatile compound was involved, pI paper suspended over bacterial cultures showed changes from 7 to 8. Cultures were grown in Czapek's broth with and without the addition of 20 g/l of peptone. Volatiles were collected from water traps and tested for the presence of ammonia using an ammonium test kit (EM Science Co., Gibbstown, NJ). Five strains of P. capacia produced 10 to 50 ug/ml of ammonia only when peptone was added to the medium.
A733

FUNGI ASSOCIATED WITH MYCELIUM OF RHIZOCTONIA SOLANI IN BARE PATCHES OF WHEAT. N. Worsnop and K. Sivasithamparam. University of Western Australia, Nedlands, W.A. 6009. Australia.

Mira cloth discs colonized by Rhizoctonia solani were incubated in soil samples collected from bare patch and non-bare patch areas of a wheat field to isolate fungi which were associated with the mycelia of R. solani. These fungi were screened for inhibition of growth of R. solani on agar. Twenty species/forms were encountered in non-bare patch soil while 50 species were isolated from patch soil. Most isolates belong to Fusarium, Trichoderma, Penicillium, Chrysosporium, Mortierella or Phoma. Trichoderma spp. were significantly more frequent within patches where they were dominant in the centre and perimeter of the patches. This may be related to the recovery seen in plants remaining in the centre and the abrupt end of the patch at the periphery. Penicillium spp. and seven other fungi, including a Trichoderma sp., inhibited growth of R. solani on PDA while only five isolates of Penicillium and one each of Aspergillus and Trichoderma showed inhibition on tap water agar.

A734

SACCHAROMYCES CEREWIAE PROTECTS MAIZE PLANTS, UNDER GREENHOUSE CONDITIONS, AGAINST COLLETOTRICHUM GRAMINICOLA, S.F. Paschoalini, S.R. da Silva*, and W.B.C. Morais*, ESAU/USP, P.O.Box 9, 13460-Piracicaba-S.P.; *Biological Institute, F.O.Box 710, 01051-Sao Paulo-S.P., Brazil.

Suspensions from washed or non-washed S. cerevisiae cells and filtrates of these suspensions, obtained from commercial baker's yeast, reduced the conidial development of C. graminicola as well as the expression of anthracnose on maize leaves treated with these preparations. When cells of S. cerevisiae were isolated from CRY and grown in PDA medium, the cell suspensions and their filtrates also reduced the development of C. graminicola and the expression of the disease. The yeast preparations and their filtrates were shown to be thermolabile. The reduction in the development of C. graminicola and on disease expression, when filtrates of S. cerevisiae were used, suggest that the presence of the yeast cell is not necessary to protect the leaves. In vitro experiments showed that S. cerevisiae cells exhibit a possible antagonistic activity against C. graminicola due to antibiosis.

A735


In a field experiment with artificial inoculation, the effect of two strains of Trichoderma viride on the incidence of onion neck rot caused by Botrytis aclada was studied. Antagonists were applied as a confidial suspension immediately after leaf topping during the harvest procedure. Rot assessed after 3 months storage at 9°C under initially favourable conditions for fungal development was reduced from 35% to 12%. Under favourable conditions (drying at 20°C for 10 days after harvest) rot was reduced from 5% to 2%. A bio-assay for a rapid selection of new antagonists was developed based on the reduction of wound infection of detached onion leaves. Of the 40 isolates tested, strains of C. simplicissimum, Trichoderma spp., Penicillium spp. and Aureobasidium pullulans were superior to those used in the field. Antagonists were also selected for interference with sporulation of B. aclada on leaf debris to reduce inoculum load in the field.

A736


In 1989 field trials at six locations in the midwest Pioneer® Hybrids 3475 and 3585 showed significantly higher early stand counts when treated with selected bacterial inoculants than when water was treated with water alone. Some treatment-hybrid combinations were better than or equal to Captain seed treatment. Analysis of variance showed that location, treatment, and the interaction of treatments with hybrid and with location were significant sources of variation. Early stand count of non-treated 3475 seed was improved 6.2% by Captain, 16.3% by water alone, and 21% by a mixture of 3 bacterial strains applied in water. Early stand count of non-treated 3585 seed was improved 11.4% by Captain, only 6% by water alone, and 11% by the mixture of 3 bacterial strains applied in water.

A738

Suppression of Phytophthora cinnamomi in vitro and in vivo by microbial antagonists isolated from a compost mix. M.P. You and K.Sivasithamparam, University of Western Australia, Nedlands, W.A. 6009.

Four fungi and one actinomycete isolated from a potting mix containing composted Eucalypt bark were found to inhibit Phytophthora cinnamomi on agar. The actinomycete (isolate A4) was deleterious to the growth of the assay plants (Antirrhinum sp.). In non-sterilized potting mix, all four fungi (isolates of Trichoderma, Aspergillus (2) and Humicola) reduced root rot with the Aspergillus sp. (isolate C236) being the most effective. The effect of these fungi and an antagonistic sterile red fungus on the survival and growth of the pathogen in sterilized and non-sterilized potting mix was investigated.

A739

EPIDEMIOLOGICAL AND HOST RANGE STUDIES OF Puccinia Jaceae, A POTENTIAL BIOCONTROL AGENT OF PURPLE STALKHISTLE. N. Shelhoff and W.L. Bruckart, USDA-ARS, Bldg. 1301, Fort Detrick, Frederick, MD 21701.

Puccinia Jaceae, Oth. is a rust fungus from Europe which infects Centaurea calcitrapa L. (purple stalkhistle), an introduced weed of California pastures. Urediniospores germinated on agar over a temperature range of 12-30°C with maximum germination after at least 8 h at 18-27°C. The greatest number of pustules developed with 8 or more hours of dew at 15-21°C. The latent period of infection was 15 days at 15°C and 9 days at 20 or 25°C. The response-surface models developed will be used to predict infection in nature using temperature and duration of dew period. Of 63 genes tested, only a few genera in the tribe Cardueae were susceptible to Puccinia Jaceae. Artichoke, safflower and a few native Cardueae species developed minor infections and became less susceptible with age.

A740

INHIBITION OF PHYTOPHTHORA VIGNAE BY SOIL BACTERIA. M.G. Fernandez and R.G. Linderman, Oregon State University and USDA-ARS Horticultural Crops Research Laboratory, Corvallis, Oregon 97330.

Phytophthora vignae, cause of stem and root rot of cowpea (Vigna unguiculata), was inhibited in vitro by (1) unidentified bacteria isolated from cowpea field soils in Sri Lanka where the pathogen was present but the disease was absent, and (2) by known biocontrol agents from other sources (Pseudomonas, Enterobacter, and Bacillus). P. vignae was inhibited on undivided plates of PDA, Kings B (KB), Tryptic soy agar (TSA), corn meal agar, and nutrient agar (NA), but only on TSA and NA or KB where divided plates were used (volatile inhibitors). Volatile inhibitors were absorbed by the medium supporting the pathogen, and pH increased therein. Substrates like tryptic soy and cowpea seed extract were necessary for production of volatile inhibitors by bacteria added to sterile soil.

Vol. 80, No. 10, 1990 1049
A742
ANTAGONISTIC MICROORGANISMS TOXIC TO MACROPHOMINA PHASEOLINA IN VITRO. P. Perdomo, E.C. Schroder, and R. Schvánez-Badel. Departments of Agronomy & Soils and Crop Protection, University of Puerto Rico, Mayaguez Campus, Mayaguez, P.R. 00709. 

Asyly Stem Blight caused by Macrophomina phaseolina has been recently reported as a severe disease of dry bean in the Caribbean region. In order to identify biological control microorganisms against M. phaseolina, we have been screening bacteria to detect potential antagonists. Three in vitro methods, the streak plate, double layer and spent culture were used to measure bacterial antagonism towards M. phaseolina. Significant reductions in radial growth rates of M. phaseolina were found with presence of an actinomycete, Pseudonocardia cepacia and Xanthomonas campestris pv. phaseoli. 

A743

Bacterial strains were isolated from several plant and plant-based sources using a variety of isolation methods to increase the probability of obtaining a diverse collection of organisms. A total of 514 strains were screened in repeated growth chamber assays on cucumber in sterile sand and vermiculite amended with Pythium ultimum. On the basis of consistency, superior performance 45 strains, mostly fluorescent pseudomonads isolated from indigenous hop plants, were selected for further testing. They were subsequently screened under greenhouse conditions in a commercial peat-based growing mix artificially infested with P. ultimum. Several strains elicited a final stand superior to the chemical control. In order to elucidate possible mechanisms, the elite strains were screened for in vitro antagonism to P. ultimum and other soilborne plant pathogens, cyanide production, direct growth promotion in sterile growth pouches and production of plant growth regulators. 

A744

Alginate pellet formulations of biocontrol fungi may control plant pathogens, weeds, and insect pests, but the time required for infection and subsequent hyphal outgrowth and sporulation may limit their efficacy under some environmental conditions. We formulated fermentor biomass of Trichoderma harzianum in alginate/bran pellets, allowed the pellets to dry only partially, primed them in a 40% aqueous solution of polyethylene glycol for 12-24 hr, then allowed them to dry. On water agar, colony radii from primed pellets were 45-98% larger after 24-48 hr (22 C) than from unprimed pellets, and conidia were produced about 48 hr sooner. Similar results were observed in steamed soil, and with the insect biocontrol fungus Beauveria bassiana. 

A745
REDUCED GROWTH OF ERYSIPHE GRAMINIS F. SP. HORDEI INDUCED BY Tilletiopsis palleccens. A.L. Klenca, S. Hippe, and S.C. Somerville1,2. 1DOE Plant Research Laboratory and 2Department of Botany and Plant Pathology, Michigan State University, East Lansing, MI 48824, U.S.A. and 7Institute of Botany, Christian Albrechts University, D-2330 Kiel 1, Federal Republic of Germany. 

The leaf epiphyte, Tilletiopsis, which possesses hyaline ballistoconidia, was found contaminating barley seedlings infected with the obligate parasite Erysiphe graminis f. sp. hordei. The contaminant was identified as an isolate of Tilletiopsis palleccens. Isolates were then tested for their morphological, physiological, and biochemical characteristics. An antagonistic relationship between E. g. hordei and T. palleccens was demonstrated on the surface of barley leaf segments. On a leaf level, T. palleccens caused severe reduction of mycelial expansion and spore production by E. g. hordei, whereas T. minor was antagonistic to a lesser extent. Low temperature scanning and conventional transmission electron microscopy showed that hyphae of E. g. hordei were collapsed and degenerated in the presence of T. palleccens. 

A746
GERMINATION RESPONSES TO VOLATILE AROMA COMPOUNDS BY TELIOSPORES OF A UROMYCES SP. FROM EUPHORBIA VIRGATA. A. R. Bennett and R. C. French, USDA-ARS, Frederick, MD 21701. 

Teliospores of a Uromyces sp. were collected from Euphorbia virgata in 1989 near Stavropol, USSR for evaluation as a potential biocontrol agent for leafy spurge (Euphorbia spp.) in the U.S. To evaluate germination potential, teliospores were exposed to volatile compounds previously known to stimulate germination of other rust spores. Of 12 compounds tested, benzonitrile induced the greatest response. Germination on agar with 50 ul/L benzonitrile was greatest between 20 and 25 C in both light and dark. In darkness, germination without stimulator was less than 20% at the optimum temperature (ca 25 C), but increased to 60% with 50 ul/L benzonitrile. Maximum germination (80%) occurred in the light without benzonitrile. These results indicate germination is favored by light and can be enhanced in the dark by exposure to benzonitrile. 

A747
CLAUVACTER MICHIGANENSIS SUBSP. SEPEREDONICUS, AN EFFECTIVE BIOCONTROL AGENT AGAINST MAJOR POSTHARVEST DISEASES OF POE FRUITS. W. Janisiewicz, USDA, ARS, Appalachian Fruit Research Station, Kearneysville, WV 25430. 

Clauvactor michiganensis subsp. seperedonicus, isolate K-5 (PFE-15-488) was isolated from mature 'Bartlett' pear after repeated washing in phosphate buffer with mild sonication. This bacterium was a top performer among 24 most promising antagonists selected after primary screening against Penicillium expansum (incitant of blue mold) and Botrytis cinerea (incitant of gray mold) on apples and pears. In tests with various concentrations of the antagonist (5 and 1.7x10^8 CFU/ml and 4.8x10^7 CFU/ml), complete control of blue mold and gray mold was achieved with 5x10^8 and 1.7x10^8 CFU/ml on pears, and with 1.7x10^8 and 4.8x10^7 CFU/ml on apples, respectively. The antagonist survived well over a 30 day period (reproduction of the experiment) at the wound site on 'Golden Delicious' apples stored at 1C, and as a wet paste preparation at 4C. 

A748
ATTACHMENT OF ANTAGONISTIC YEAST TO FRUIT ROTTING FUNGI: FURTHER CHARACTERIZATION OF BIOCONTROL ACTIVITY AND CHARACTERIZATION OF POSSIBLE INHIBITORY ACTION, C. Bles, M. Wissinkiewski, C. Wilson, and R. McLaughlin. USDA-ARS, AFPS, Kearneysville, WV 25430. 

Yeast isolate US-7 protects apples and peaches from postharvest fruit rotting fungi (Botrytis cinerea & Penicillium expansum). In order to examine the yeast-fruit interaction, fungal growth was monitored on agar plates overlayed with cell-free yeast isolates applied to the plates 24 hr later near the young hyphal growth. Samples were taken 24 hr later from the section where the fungus and yeast had interacted. Light microscopy revealed a general attachment of the effective biocontrol agent US-7 and non-effective isolate 117. Scanning electron microscopy indicated that both isolates attached to the fungal hyphae, but the US-7 isolate attached more tightly. Twenty-four hours after applying the US-7 isolate to B. cinerea and P. expansum, pitting and hyphal collapse were observed. Previously the mode of antagonism was suggested to be nutrient competition. However, this research indicates other mechanisms may also play a role in biocontrol. 

A749
EFFECT OF APPLE FRUIT TISSUE CALCULI ON POSTHARVEST BIOCONTROL EFFICACY OF CRYPTOCCOCCUS LAURENTII AGAINST BOTRYTIS CINEREA, R. G. Roberts and J. T. Raese, USDA, ARS, Tree Fruit Research Laboratory, Wenatchee, WA 98801.
Golden Delicious' apple trees were sprayed to runoff four times in June, July, and August with either CaCO₃ at 2.4 g/L, CaNO₃ at 1.0 g/L, or were unsprayed (calcium controls). Fruit were harvested from all parts of the canopies of five single tree replicates per treatment, then surface disinfested by immersion in cold potassium. Fruit cortex and peel tissue samples from 40 fruit per tree were pooled for mineral analysis. Ten fruit per replicate per treatment were wounded once, then 10 μl of a 10⁴ conidial/ml suspension of Botrytis cinerea. Pathogen controls received only Botrytis conidial suspensions. Inoculations were repeated twice, and data from all three trials were pooled for analysis. Percentages of wounds with Botrytis lesions were determined after storage for 12 days at 16°C. Fruit treated with CaCO₃ had significantly (p=0.05) greater calcium content (238 ppm) than did CaNO₃-treated (206 ppm) or untreated (196 ppm) fruit, and had 28% less decay among the pathogen controls. Biocontrol efficacy of C. laurentii did not differ between the calcium treatments, however, as only one of 450 fruit treated with the yeast became infected.

A750

BIOCORAL CONTROL OF MUCOR ROT OF PEAR BY CRYPTOCOCCUS LAURENTII, C. FLAVUS AND C. ALBIDUS. R. G. Roberts, USDA, ARS, Tree Fruit Research Laboratory, 1104 N. Western Avenue, Wenatchee, WA 98801.

Four strains of Cryptococcus flavus (RR89-154, RR89-156, RR89-160, RR89-211) and one strain each of C. albidos (RR89-212) and C. laurentii (RR89-129) isolated from pear leaves and fruit gave effective biological control of Mucor rot of pear fruit. Biocontrol efficacy of these strains against Mucor rot was evaluated in ripe and non-ripe 'Anjou' pears by treating artificial wounds with 10 μl of 10⁴ conid/ml buffer-washed yeast cell suspensions, then immediately challenging the wounds with 10 μl of a 10⁴ conid/ml suspension of Mucor conidial suspensions. Conidial wounds received only buffer and Mucor spores. Ten fruit per each of four repays per yeast strain were stored for 5-12 days at 5, 10, or 15°C, then percentages of wounds with lesions were determined. Each experiment was repeated once. Reduction in percentages of wounds in treated fruit that became infected relative to controls in ripe pears varied with incubation temperature and yeast strain. The ranges and means (in parentheses) of percentage reductions in ripe pears from all trials were: 12.8 (22.7)-35.9 at 15°C, 17.5 (49.5)-60.5 at 10°C, and 37.5 (61.7)-96.0 at 5°C. In non-ripe pears, percent reductions were 87.0 (94.0)-100.0 at 15°C, 97.6 (98.6)-100.0 at 10°C, and 100% for all strains at 5°C. Repeated trials gave similar results.

A752

PROTECTION OF COTTON SEEDLINGS AGAINST RHIZOCTONIA SOLANI AND PYTHIUM SPP. BY BACTERIAL SEED TREATMENT. M.L. Courtney and J.C. Rupe. Department of Plant Pathology, University of Arkansas, Fayetteville, AR. 72701.

Bacteria were isolated from the hypocotyls of healthy cotton seedlings grown in soil naturally infested with pathogenic Pythium spp. and Rhizoctonia solani. Cotton seeds were treated with any of 139 bacterial isolates and planted in naturally infested soil. Pots were incubated at 21°C for 14 days. Seedling mortality was measured, and seven isolates improved stands significantly (p < 0.05) relative to non-treatments. Control of Pythium seedling blight was achieved by one isolate (Arthrobotrypia globiformis) provided protection in soil where R. solani was the principal pathogen and another isolate (unidentified) provided some protection in Pythium-inoculated plots as the principal pathogens. No one isolate was effective against both pathogens.

A753

BIODOLOGICAL CONTROL OF RHIZOCTONIA ROOT ROT OF WHEAT BY VERTICILLIUM BIGUTTATUM AND A STERILE RED FUNGUS. R.B. Cowling and K. Sivasithamparam. The University of Western Australia, Nedlands, W.A., Australia. 6009.

A Western Australian isolate of Verticillium bigutatum controlled root rot in wheat caused by a patch strain of Rhizoctonia solani Kuhn. V. bigutatum reduced disease in R. solani-inoculated pots containing nutrient-poor white sand or soil from a wheat field with

A754

EFFECT OF PSEUDOMONAS FLUORESCENS ON GROWTH AND PROLIFERATION OF TRICHODERMA HARZIANUM IN STEAMED AND RAW SOIL. Li Bin, G. R. Knudsen, and D. J. Eschen. Plant Pathology, PSES, University of Idaho, Moscow, 83843.

Alginate pellets of T. harzianum (Th) were buried in steamed or raw soil (matric potential -1 or -5 bars) with P. fluorescens (Pf) strain 2-79 (at 0, 10⁴, or 10⁵ cfu/g of soil). Trichoderma and Pf were enumerated in both soils after 7 and 14 days: hyphal growth and density of Th were measured in steamed soil. Pf populations remained unchanged in steamed soil, and inhibited Th; mean cfu/g of Th increased from 0 to >10⁴ with Pf absent, to 3.5 x 10³ with 10⁵ cfu/g of Pf. Pf also reduced hyphal density. In raw soil, Pf declined exponentially (mean = 1.6 logs/day). Trichoderma remained at the background level of about 200 mean cfu/g whether Pf was present or not.

A755

BIOCONTROL OF RHIZOCTONIA ROOT AND CROWN ROT OF SOYBEANS BY BACILLUS MEGATERIUM ATCC-55000, Z. L. Liu and J. B. Sinclair. Dept. of Plant Pathology, University of Illinois, Urbana, 61801.

B. megaterium ATCC-55000 was studied as a potential biocontrol agent on soybeans in the field. Seeds treated with 55000 resulted in a significantly lower disease index caused by R. solani 65L-2 at 100 mg/ml soil applied in furrow compared to Bacillus subtilis AT13 and CM. Significant (p<0.25) yield increase was recorded for 2 yr in successive treatments, but not the second after 1 yr treatment in the presence of R. solani. A significant reduction in recovery of R. solani was obtained after in-furrow treatment with 55000. Yield increase (p>0.05) was recorded in 30-cm clay pots containing soil treated with 55000 + R. solani. Strain 55000 at 10⁵ cfu/g soil was required for disease control in the rhizosphere. Yield from plants treated with 55000 were significantly higher than the untreated control and V. zea cv. Williams 82 at Urbana. No significant yield differences were recorded in three other field plots in heavier soils in central and northern Illinois.

A756


Aspergillus flavus strains were examined for their ability to secrete aflatoxin (AF) pathway metabolites during liquid fermentation. An A. parasiticus mutant (SRR 163) blocked in an early stage of aflatoxin conversion to averufin produced the AF pathway metabolites but retained about 80% of the total averufin produced inside the fungal mycelia. In contrast, another non-aflatoxicogenic strain of A. parasiticus (SRR 2043), which accumulates the AF pathway intermediates, O-methylsterigmatocystin (an AF precursor) and dihydro-O-0-methylsterigmatocystin (an AFBP precursor), secreted greater than 50% of these metabolites. Co-fermentation of these non-aflatoxicogenic strains, SRR 163 (blocked early in the pathway) and strain SRR 2043 (blocked late in the pathway), resulted in AFBP and AF synthesis. The results indicate that, during co-fermentation, certain AF non-producing strains can secrete and exchange AF pathway metabolites and produce aflatoxins.

A757

Low doses of ultraviolet light (254nm UV-C) irradiation reduced postharvest rot of pome, stone and citrus fruits. Brown rot (Alternaria fructicola) of Elberta and Loring peaches was significantly reduced by UV-C 10 and 20 days respectively, after irradiation. The UV-C levels which gave the best results for Loring and Elberta were 7.5 to 40x10^7 ergs/mm^2 and 4.5 to 2x10^7 ergs/mm^2, respectively. Thirty days after treatment with 7.5x10^7 erg/mm^2 of UV-C Applies showed rotting due to Alternaria spp M. fruticola and bacteria soft rot at 9.5, 0 and 10 respectively; whereas, controls showed 3x, 12 and 12 rots, respectively. The application of UV-C was effective in controlling green mold rot (Penicillium digitatum), stone end rot (Alternaria citri), as well as sour rot (Geotrichum candidum) of nancy tangerines 18 days after irradiation. The optimum dosage levels for controlling sour and stem rot were 0, 0, 10^7 ergs/mm^2 to 2x10^7 ergs/mm^2.

Acremonium coenophialum, an endophytic fungus associated with tail fescue, produced alkaloids which play a major role in fescue toxicosis of grazing animals. The objective of this research was to develop an immunosay for detection of these alkaloids in forage samples. Various protein conjugates of an endophytic alkaloid derivative were synthesized in 125 conjugates and used to immunize rabbits. The antisera which was selected for further study had a high titer for binding a non-immune conjugate and was sensitive to competition by both ergonovine and ergometrine. When used in a direct competitive ELISA, this antisera distinguished between endophyte-infected (E+) and endophyte-free (E-) tail fescue.

A762
ULTRASTRUCTURAL CHANGES IN DORMANT MONILINIA FRUCTICOLA CONIDIA WITH HEAT TREATMENT. D. A. Margoan and B. J. Phillips, USDA, ARS, FWA, HCRL, 2021 South Peach Avenue, Fresno, CA 93727

Monilinia fructicola conidia were heated in 52°C water for 0, 0.5, 1.0 or 2.0 min. Germination after 24 hr on water agar was 94.8, 65.6, 2.3 and 0.3%, respectively. Earliest conidial outgrowth occurred on 2 min. Low temperature (0.5 min.) longer treatment times resulted in further disruption of mitochondrial cristae, matrices, and outer mitochondrial membranes, disruption of vacuolar membranes, and gaps in the conidial cytoplasm. No ultrastuctural changes were observed in the nuclei or cell wall after treatment, as reported for germinated conidia. The site of heat lethality in dormant M. fructicola conidia appears to be located in the mitochondria, probably in the inner membrane.

A763

Limited availability of ergovaline, the predominant ergopeptide alkaloid produced by Acremonium coenophialum, hinders research on its role in fescue toxicosis. Isolates of the fungus were obtained from a single seed lot and grown in both liquid fermentation and solid culture. Extracts obtained from liquid (mycelium and filtrate) and solid cultures with 2% tartaric acid and acetic acid (30:70), were partially purified by liquid-liquid partition extraction. Extracts, fluorescent under long wave UV light and positive with p-dimethylaminobenzaldehyde reagent, were separated by reverse phase HPLC using fluorescence (235 excitation, 435 emission) detection. A major fluorescent peak which was observed in all extracts coeluted with authentic ergovaline.

A764
PLANT AND ANIMAL TOXICITY OF CULTURE FILTRATES OF PHOMOPSIS LONGICOLLA P1 AND P12 TO SOYBEANS AND CHICKEN EMBRYOS. S. Z. Shah, Z. L. Liu, and J. B. Sinclair, Dept. of Plant Pathology, Univ. of Illinois at Urbana-Champaign, Urbana, IL 61801-4709.

Isolates P1 and P12 of Phomopsis longicolla, cause of soybean seed decay, from field-grown soybean seeds cv. Williams 82, were studied for their toxicity to soybean seedlings and embryos. The toxic components of 21-day-old culture filtrates of each isolate were tolerant to heat for 1 min at 60, 75, 90, and 100°C; to pH 3.4; and to intensive fluorescent light for 24 hr. Isolate P12 caused more severe cotyledonary browning than isolate P1 to 12-day-old soybean seedlings. Extracts of 2-day-old cultures were tested in culture filtrates. Both isolates significantly inhibited germination of soybean seeds in culture and soybean root development compared to potato-dextrose broth or deionized distilled water controls. Seedlings suspended in 125 culture filtrates of each isolate in distilled water were dead in 6.0 days. Chicken embryos were killed within 6.0 days at 37°C when incubated with 2.0 ml of crude culture filtrates.

A765
DO CELEST HYDROLYSASES PROVIDE RESISTANCE AGAINST THE FUNGAL PATHOGEN PUSARIUM OXYSPORA F. SP. APII. S. L. Krebs and R. Grumet, Department of Horticulture, Michigan State University, East Lansing, MI 48824.

Specific activities of 2 plant hydrolytic enzymes, endochitinase (CHI) and 2,4-lucanase (P13C), were determined following germination of celery seeds ('Pel 683') on soil containing either: a non-pathogen (F.o. f.sp. cepae), P.o. f.sp. apii (incompatible) P.o. f.sp. apii (compatible) or no inoculum (control). In all treatments except the control, CHI activity was detected 14 days after germination (DAG), peaked at 21 DAG, and then declined. At 28 DAG, leaf yellowing and wilting was observed in the compatible interaction.
Peak CHT activities in roots and shoots were lowest in control and non-pathogen treatments. 2X higher in the resistant interaction, and 6X higher in the susceptible interaction. 713G activity was localized in roots, and levels of induction were similar for both compatible and incompatible pathogens (2X over control). Treatment of celery roots with chitosan solutions (25 µg/ml) resulted in a 6X induction in CHIT activity, and a 2X increase in 713G activity. Current experiments are testing whether chitosan-treated ‘FL 663’ seedlings show enhanced resistance to race 2 (compatible).

A766
CHEMICAL ANALYSIS OF THE AROMA EMITTED BY PYCNIA OF THE CANADA THISTLE PATHOGEN, PUCINELLIA PUNCTIFORMIS. R. C. French and W. J. Connick, Jr., USDA-ARS, Beltsville, Md. 20701, Pri. Detrick, Frederick, Md. 21701 and USDA-ARS, P.O. Box 19637, New Orleans, La. 70117

Pycnia of the Canada thistle pathogen, Pucinella punctiformis (Straw's) Koehl, produce a highly scented nectar which may attract insects for cross fertilization. Volatile compounds emitted from the yellow colored pycnia of systemically infected thistle shoots were collected on Tenax columns, eluted, and analyzed by GC-MS. Benzaldehyde, phenylacetaldehyde, phenethyl alcohol, and indole were identified as the predominant compounds from the rusted (flowerless) thistle shoots. Volatiles from flowerless flowers contained the same compounds, minus indole. None of these compounds stimulated the germination of aeciospores or teliospores of P. punctiformis. The identified compounds may be useful in biocontrol procedures to attract insect predators to young Canada thistle [Cirsium arvense (L.) Scop.] and perhaps aid in rust dispersal.

A767
BIOTRANSFORMATION OF MONOTERPENES BY GLOMELLA CINCULATA Mitsuo MIZAZARA, Hiroshi NAKAO, Mitsuhiro HAIRAKI, and Hiroshi KAMOKA, Department of Applied Chemistry, Faculty of Science and Engineering, Niigata University, Higashioku-ku, Niigata, 950 JAPAN, Lab. of Plant Disease Science, Faculty of Agriculture, Gifu University, Yanozono, Gifu, 501-11 JAPAN

The microbial transformation of 1,8-cineole (1) and camphor (7) by Glomella cinículata were studied. 1,8-Cineole (1) was transformed into a mixture consisting of 2-exo-hydraxy-1,8-cineole (2), 2-endo-hydraxy-1,8-cineole (3), 3-endo-hydraxy-1,8-cineole (4),3-exo-hydraxy-1,8-cineole (5) and (1R, 2R, 4S)-2-exo-hydraxy-1,8-cineole (6). Pathway for oxidation of 1,8-cineole have been proposed based on the structural evidence and time course change. On the other hand, camphor (7) was transformed to a major metabolite (hydroxy camphor) by G. cinículata.

A770
ELISA AND IMMUNOCHEMICAL DETECTION OF Fusarium SOLAN-sti-PAPHERINOCHONINUS CITRUS TREES IN GROVES WITH BRIGHT. S. Nemec, S. Jabaji-Hare, and P. M. Charret, USDA, ARS, Orlando, FL 32837, and Dept. Phytopathology, Universites’ Laval, Ste-Foy, Canada.

Xylem fluid of symptomless 1-2.5 cm dia. scaffold roots and branches of healthy-appearing and blight-diseased citrus trees in ridge and flatwoods Florida groves contained naphthoquinone toxins of F. solani by competitive ELISA analysis. Blighted tree roots contained two to five times more toxin than that of healthy-appearing trees. Concentrations were as high as 100,000 ng/ml and fluctuated seasonally. Concan. in blighted trees roots were less than those in roots of trees on adjacent blight-conductive soils. In F. solani-infected roots, fungal cell walls and vacuoles were labeled by colloidal gold and intense labeling of the outer fungus walls suggested that toxins were secreted into the vessel lumens. The fungus probably synthesizes these toxins when it causes root rot on fibrous roots, and from there are translocated to other plant parts.

A771

We are examining maize seeds for proteins that are induced to play pathogenic fungi. Our work has focused on the identification of several small, acid–soluble, basic peptides with antimicrobial properties and comprising approx. 0.3% of the soluble protein of mature maize kernels (milled tissue). One of the peptides (CM-III) was purified to homogeneity by cation exchange FPLC. CM-III inhibits spore germination of the phytopathogenic fungi, Aspergillus nidulans and Sclerotinia sclerotiorum. It has a MW of ~4000 on SDS-PAGE, is rich in arginine and gluamine, and two out of four sequences homologous to the thionins, a conserved group of cysteine-rich peptides with antimicrobial activity found in other cereals and some dicots.

A772

We have isolated and characterized six chemically-induced mutants of the haploid, filamentous fungus Aspergillus nidulans that are resistant to the experimental fungicide 8-chloro-4-(2-chloro-4- fluoroxyquinoline, LY214352). The mutants are 13- to 430-fold more resistant to LY214352 than a parental strain and one of the mutant strains requires LY214352 for maximal growth. The resistance trait is controlled by a single dominant or partially dominant gene in each mutant and it is likely that all of the mutations are allelic. The mutants were not cross-resistant to other fungicides including myclobutanil, tebuconazole, and propiconazole.

A773
OPINE-INDUCED Ti PLASMID CONJUGATION IS SUBJECT TO NEGATIVE AND POSITIVE REGULATION. S. Beck von Bodman, S-W. Qin, S. W. Allen, and S. K. Farrand, Department of Plant Pathology, and Department of Microbiology, University of Illinois at Urbana-Champaign, Urbana, Illinois, 61801.

EcoRI fragment 26 of pTiC58 encodes a central repressor function that negatively coregulates expression of pTiC58 agrocinopine catalase and agrocinopine-induced conjugational transfer. The repressor responds to crown gall-specific agrocinopine opines with the consequence of full expression of both phenotypes. Comparative DNA sequencing analysis of EcoRI fragment 26 from the wild-type Ti plasmids of corresponding DNA from a spontaneous constitutive mutant, pTiC58traR0. Identified a five basepair deletion in the mutant genome that contributes to the constitutive phenotype. Isolation of a Tr5-induced tra mutant that remains responsive to opine catalase and tra expression indicates that conjugational transfer is controlled at a second level. Specifically, the Tr5-specific and spontaneous Tr5 mutant Ti plasmids express opine reporter genes within Tr5 region II. In contrast, Tr5-induced Tra mutants in Tr5 region I fail to express these same reporter functions, thus indicating that a tra-I-specific gene product, i.e., an activator, is essential for
transcription of Tra II-related genes. Accordingly, conjugal transfer of pTCS8 is subject to primary negative regulation through an agronomically-sensing mechanism, and a positive secondary level of regulation through a Tra region I-encoded activator function.

A774
REGULATION OF PAPILLA FORMATION AND INDUCED CELLULAR RESISTANCE. K. Yokoyama, J. R. Atst, and C. J. Bayles, Department of Plant Pathology, Cornell University, Ithaca, NY 14853.

Papillae are localized plant cell wall appositions that can stop penetration attempts by parasitic fungi. We have discovered a papilla-regulating factor (PRF) in aqueous extracts of barley leaves prepared by autoclaving. The PRF induces papilla formation, increased papilla frequency from 65% to 95%, and reduced the penetration efficiency of the powdery mildew fungus from 80% to 2.5%, all in susceptible barley. Moreover, the PRF induced lignification in both barley and radish cell walls. Application of lignification preparations similarly from several plant sources also induced papilla formation and resistance in susceptible barley. These results suggest that the PRF is an elicitor. The PRF had no apparent, direct deleterious effect on either the host or the parasite. We will identify the PRF and clarify the mechanism by which it regulates papilla formation.

A775
AN INHIBITOR OF PECTINLYASE FROM SUGAR BEET. W. M. Bugbee, U.S. Department of Agriculture, Northern Crop Science Laboratory, P.O. Box 5677, Fargo, ND 58105-5677.

Pectin lyase (PNL) is the major pectolytic enzyme produced by Rheozotinia solani AG 2-2 in culture and in infected sugar beet crowns and roots. A constitutive inhibitor of PNL (PNLI) was extracted from beet root and beet leaf. The PNL was purified by cation exchange, affinity, and gel filtration chromatography. The PNLI is a protein with a molecular weight estimated at 43 kD. Inhibitory activity was most effective at pH 6.5. The average content of PNLI for crown, hypocotyl, and root tissue was 40% higher in a root resistant germplasm line than in a susceptible cultivar and was higher in the root than the crowns of the resistant cultivar. PNLI partially protected cells from damage caused by PNL. Growth of R. solani in liquid culture was not inhibited by PNLI.

A776
USE OF FLUORESCENT DYES TO MONITOR THE EFFECTS OF Oligogalacturonides on ACTIVE OXGEN METABOLISM. E. W. Orlandi, & C. J. Baker, Department of Botany, University of Maryland, College Park, MD, 20742 and U.S.D.A., A.R.S., Microbiol. & Plant Path. Lab., Beltsville, MD 20705.

Phytoalexin elicitors have been hypothesized to bind to cell membrane receptors which, in turn, stimulate the production of H2O2. The subsequent peroxidase-mediated reduction of the H2O2 has been reported to result in the oxidation of certain chlorophyll cay dyes, with far red light emitted. We have found similar results in cell-free conditioned medium from soybean suspension cultures. The introduction of oligogalacturonides to this conditioned medium stimulates the oxidation of fluorescent dyes, indicating an increase in H2O2. This indicates a relationship between the elicitor and certain extracellular enzyme systems which are not membrane-mediated. On the contrary, the oligogalacturonide appears to have a direct effect on one or more of the enzyme or substrate components, resulting in the bleaching of the fluorescent dyes.

A777

Soybean (cv Mandarin) cell suspensions were treated with pectic oligoaxarichides, Pseudomonas syringae pv glycinea race 6 which causes a hypersensitive reaction in plant and with race 4 which is a pathogen. The role that active oxygen plays in the early stages of interaction was investigated. Active oxygen production was followed using a luminometer to measure the luminescence emitted by chemiluminescent reaction with the active oxygen species H2O2 and/or O2 (converted to H2O2 with superoxide dismutase). Treatment of cells with pectic oligoaxarichides resulted in an immediate and transient increase in active oxygen levels. Treatments with bacteria resulted in active oxygen levels lower than untreated controls. These results suggest the presence of a H2O2 scavenging mechanism in bacterial treatments.

A778

Protoplasts isolated from tobacco suspension cells were treated with pectic fragment, NaDHP or Pseudomonas syringae pv syringae which causes a hypersensitive response (HR) in tobacco and a non-PR-causing mutant, B7. O2 - was detected by monitoring the oxidation of epinephrine at pH 7.6. The highest levels of O2 - production were seen in protoplasts treated with NaDHP or pectic fragment, while levels in protoplasts treated with bacteria were comparable to untreated controls. Exogenous NaDHP was not required to stimulate superoxide dismutase-inhibitable oxidation of epinephrine in tobacco protoplasts. These results suggest that O2 - production and regulation in plant cells may occur by different mechanisms involving various plasma membrane and/or cell wall components.

A779

By Cladosporium TLC bioassay, antifungal activity was detected in chloroform soluble fractions of methanol extracts prepared from Arabidopsis thaliana leaves inoculated with the pathogen Pseudomonas syringae pv syringae. Little or no antifungal activity was detected in extracts prepared from leaves infiltrated with phosphate buffer, Xanthomonas campestris pv. campestris or an axenic mutant of E. coli. Antifungal activity increased rapidly between 12 and 48 hours after inoculation with P. syringae and reach maximum activity between 24 and 48 hours post inoculation. Phytoalexin activity was also induced by AgNO3 or ZnCl2 treatment. Because Arabidopsis thaliana is amenable to many genetic and molecular techniques including T-DNA insertional mutagenesis and chromosome walking using a YAC library, this crucifer may prove to be a useful model host for studying the role of phytoalexins in disease resistance.

A780
FACTORS INFLUENCING THE PRODUCTION OF PECTINOLYTIC ENZYMES BY Pseudomonas Solanacearum. Jerry Leasow, Caitlyn Allen and Luisa Segura. Department of Plant Pathology, University of Wisconsin, Madison 53706 U.S.A.

The production of extracellular endopolygalacturonase (PG) in 9 defined medium by P. solanacearum was increased by adding low MW factors from plant tissues. The effects of nutritional and other components in plant extracts on induction of PG was examined. Bacteria grown on rich media produced only small amounts of PG (pl 9). However on minimal media a significant increase in (pl 8) PG activity was observed at late logarithmic phase. PG activity was further stimulated by the addition 0.1% galacturonic acid (GA). Chloroform soluble compounds isolated from tobacco leaves significantly increased induction by GA. In addition low MW compounds released by suspension-cultured tobacco cells inhibited the production of pectinmethylesterase (PME) by the bacteria.

A781 Withdrawn

A782
INDUCTION OF LOCAL RESISTANCE TO Fusarium dry rot in POTO TUBER DISCS by NON-PATHOGENIC FUNGUS Cladosporium cucumerinum. Y. Zeng and R. Hamerschmidt. Botany and Plant Pathology, Michigan State University, East Lansing, MI 48824.

The induction of local resistance of potato tuber tissue to Fusarium auburn by Cladosporium cucumerinum was studied. Resistance to infection by P. auburn was evident within 48 hr of inoculation with C. cucumerinum. Previous work has suggested that lignin synthesis was part of the resistance of potato to C. cucumerinum. It has thus played a role in the induced resistance response. Tissue inoculated with C. cucumerinum developed levels of PAL that were, 24 hr after inoculation, 5 fold greater than the wounded controls. Peroxidase, especially acidic isozymes, were induced more rapidly by C. cucumerinum than by wound. Lignin deposition, as reported earlier, was induced within 12 hr of C. cucumerinum, however, did not stimulate CAD activity or accumulation of chlorogenic acid over control levels. Examination of PAL gene expression in response to wounding and Cladosporium inoculation is in progress.

1054 PHYTOPATHOLOGY
A784
SYNTHESIS OF INDOLE-3-ACETIC ACID BY USTILAGINO MADIS. D. A. Navarro and K. E. Damann. Department of Plant Pathology and Crop Physiology, Louisiana Agricultural Experiment Station, L.S.U. Agricultural Center, Baton Rouge, La. 70803-1720.

Corn infected with Ustilago maydis often forms galls. Bioassays were used to screen for U. maydis phytomembrane production, and compounds with cytokinin or auxin activity were detected. XAD-7 chromatography and silica gel TLC was used to purify indole-3-acetic acid (IAA) from submersates of U. maydis cultures supplemented with tryptophan. The mass spectrum of putative IAA from U. maydis corresponded to that of authentic IAA. IAA synthesis by U. maydis was monitored over time using a modified Salkowski reagent. IAA began accumulating in early log phase and peaked in the stationary phase. The IAA concentration peaked at 100 pg/mL after 7 days of incubation. Different precursors converted indole-3-pyruvic (IPY-A) to IAA, but not tryptamine or indole-3-acetic acid. Southern analysis of U. maydis DNA did not reveal detectable homology with auxin probes (IAA and IAAh) from Pseudomonas savastanoi. Auxin synthesis by perigerminal cells was stimulated by the addition of α-ketoglutarate and pyridoxal 5-phosphate. We suggest that in U. maydis IAA synthesis proceeds from tryptophan through IPY-A and indole-3-acetaldehyde.

A785
COLD ACCLIMATION AND RESPIRATION IN MILDEWED WINTER BARLEY. M. R. McInnes, P. G. Arness, W. D. Paul, and A. M. Hetherington. Division of Biological Sciences, University of Lancaster, Lancaster LAI 4YQ, U.K.

Winter barley was grown in cold acclimating conditions, 9°C (8h day)/4°C (night), and infected with a compatible race of powdery mildew (Erysiphe graminis f.sp. hordei). Controls were grown at 20°C (16h day). Treatment effects were similar to whether oxygen uptake was measured on whole leaves or isolated mitochondria. Infection stimulated total respiration in cold grown plants, with a high healthy respiration rate, and controls, with a lower rate. Whereas in healthy cold-grown plants the cyanide-insensitive component of total respiration increased significantly at temperature increased, in comparable mildewed plants it remained constant, cyanide-sensitive respiration being the dominant component. The altered respiration pattern in leaves was related to carbohydrate availability, but changes in mitochondrial activity in the presence of exogenous substrate suggest that functional efficiency was also impaired.

A786
A ROLE FOR CUTINASE IN THE EXPRESSION OF TISSUE SPECIFICITY BY FUNGAL PATHOGENS. Frances Trail and Wolfram Köeller, Department of Plant Pathology, Cornell University, New York State Agricultural Experiment Station, Geneva, NY 14456.

Previous studies indicated a role for cutinase in the expression of tissue specificity by directly penetrating fungal pathogens. Leaf-specific pathogens had cutinases with plating points distinct from those of non-pathogens, which infected both stems and leaves secreted both cutinase types. The presence of different types of cutinase secreted by these pathogens was substantiated by treatment of the culture filtrates with [3H] dioxopropyl fluorophosphate, followed by electrophoresis of the labelled proteins. A bioassay has been developed to explore the role of these two cutinases in penetration by a stem-specific isolate of Rhizoctonia solani that is nonpathogenic on leaves. Inoculum amended with cutinase from a leaf-specific pathogen penetrated the bean leaf cuticle and produced disease symptoms, whereas inoculum amended with cutinase from a stem-base pathogen remained nonpathogenic. The ultrastructure of colonization of these leaves was investigated by scanning electron microscopy. These results are the first evidence for a role of cutinase in the expression of tissue specificity by fungal pathogens.

A787
A TECHNIQUE FOR DETECTING CHITINASE, β-1,3-GLUCANASE AND PROTEIN PATTERNS ON POLYACRYLAMIDE ELECTROPHORESIS OR ISOELECTROFOCUSING GELS. S. S. Pan, X. S. Ye and J. Kuc, Department of Plant Pathology, University of Kentucky, Lexington, Ky 40546.

A procedure is described to assay chitinase and β-1,3-glucanase isozymes and protein patterns on polyacrylamide electrophoresis (PAGE) or isoelectrofocusing gels. After electrophoresis or isoelectrofocusing, an overlay gel containing glycine, chitin and sulphanilic acid in citrate buffer was incubated in close contact with the resolving gel. Chitinase isozymes were revealed by UV illumination after staining the overlay gel with fluorescent brightener 28. The assay appeared sensitive and was shown to be highly specific on PAGE gels. After the resolving gel was incubated with laminarin, β-1,3-glucanase isozymes were detected with 2,3-diphenylindolotetrazolium bromide and could be quantified on PAGE gels. The resolving gel with β-1,3-glucanase bands was stained with Coomassie blue to reveal protein patterns. If both resolving and overlay gels are properly marked, chitinase and β-1,3-glucanase can be identified on gels stained with Coomassie blue.

A788
MANIPULATION OF HOST SUSCEPTIBILITY TO GRAY MOLD BY CALCIUM NUTRITION AND INHIBITORS OF ETHYLENE PRODUCTION OR ACTIVITY. Y. Eliad, Dept. Plant Pathology, The Volcani Center, Bet-Dagan 50250, Israel.

Supplemental fertilization with Ca(NO3)2 (1.2-MM) of tomatoes, cucumbers and roses in greenhouses reduced incidence and severity of gray mold (Botrytis cinerea). Calcium suppressed disease development in cut flowers of rose incubated at 100 more than those incubated at 40 or 20°C. Leaves from calcium-treated plants exuded less nutrients than from untreated plants. Calcium inhibited the activity of pectolytic enzymes of B. cinerea and the production of ethylene by plants. Ethylene increased susceptibility of these and other plants to gray mold. Disease was controlled by inhibitors of ethylene production or activity (Ag-IBA, polyamines, O3H, norbornadiene, benzylammonium, Ag+); by an inhibitor of polyamines synthesis (IPMO) and by radical scavengers. All these compounds may have other indirect effects.

A789
THE STIMULATORY EFFECT OF AMINO ACIDS ON OSPORE GERMINATION OF PHYTOPHTHORA CACTORUM IN VITRO. J. Jiang and D. C. Erwin, Dept. of Plant Pathology, Univ. of California, Riverside, CA 92521.

Mature oospores of Phytophthora cactorum (30 days old) were induced to become dormant by incubation in distilled H2O at 2°C for 20 days. Germination of dormant oospores was increased significantly by 22 amino acids compared to glass distilled H2O and the buffers used in the amino acid solutions. Germination % varied with amino acid, but all germinated within the range of 35-78% at a concentration of 10.0 mM. The minimum concentration of alanine and glycine which stimulated oospore germination was 0.1 mM. Several sugars, organic acids and salts did not stimulate oospore germination at 10.0 mM. When 1.0 mM alanine was mixed with 1.0 mM sucrose or mineral salts, the germination % was higher than with alanine alone. When oospores were incubated in 10.0 mM sucrose solution for 4 days, no germination occurred, but when the sucrose was replaced by alanine (10.0 mM) on day 4, about 40% germinated by the following day. Germination of dormant oospores was increased significantly also by alfalfa root exudate, soil extract, and oospore exudate.

A790
ANTIFUNGAL ACTIVITY FROM THE FRIGAINS OF CORN, SORGWHM AND WHEAT. Darnett, J. F. Leslie and S. Muthukrishnan. Dept. of Plant Pathology, and Dept. of Biochemistry, Kansas State University, Manhattan, Kansas 66506.

Crude protein extracts were made from grains of 12 cultivars each of corn, sorghum, and wheat. These preparations were fractionated on 10% SDS polyacrylamide gels, western blotted and bands corresponding to chitinase and β-glucanase identified. In sorghum, a major chitinase band (mol. wt. 27 kD) and at least two minor bands (25-28 kD) were seen. In corn and wheat at least, two major chitinase bands were observed. For β-glucanase from corn seed, at least three bands were identified per cultivar and there was extensive polymorphism for band size. Isolates from 43 fungal species were grown on carrot juice agar and tested for their sensitivity to these protein preparations. Of the 43 species tested, isolates from 21, including Oomycetes, Ascomycetes and Deuteromycetes, had inhibited growth. Alternaria alternata, Trichoderma viride, and binucleate Rhizoctonia from AG-A, AG-G, and AG-I were particularly strongly inhibited.
A792

DRRG49 AND DRRG26 GENES ARE STRATEGICALLY ACTIVE IN PEA SEEDLINGS RESISTANT TO RACES OF FUSARIUM OXYSPORUM F. SP. PISI. L. A. Hadwiger, C. Chiang, and D. Horovitz, Department of Plant Pathology, Washington State University, Pullman, WA 99164-6440.

Pea lines M410 (Sus) and Vantage (Res) have common genetic backgrounds except for the single-factor resistance to Race 1 of F. oxysporum f. sp. pisi. We acquired DRRG49 and 206 from a cDNA library of Alaska pea pods expressing non-host resistance to F. solani f. sp. phaseoli. These genes are expressed as pea seedlings develop in Race 1-infested soil, but the mRNA accumulates most intensively in Vantage (Res) a few days prior to the corresponding appearance of severe wilt symptoms in M410 (Sus). These results indicate the "master" single Mendelian resistance traits may potentiate multiple "slave-like" gene responses to resist multiple pathogen challenges. The combined activity of DRRGs, such as gene 49 which codes for a major portion of the inducible pea proteins and more recently has been found in many other plants, may provide major functions in resistance generated against multiple pathogens.

A794

SCREENING FOR GENES INDUCED IN A GRASS/FUNGUS SYMBIOSIS. H.-F. Tsai, C. L. Schardl, and M. R. Siegel. University of Kentucky, Lexington KY 40546-0091.

Acremonium coenophialum is a mutualistic, seed-dissimilated endophyte of tall fescue (Festuca arundinacea), an important pasture, forage and turf grass. We wish to identify cDNA clones of plant and fungal genes that are modulated in symbiosis. Poly(A)RNA was isolated from cultured endophyte, uninfected grass, and the grass/fungus symbiote. The grass and symbiote mRNAs were from the meristems and surrounding leaf sheaths, where the endophyte is normally concentrated. The cDNAs were cloned in AluI-ZAP XR (Stratagene), a hybrid phage/phagemid vector. The number of primary plaques in each library were: 4 x 10^2 from the endophyte, 1.4 x 10^6 from the grass, and 2 x 10^5 from the symbiote. The fungus and plant libraries are being used as control competitors in a subtractive-hybridization screening to identify clones of mRNA species whose expression is enhanced in the symbiotic interaction.

A796

MUTANTS OF COCHLIOBOLOS HETEROSTRUPS WITH ALTERED ABILITIES TO SECRETE CELL WALL DEGRADING ENZYMES. L. E. Lynholme, C. A. Spike, and C. R. Bronson, Iowa State University, Ames, Iowa 50011.

The goal of this research is to determine the role of cell wall degrading enzymes in the pathogenicity of C. heterostrophus. An efficient mutagenesis protocol has been developed based on UV irradiation of protoplasts. The survivors are being screened for more, less, or no secretion of 4 enzymes relative to wildtype. To date, 7 protease, 5 β-xylosidase, 3 polygalacturonase, and 2 xylanase mutants have been tentatively identified. Segregational analysis has confirmed the genetic control of the mutant phenotypes of 2 low and 4 non-producers of protease and 1 high producer of β-xylosidase. The remaining putative mutants are being tested. Six of the protease mutants have been inoculated onto maize and found to remain pathogenic. After backcrossing, the pathogenicity of the mutants relative to wildtype will be measured quantitatively by determining infection efficiency and lesions size on maize.
Segregation for virulence/avirulence was studied in *Uromyces appendiculatus* for 33 single-uredial isolates representing nine field populations. Three forms were isolated from infected bean leaves: a non-virulent form, a virulent form, and a mixed form. The mixed form segregated in 86 of 237 host parental isolate combinations (36%). Of 116 avirulent line/iso- late combinations, 50 segregated, suggesting recessive virulence in the mass-selfed progeny. However, 36 of 121 virulent line/iso- late combinations also segregated after mass-selfing, suggesting dominant virulence (studies are not complete to determine how many actual virulence genes are segregating in these 100 collections). All nine collections were polymorphic for virulence on from five to eight of the bean lines, and all had members that segregated for dominant virulence on from two to six of the lines.

### A800

**INTER- AND INTRA- SPECIES HYBRIDISATIONS BETWEEN PATHOGENIC *FUSARIUM* SP. BY PROTOPLAST FUSIONS AND HYPHAL ANASTOMOSIS**

**C. Madhoshingh, Agriculture Canada, London, Canada, N6G 2V4.**

Pathogenic isolates of *Fusarium graminearum* Schwabe (FG), *F. oxysporum* f.sp. *lycopersici* (Sac.) Snyder & Hansen (FOL) and f.sp. *radicis lycopersici* Jarvis & Shoemaker (FORL), treated with the mutagen nitrosog-uanidine presented a number of xylem isolates of the myco- statin (M) resistant mutants. Protoplast fusions and hyphal anastomoses were promoted between FOL (C), FORL (N) and FG (C) in liquid and agar cultures. Samples from these cultures were inoculated on medium contain- ing 'resistant levels' of both antibiotics. Isolates growing on the double antibiotic medium, were consid- ered hybrids. Hyphal tip isolations from the hybrids were maintained on double antibiotic plates. The hyphal hybrids demonstrated differences from the parents in their protein and enzyme patterns, pathogenicity, growth and morphology.

### A801

**INHERITANCE OF STRIPED RUST RESISTANCE IN EIGHT WHEAT CULTIVARS POSTULATED TO HAVE RESISTANCE GENES AT Yr3 and Yr4 LOCII.**

**Xiaoming Chen and Roland F. Line. USDA/ARS, Dept. of Plant Pathology, Washington State Univ., Pullman, WA 99164.**

Cappelle Desprez (CD), Drucamp (DR), Hybrid 46 (H46), Minister (MIN), Nord Desprez (ND), Stephens (STE), Vilmarin 23 (V23), and Yamhill (YAM) have been postulated to have resistance genes at the Yr3 locus and/or Yr4 locus. Seedlings of parents and F1, F2, and BC1 progeny from reciprocal, diallel crosses among the cultivars and of the eight cultivars with Chinese 166 (Yr2) were tested for resistance to selected North American races of *Puccinia striiformis*. Each remaining cultivar has two genes. No common loci were detected for crosses of H46 with DRU, MIN, and STE and of YAM with STE. MIN, CD, DRU, ND, and STE have genes at the Yr3 locus, and the gene in MIN is different from the gene in the other cultivars. H46, V23, and YAM have genes at the Yr4 locus, and the gene in H46 is different from the gene in V23 and YAM. The second gene in H46 is not at the Yr3 locus, as previously reported.

### A802

**ADAPTING A SINGLE-LOCUS, OVERDOMINANCE SELECTION MODEL TO THE AUTOECIOUS, MACROCYCLIC LIFE CYCLE OF *Uromyces appendiculatus*.**

**D. C. Hansen and J. V. Groth, Department of Plant Pathology, University of Minnesota, St. Paul, MN 55108.**

Sexual populations of the bean rust fungus are frequently characterized by high percentages of isolates heterozygous for "unnecessary" virulence genes. Overdominance is one possible explanation for the high frequency of virulence heterozygotes in the single locus, overdominance selection model of classical population genetics was adapted to the autoecious, macrocyclic life cycle of the bean rust fungus, and used to investigate the influence of the size of the virulence heterozygote population on selection coefficients and number of repeating cycles per year on the change in frequency of virulence heterozygotes both within and between years. The results of computer simulations were used to investigate the effect of varying numbers of repeating cycles per year on the frequency of heterozygotes. Proof of overdominance may be obtained by sampling early and late in severe epidemics and observing an increase in heterozygote frequency for several virulence genes.

### A803

**IS801, AN UNUSUAL TRANSPOSABLE ELEMENT OF *PSEUDOMONAS SYRINGAE* PATHOVAR *PHASEOLICOLA*.**

**M. Romantschuk*, G. Richter, and D. Mills. *Department of General Microbiology, University of Helsinki, SF-00100, Helsinki, Finland, and Department of Botany and Plant Pathology and Genetics Program, Oregon State University, Corvallis, Oregon, 97331-2902.**

A transposable element, designated IS801, has been isolated from strain 18700 of *P. syringae pv. phaseolicola*, a pathogen of bean. Partial and complete copies of the element reside on a cryptic plasmid of 18700, and in the genomes of other *P. syringae* pathovars. Two copies of IS801 that had transposed into different sites of an entrapped plasmid, pUCK800, as well as a third copy which has not yet been observed to transpose, have been cloned. IS801 generates a duplication of a five base pair (Sp) target site. The element is 1537 bp in length, contains two open reading frames of 1230 bp and 597 bp on opposite strands, and is unusual in that it contains no direct or inverted repeats at its termini.

### A804

**NITRATE NONUTILIZING MUTANTS OF COLLETOTRICHUM.**

**Brooker, N. L., Leslie*, J. F., and Dickman, M. B. Department of Plant Pathology, University of Nebraska, Lincoln, NE 68583-0722 and Kansas State University, Manhattan, KS, 66506.**

Colletotrichum spp. represent a diverse and complex group of economically important fungal pathogens. Methodologies used for analyzing relatedness of species and subspecies have been com- plex, and in many cases contradictory. An alternative approach is based on vegetative compatibility. Mutants unable to reduce NO3 to NH3 (nit mutants) arose spontaneously as sectors resistant to KC10, in strains isolated from mango. Nit mutants could be divided into four phenotypic classes. These classes presumably represent mutations at a nitrate reductase structural locus (niti), global nitrogen regulatory locus (nni), a nitrate assimilation pathway-specific regulatory locus (niti), and several loci that affect the assembly of a molybdenum-containing cofactor (Nit M). Frequencies of nit mutations, mutant morphologies, physiological complementation of the nit mutants, and preliminary vegetative compatibility studies suggest that the isolates examined are all genetically distinct.

### A805

**VEGETATIVE COMPATIBILITY AMONG ISOLATES OF COLLETOTRICHUM GLOEOSPOROIDES F. SP. ASECCHROMENAE.**

**R. L. Chaudo, J. C. Correll, G. J. Weidemann and D. O. TeBeest. Department of Plant Pathology, University of Arkansas, Fayetteville, AR 72701.**

Field isolates of Colletotrichum gloeosporioides f. sp. asecchromenae (CGA) from Northern Jointvetch and Indiana Jointvetch in Arkansas and Louisiana were examined for vegetative compatibility. Nitrate non-utilizing (nit) mutants were generated from isolates using minimal medium amended with potassium chloride. Auxotropic mutants and nit mutants derived from auxotropic mutants also were generated from several isolates and paired to study heterokaryosis. Pairs of phenotypically distinct Nit and NIm mutants as well as several auxotropic mutants suggest that all isolates are vegetatively compatible. Mycelial transfers of wild type isolates grown on water agar plates were examined microscopically and hyphal fusions were observed in all strains. Analysis of mycelial blocks taken from heterokaryotic colonies suggests localization of the heterokaryotic region within the central portion of the colony. Results indicate that CGA isolates from different geographic locations are vegetatively compatible.
Strains of Xanthomonas campestris cause bacterial canker (X. c. citri) and bacterial spot (X. c. citri var. coccundefined) of citrus, respectively. Strains of both pathovars were compared for their capability to grow endophytically and epiphytically. These capabilities were positively correlated with aggressiveness. Endophytic populations were indicative of epiphytic populations, and might be used to predict field spread of a particular aggressiveness type. Strain X cultivar interactions occurred when aggressive strains of X. c. citri var. coccundefined were compared on cultivars Swingle citrumelo and Duncan grapefruit. X. c. citri and the aggressive strain of X. c. citri var. Swingle citrumelo were the only strains capable of significant growth in citrus leaves.

A808

CHARACTERIZATION OF XANTHOMONADS FROM ARACEAE BY FAT-ACID ANALYSIS. M. C. Hudspeth, A. R. Chase, and R. E. Stall. Plant Pathology Department, University of Florida, Gainesville, FL 32611.

Based on analyses of fatty acid profiles of xanthomonads from different genera of Araceae 150 strains were divided into subgroups. Strains from Ageratum, Anthurium, Colocasia, Dieffenbachia, Epipremnum, Philodendron, and Sympodium all had similar ratios (2:1) of the predominant acids 15:0 iso and 15:0 anteiso, respectively. Subgroups were based on quantitative differences among other unsaturated and hydroxy fatty acids. None of the subgroups was consistently associated with strains from a particular host. The profiles of 12 strains from Xanthosoma differed from the strains isolated from other aroids by the unique 1:2 ratio of the 15:0 iso to 15:0 anteiso fatty acids. The strains from Xanthosoma were pathogenic to other aroids.

A810

ISOLATION OF EXTRACELLULAR POLYSACCHARIDES PRODUCED BY Clavibacter michiganensis subsp. sepedonicus. A. Westra and S. A. Slack, Department of Plant Pathology, Cornell University, Ithaca New York 14853.

Fluidal strains of Clavibacter michiganensis subsp. sepedonicus (Cms), the causal agent of bacterial ring rot of potato, were found to produce, in vitro, four extracellular polysaccharide components that could be separated on the basis of their size and charge. Components I and II were large (≥2 x 106 and 4.5 x 106 daltons, respectively), acidic polysaccharides that appear to be aggregates of a small fraction of a dalton, component III. Components I, II, and III were of similar neutral sugar composition (1 fucose: 5 mannose: 1 galactose: 1 glucose) and reacted similarly with polyclonal antisera specific for whole Cms cells. Components I and II, when treated with 0.1% SDS, dissociated into a subset of a size and composition similar to that of component III. A fourth component, IV, differed considerably from the other three components in that it was neutral, composed primarily of mannose, and did not react with polyclonal antisera specific for whole Cms cells. All components were found to be homogenous based on ion exchange or gel permeation chromatography, compositional analysis, and reaction with polyclonal antisera in Oscherteryo agar double diffusion scroloty. No evidence for the presence of glycopeptides was found.

A811

THE ORIGIN OF CONJUGAL TRANSFER OF THE Agrobacterium tumefaciens Ti PLASMID pTiC58. D. M. Cook and S. K. Farrand, Department of Plant Pathology, University of Illinois at Urbana-Champaign, IL 61801.

Ti plasmids of Agrobacterium tumefaciens are conjugational plasmids and their transfer is induced by opines secreted from galled cells. In well-characterized conjugational plasmid systems such as F, the process of conjugation initiates at a cis-acting site known as the origin of conjugational transfer or oriT. We have localized an oriT on the A. tumefaciens plasmid pTiC58 to a region encoding conjugational transfer loci TraI and

A812

TRANSFORMATION OF A XYLEM-LIMITED BACTERIA (Clavibacter xyli subsp. cynodontis) OF BERMUDA GRASS TO EXPRESS INSECTICIDAL A. thuringiensis TOXIN. M. C. A. Meister, T. A. Chen. Martin Hall, Cook College, Rutgers University, New Brunswick, N.J. 08903.

Clavibacter xyli subsp. cynodontis is a gram-positive coryneform bacteria which lives non-pathogenically in the xylem of bermuda grass. Our goal is to transform the bacteria with a gene coding for one of the insecticidal proteins from Bacillus thuringiensis. Such a bacteria could then be transferred back into the host and would presumably confer upon it insect resistance. We have developed a procedure for transforming the bacteria with a plasmid using electroporation, and are currently cloning a Bt toxin gene into the plasmid in preparation for transforming it into the bacteria. The transformed bacteria will then be transferred into uninfected bermuda grass and the grass tested for insecticidal properties. In addition, we are infecting various other turf grasses with the bacteria to determine if it can grow in other hosts.

A813

PHENOTYPIC PLASTICITY AFFECTING EPIPHYTE SURVIVAL IN PSEUDOMONAS SYRINGAE. M. Wilson and S. E. Lindow, Department of Plant Pathology, University of California, Berkeley, CA 94720.

P. syringae cells cultured in either solid or liquid media, or harvested from inoculated bean plants, were sprayed onto greenhouse-grown or field-grown bean plants and then incubated, either in a growth chamber at low relative humidity and high light intensity, or in the field. Recoverable P. syringae populations decreased 10-50 fold in the first 12 h, then increased again in the following 24 h. In the growth chamber, the population of liquid-cultured cells decreased on average 250-fold, compared to the plant-harvested and solid-cultured cells which decreased on average only 90-fold and 50-fold, respectively. In the field, similar patterns of survival were observed, but in addition the plant-harvested cells started to multiply earlier and reached a higher final population than other cell types. These results suggest that studies of bacterial epidemiology using cells cultured in vitro will not be accurate predictors of field behavior.

A815

DEVELOPMENT OF AN INDEXING SYSTEM FOR CONTROL OF BACTERIAL BLIGHT OF ANTHURIUM. D. Norman, A. Alvarez, and A. Benedict. University of Hawaii, Honolulu, HI 96822.

Production of disease-free plants and the development of an indexing system to detect latent infections in asymptomatic plants is a first step in controlling anthurium blight, a widespread disease that has caused significant losses to the anthurium industry. A diagnostic system for symptomless plants potentially infected by Xanthomonas campestris pv. dieffenbachiae (Xcd) was developed. Indexing involved assays of tissue
A816


Studies are being initiated to determine if streptomycetes can have inhibitory effects on the white rot wood decay fungi Armillaria ostoyae (NABs E) or A. bulbosa (NABs VII). The five streptomycete morphotypes being tested were originally isolated from the mycorrhizoplane of healthy red pine seedlings growing in four year old plantations which have experienced approximately 12% mortality due to root rot caused by A. ostoyae. However, A. bulbosa is also abundant in the same plantations. The streptomycetes selected have demonstrated inhibitory effects in vitro on growth of Laccaria and Thelohaphora spp. In these studies, an Armillaria isolate is inoculated onto MMN agar previously inoculated with a single streptomycete morphotype. Radial mycelial growth away from the streptomycete culture was measured. Three clones representing each Armillaria sp. have been tested. Compared to controls, inhibition ranged from 10-100%, depending on the streptomycete morphotype used. The effects of these streptomycetes on rhizomorph growth are also under study. Further work may involve characterization of the inhibitory compounds.

A820

PROFESSIONALISM IN PLANT PATHOLOGY - CAN IT BE TAUGHT? C. J. D'Arcy and W. L. Pedersen, Department of Plant Pathology, University of Illinois, 1102 S. Goodwin Ave., Urbana, IL 61801.

Professionalism has several aspects, which include the methods, character and standards of individuals in the field. Since we believe that some aspects of professionalism can be taught, in 1982 we initiated a course for graduate students on professionalism in plant pathology. The objectives of the course are to convey practical information about our field and to encourage development of interpersonal skills. The ungraded course is totally voluntary. Many guest speakers are invited to share their expertise and experience on a wide range of topics. Some topics which have been included are scientific writing, authorship, editorial reviews, manuscript reviewing, job search and applications, types of jobs, interviews, benefits, seminar presentation and evaluation, grant funding sources and review processes, and teaching methods and evaluation. Sample "scenarios" which are used to generate class discussion will be displayed.

A817

CALCIUM CHLORIDE ALLEVIATION OF SALT STRESS IN RHIZOBIUM LEMMINSOARIS BIOVAR VICTAE. C. Chien, R. Rupp, and C. S. Orser. Department of Bacteriology and Biochemistry, University of Idaho, Moscow, Idaho 83843.

R. lemnisarivus biovar vicvariae strain C1204b exhibits a dramatically reduced growth rate when exposed to 200 mM NaCl. Common osmoles, such as proline, glycine betaine, choline, glutamate and trehalose, do not relieve the sodium toxicity. However, the addition of calcium chloride relieves the inhibition of growth caused by salt stress to the free-living microsymbiont. The growth rate of C1204b in the presence of NaCl steadily increased with increasing CaCl2 concentration from 1 to 6 mM. Moreover, other divalent cations (SrCl2, MgCl2, BaCl2) are also able to ameliorate salt toxicity, although to a lesser degree than CaCl2. Thg-induced mutants were generated which no longer respond to CaCl2 alleviation of NaCl toxicity. The mutants also do not respond to other divalent cations. Complementing cosmid clones have been isolated for the CaCl2 mutants from a genomic bank of C1204b.

A821

AN IMPROVED TECHNIQUE FOR CREATING AND MAINTAINING LEAF WETNESS BY USE OF ULTRASONIC HUMIDIFIERS. B. J. Steffenson and T. C. Fetch, Jr. Dept. of Plant Pathology, North Dakota State Univ., Fargo, ND 58105.

Many plant pathogens require free moisture for infection. Laboratory studies of this requirement indicate that improvements for inducing leaf wetness can be made using ultrasonic humidifiers. UHS produce a fine mist (about 5um diameter droplet) by means of a piezoelectric transducer, and in our studies, have proven effective in creating and maintaining uniform leaf wetness on barley and wheat. Using UHS in plexiglass-stained professional attitudes. The ungraded course is totally voluntary. Many guest speakers are invited to share their expertise and experience on a wide range of topics. Some topics which have been included are scientific writing, authorship, editorial reviews, manuscript reviewing, job search and applications, types of jobs, interviews, benefits, seminar presentation and evaluation, grant funding sources and review processes, and teaching methods and evaluation. Sample "scenarios" which are used to generate class discussion will be displayed.

A822

A leaf-disk inoculation technique for evaluation of sunflower downy mildew (Plasmopara halstedii) resistance. T. J. Gulya. USDA Northern Crop Science Laboratory, Box 5677, Fargo ND 58105.

A leaf-disk inoculation (LDI) technique was developed which permitted determination of susceptibility within 7 days, compared to 14 days for conventional seedling inoculation. Leaves were harvested in microwaves, dusted with 400 mesh carbon cowdium powder and rubbed gently. Leaf disks (1.5 cm diam) were cut, avoiding main veins, and were vacuum-infiltrated with distilled water. The water-soaked disks were immersed in a zoosporangia suspension (2 x 10^3/ml) for 3-5 sec and then thoroughly dried on 75% water agar plates, and incubated in growth chambers at 15 C with 16 hr photoperiod of 150 micromolm/sec. Sporulation, predominantly around disk edges, was observed with a dissecting microscope. The cowdium-carbon and water-wounding and water-soaking greatly increased the number of disks with sporulation and sporulation intensity. Leaf disks from mildew-resistant genotypes exhibited sparse sporulation on 5% or less of the disks. Results from LDI corroborated well with those from conventional seedling inoculations.

A823


The double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) has been used extensively for the detection of citrus tristea
A824

A COMPARISON OF THREE SELECTIVE MEDIA FOR ENUMERATION OF SCLEROTIOSONA MACROPHAGIA AND PHASEOLINA CELLS. C.L. Cloud and J.I. Rup. University of Arkansas, Fayetteville, 72701.

Selective media for enumeration of sclerotia of *Macrophomina phaseolina* have temporal limitations in relation to incubation periods. The selective chemical compounds used also either highly toxic or difficult to obtain. A new selective medium (PK) consisting of 30 mL of potato dextrose agar, 100 mg/L rifampin, and 224 mg a.i/L methyl acetate is compared against two other selective media (MP, MS) used commonly to enumerate *M. phaseolina* sclerotia.

Two techniques used in *Macrophomina phaseolina* infected pea plants were assayed as well, as sterilized soil infected with sclerotia produced on vermiculitic prepared on vermiculitic and then diluted serologically to 1/4 w/v. PK had an overall significantly higher (P<0.01) sclerotial count in natural field soil and did MP and MS. Sclerotial counts in 7 out of 20 natural soil samples were significantly higher (P<0.01) on PK than on MP or MS. No significant differences in sclerotial counts among the three media were observed in the artificially infected soil. PK is a preferred medium because it is efficient in enumerating sclerotia in natural soil, contains fewer chemical compounds, and the incubation period for PK ranges from 4 to 14 days compared to no more than 4 days for MP and after 7 days for MS.

A828

DECREASED WINTER SURVIVAL OF SOUTHERN CALIFORNIA INFECTED WITH SOUTHERN CALIFORNIA RED LEAF VITUS. M. R. Maughan, USDA, ARS, Crop Science Research Laboratory, Forage Research Unit, P.O. Box 5367, Mississippi State, MS 39762-5367.

*Tritonum subterraneum* L. plants were transplanted to the field in 6 wk-old seedlings in mid-Oct. 1989 in an experiment to test the hypothesis that virus infection predisposes plants to winterkill. Plants were set 15 cm apart in a 4 x 4 grid within micropots mulched with 75-cm squares of DuPont TYPAR landscape fabric separated by 60-cm-wide rescue borders. Treatments consisted of two levels of virus inoculation across two cultivars in a 2 x 2 factorial experiment (RCB) with 4 replicates. Inoculations were made in late Nov. with a Mississippi isolate of *subterraneum* clover red leaf (soybean dwarf) luteovirus (Phytopathology 78:1584), using viruliferous *Acrithosiphon pisum.* Freezing temperatures in late December reduced stands by more than 50%. Counts of plants surviving in mid-Feb. 1990 were subjected to ANOVA. Significantly fewer (p=0.05) inoculated than noninoculated plants survived (23% vs 44%), supporting the hypothesis.

A829

AN OUTBREAK OF MAIZE CHLOROTIC MOTTLE VIRUS IN HAWAII AND POSSIBLE ASSOCIATION WITH THrips. X. Q. Jiang, D. R. Wilkinson, and J. A. Berry, Pioneer Hi-Bred International, Inc., 7250 NW 62nd Ave., P.O. Box 1004, Johnston, IA 50131.

Maize Chlorotic Mottle Virus (MCMV), a viral component of the CN (corn necrosis) complex, was first recorded for the first time in Hawaii using ELISA. The disease spread quickly on the island of Kauai and appeared closely related to an increasing population of thrips which could not be controlled by spraying due to a long period of rain. Biotin-labelled indirect ELISA verified that the virus was associated with the thrips. Thrips transmission studies are currently in progress. Although MCMV has been reported to be non-seed transmissible, all parts of immature ears (silk, pericarp, embryo, cob, and whole seed) were shown positive to MCMV antibody. Subleaves are currently underway to survey other possible vectors and to test mature seeds.

A830


A virus causing mosaic disease of *Vigna unguiculata subsp. sesquipedalis* on Guam was isolated and partially characterized. Systemically-infected bean plants develop mosaic, vein-banding, and leaf deformation. In host-range tests, 49 plant species, cultivars, or breeding lines were sap-inoculated. Thirty-nine developed viral symptoms or were shown to be infected by return inoculations on *Chenopodium amaranticolor,* a local-hostion. The virus was transmitted in a non-persistent manner by the aphid *Aphis craccivora.* In *Psylliodes chrysripus* larvae, enzyme-linked immunosorbent assay (F-AS ELISA) sap from virus-infected plants reacted negatively with antiserum for strain W of *blackeye cowpea mosaic virus* (BCMV). Biological and serological tests putatively identify the virus as a strain of BCMV.

A831


Inoculation of sap from yellow ironwood (Verbesina alternifolia) exhibiting mosaic symptoms resulted in necrotic local lesions in *Nicotiana rustica.* Ultrastructural studies of these two hosts revealed large spherical particles (70-120 nm) similar to tomato spotted wilt virus.
(TSWV). Unlike TSWV, the particles were individually encased in a membra
dane which originated from either rough ER or the outer
membrane or Golgi bodies. The particles were
associated with two types of cytoplasmic inclusions
distinct from those induced by RNA virus. Two
immunoperoxidase tests indicated no serological rela-
tionship to common TSWV isolates or the impatiens isolate of
TSWV described by Law and Moyer (Phytopathology
79:1187).

A832
TRANSLLOCATION OF BARLEY YELLOW DWARF VIRUS-PAV-IL IN TOLERANT
AND SUSCEPTIBLE SISTER OAT LINES. H. M. Foulk and C. J. D'Arcy,
Department of Plant Pathology, University of Illinois, 1102 S.
Goodwin Ave., Urbana, IL 61801.

Translocation of barley yellow dwarf virus PAV-IL (BYDV-PAV-IL) in
two pairs of sister oat lines tolerant and susceptible to the
virus was measured by triple antibody sandwich enzyme-linked
immunosorbent assay. Two-week-old oats, grown in aeroponic
culture, were inoculated with viruliferous Rhopalosiphum padi L.
Plants were collected at 2-day intervals and dissected into
four parts: the shoot and three root segments (basal, central
and apical). Symptoms of BYDV-PAV-IL were observed only on
susceptible oat lines 9 days after inoculation. Virus was
detected in shoots and all root segments of each line from 2 to
15 days after inoculation. There were no differences in virus
levels in shoot or roots of tolerant and susceptible oat
lines. Virus levels were higher in apical root segments
than in those closer to the crown. For these oat lines,
there is no evidence that differences in virus translocation
are responsible for differences in susceptibility to BYDV-PAV-IL.

A833
ELIMINATION OF SWEET POTATO FEATHERY MOTTLE VIRUS FROM
SWEET POTATO USING IN VITRO CULTURE OR PROPAGATION
OF APLICAL BUDS UNDER GREENHOUSE CONDITIONS. H.M. Griffiths and S.A.
Snow, Dept. Plant Pathology, Cornell University, Ithaca, NY 14853.

Auxiliary buds (3.5 mm) from sweet potato plants cv. Georgia Red infected with
sweet potato feathery mottle virus (SPFMV) were excised from plants in vitro on MS
medium supplemented with 20 mg/l ribavirin (MSR), heat treated under a 4-h alternating
35°C light/31°C dark regime for 28 days, and tested for virus freedom using dot-blot
ELISA. SPFMV-free cultures were confirmed negative by ribavirin-free MSR media.
Culturing buds from proximal regions on MSR resulted in the highest proportion
(85%) of SPFMV-free plants. An alternative method for obtaining SPFMV-free
plants was to excise apical buds (~7 mm) from infected greenhouse plants, to
induce rooting in vitro, and then to transfer plants to greenhouse conditions for
continued growth. For cv. Georgia Red, 12% of plants established from apical buds
were SPFMV-free and for cv. Jewel, 65%. These two studies show that SPFMV-
free plants can be obtained either by in vitro culture procedures using ribavirin
and heat to excise apical buds from greenhouse plants which were rooted in vitro
prior to transferring to the greenhouse.

A834
INCIDENCE OF BARLEY YELLOW DWARF VIRUSES (BYDV) IN WHEAT AND
OTHER HOSTS IN ARKANSAS. T. Mahwood, R. C. Gerlicher and C.
J. D'Arcy, Deps. of Plant Pathology, Univ. of Arkansas,
Fayetteville, AR 72701, Univ. of Illinois, Urbana, IL 61801.

Leaf samples of symptomatic wheat (Triticum aestivum L.)
were collected from commercial plantings throughout Arkansas
in the spring of 1989 for BYDV testing. Samples were assayed
by indirect ELISA using polyclonal antisera for trapping
and monoclonal antibodies for detection of the PAV,
M4V, and RPV serotypes of BYDV. Of 588 wheat samples tested,
two were positive for the PAV serotype, 13 for RPV, and
one for MAV. Suspect wheat samples from the summer of 1989 showed that fescue is a potential overwinter-
ning host for the serotype of BYDV. Three-year-old plots
of 'Kentucky 31' fescue, which were free of the endophyte
Acremonium coenophialum, had a significantly higher incidence
of BYDV (86%) than endophyte-infected plots (42%).

A835
1989 SURVEY FOR THREE SEROTYPES OF BARLEY YELLOW DWARF VIRUSES
IN OAT AND WHEAT FIELDS IN ILLINOIS. C. J. D'Arcy, Department
of Plant Pathology, A. D. Rewings, USDA ARS, Crop Protection
Research Unit, and A. H. Easterling, Natural History Survey,
University of Illinois, 1102 S. Goodwin Ave., Urbana, IL 61801.

Fifty leaf samples were collected in a random pattern from each of
11 oat and seven wheat fields in Illinois during May and June
1989. Samples were assayed for three serotypes of barley yellow
dwarf viruses (BYDV) in two types of enzyme-linked immunosorbent
assay (ELISA). Double antibody sandwich ELISA systems used
polyclonal antibodies for virus detection; triple antibody sand-
wich ELISA systems used monoclonal antibodies for detection.

Incidence of BYDV-PAV serotypes were 0-42% in oat fields and
0-42% in wheat fields. Incidence of BYDV-M4V serotypes were 0-
42 and 0-42 in oat and wheat fields, respectively. Only one BYDV-
M4V serotype was detected. Results from the two BYDV-PAL ELISA
tests were in agreement for over 98% of the 900 samples;
however, there was less agreement between the two BYDV-HPV
ELISA systems. Differences in disease incidence were noted among oat
cultivars and across geographic regions of Illinois in 1989.

A836
EFFECT OF DPX-V9360 AND PRIMISULFURON IN MAIZE DWARF MOSAIC
VIRUS SEVERITY IN CORN. N. J. VanGessel, K. R. Zagula, S.
A. Lomel1, and H. D. Debeljak. Dep. of Crop Science, and
Dept. of Plant Pathology. North Carolina State University, Raleigh, NC 27695-7620

Greenhouse studies were conducted to evaluate the effect of the herbicides DPX-V9360 and primisulfuron on maize dwarf mosaic
virus (MDMV-A) infection in corn (Zea mays L. cv. Pioneer
3676). Both herbicides applied 24 hours after inoculation with MDMV-A suppressed visual symptoms when raced 17 days after treatment. Viral concentration, as determined by indirect ELISA, was significantly reduced when either herbicide was applied 24 hours before inoculation and tissue collected 17 days after treatment. Thus it appears either herbicide is reducing the severity of MDMV-A infection.

A837
SUGAR CONTENT, ANTIOXYCIN PRODUCTION AND REDISTRIBUTION IN
SUBTERRANEAN CLOVER RED LEAF (SOYBEAN DWARF) LUTEROVIRUS-
INFECTED SUBTERRANEAN CLOVER LEAVES. A. E. Zipf and F. A.
Redin. USDA, ARS, Crop Science Research Laboratory,
Mississippi State, MS, 39762-5367.

Symptoms of subterranean clover red leaf (soybean dwarf)
luteovirus infection of Trifolium subterraneum cv. Mt. Barker and
Geraldton include distinctive sugar accumulation and leaf
yellowing which extends from the margins inward. The bright reddening
is restricted to the upper and lower epidermal cells. Increases
in sucrose and fructose content of leaf chromatography
coincided with increased reddening of Infection Mt. Barker leaves.
Feeding of detached Geraldton leaves with 3% solutions of fruc-
tose, sucrose, and glucose produced reddish-purplish discolora-
tion within 5 days. The relative increases in sugars between
anthocyanin pigments isolated from virus-infected leaves or
sugar-fed leaves.

A838
DISEASES OF PEPEROMIA, IMPATIENS AND HIBISCUS CAUSED BY
CUCUMBER MOSAIC VIRUS. Stanislav Flasinski, Simon Scott,
J. Q. Xia, Chao Sun, and O. W. Barnett, Department of Plant
Pathology and Physiology, Clemson University, Clemson, SC 29634-
0377.

A disease of Peperomia characterized by large, black ringspots
was caused by an isolate of cucumber mosaic virus (CMV-ppp).
Various Peperomia cultivars exhibited chlorotic mottling,
chlorotic ringspots, systemic spots, and/or necrotic rings after
inoculation with CMV-ppp and most plants were stunted
relative to uninfected plants. CMV-ppp formed a purplish
morlet rop boxyelodery serology tests with D and S serotypes. An S
serotype isolate of CMV was found in Impatiens hawkeri-type with
mosaic and strawleaf symptoms, the first of this serotype found
in North Carolina. Moso disease of Peperomioides pre 0 in California
also was due to a S serotype. Host reactions, agarose gel electrophoresis of
virus and RNAs, and polypeptide gel electrophoresis of the proteins and
dRNAs showed minor differences among the isolates.

A839
TOLERANCE OF Fusarium SPECIES TO HYGROMYCIN AND BENOMYL.
L.L. Thompson and T. Kenedahlah, Department of Plant Pathology, University of
Minnesota, St. Paul, MN 55108.

To study the feasibility of using the hygromycin and benomyl
resistance genes cry1Ac, cry1Ab, and cry1B from Bacillus thuringiensis
were tested for tolerance to hygromycin and 13 species (59
isolates) were tested for tolerance to benomyl. Growth
was detected in 6 of the 13 species in a medium containing 100
µg/ml of hygromycin, while only 3 of these 6 grew on 150 µg/ml. Tests
with a benomyl-amended medium revealed that all species grew at
1 µg/ml, 10 at 3 µg/ml and 4 at 5 µg/ml. All isolates grew more
slowly on the selective medium than on unamended potato-dextrose
agar and all 21 F. moniliforme isolates grew more slowly on

Vol. 80, No. 10, 1990 1061
A REVERSE GENETICS APPROACH TO CLONING A PHYTOLACXIN-DETOXIFICATION GENE FROM Fusarium solani f. sp. pisi. Phascolus vulgaris (French bean) produces three major phytolacxins, phascolalin, phascolinsulfavon and kievitone, all of which are enzymatically detoxified by the pathogen Fusarium solani f. sp. pisi (Fsp). The secreted glycosylated kievitone hydrazase (KHy) has been purified and the N-terminal amino acid sequence determined. In order to identify clones of the Fsp gene, two genomic libraries were prepared. Eco DNA was partially digested with Sau3AI and fractionated by rate zonal centrifugation. Fragments of 8-23 kb were ligated into SFIH1 phage vector to produce a library of 5 x 105 primary plaques. Fragments of 30-42 kb were ligated into cosmids pKBH2, an E. coli/A. nidulans shuttle vector, giving rise to 5 x 106 colonies. Oligonucleotide mixtures, based on the KHy N-terminus, are being used as probes. Putative positives will be analyzed by Southern blot and by heterologous expression in KHy-deficient fungi.

A REVERSE GENETICS APPROACH TO CLONING A PHYTOLACXIN-DETOXIFICATION GENE FROM Fusarium solani f. sp. pisi. Phascolus vulgaris (French bean) produces three major phytolacxins, phascolalin, phascolinsulfavon and kievitone, all of which are enzymatically detoxified by the pathogen Fusarium solani f. sp. pisi (Fsp). The secreted glycosylated kievitone hydrazase (KHy) has been purified and the N-terminal amino acid sequence determined. In order to identify clones of the Fsp gene, two genomic libraries were prepared. Eco DNA was partially digested with Sau3AI and fractionated by rate zonal centrifugation. Fragments of 8-23 kb were ligated into SFIH1 phage vector to produce a library of 5 x 105 primary plaques. Fragments of 30-42 kb were ligated into cosmids pKBH2, an E. coli/A. nidulans shuttle vector, giving rise to 5 x 106 colonies. Oligonucleotide mixtures, based on the KHy N-terminus, are being used as probes. Putative positives will be analyzed by Southern blot and by heterologous expression in KHy-deficient fungi.

CHARACTERIZATION OF EXTRACELLULAR ENZYMES OF COLLETOTRICHIUM SPECIES. R. S. Redman, A. Schlemmer, and R. Rodriguez, Department of Plant Pathology, University of California, Davis, CA 95616.

Extracellular protease and endoglucanase from C. lindemuthianum, C. coccodes, C. acutatum, C. frigariae, C. gloeosporioides, C. musae, and C. graminicola were characterized. A high degree of variation was observed in the biochemical regulation of these enzymes among different isolates of each species. Four active forms of the endoglucanase were separated by SDS-PAGE. One of the active forms of endoglucanase (EG-1) was purified to homogeneity and found to belong to a large D-linked glycoprotein. An oligonucleotide probe was constructed based on a partial protein sequence from EG-1; genomic clones were isolated by hybridization to the oligonucleotide probe. Characterization of these clones will be discussed.

A RECURRENT TRANSLOCATION ASSOCIATED WITH HOST-SPECIFIC VIRULENCE IN COCCILIOBULUS HETEROSTROPHUS. H. ZHANG and C. R. Bronson. Department of Plant Pathology, Iowa State University, Ames, IA, 50011.

The host-specific virulence of race T of Coccillobulus heterostrophus to T-cyttoplasm maize is controlled by the locus Tox. We have tested the hypothesis that race T (tox+) and race O (tox-) isolates of C. heterostrophus differ by a chromosome rearrangement with its breakpoint at or near Tox. The chromosomes of 16 near-isogenic laboratory strains (8 tox+ and 8 tox-) and 16 unrelated field isolates (8 tox+ and 8 tox-) were separated by pulsed field gel electrophoresis and hybridized with probes known to map around Tox. The hybridizations demonstrated that the two chromosomes associated with Tox in the tox- isolates are reciprocally translocated with respect to their homologous chromosomes in the tox+ isolates. In addition, the tox+ field isolates had fewer chromosome size polymorphisms than the tox- field isolates. These results suggest that race T may have evolved from race O by a reciprocal translocation event.
A848
IDENTIFICATION OF A GENE ENCODING PISATIN DEMETHYLATING ABILITY FROM Fusarium oxysporum F. Sp. Pisi. J. N. Delserone and H. D. VanEtten, Departments of Plant Pathology, Cornell University, Ithaca, NY 14853 and University of Arizona, Tucson, AZ 85721, respectively.

Previous studies have established that the ability to rapidly demethylate, and thereby detoxify, the pytoalexin pisatin is required by Nectria haematoscoeca for high virulence on garden pea (Pisum sativum). Preliminary studies suggest that this also is true for Fusarium oxysporum F. sp. pisi. To determine whether there is a relationship between genes encoding pisatin demethylating ability (pda) in both fungi, genomic DNA of F. oxysporum was probed with a cloned PDA gene from N. haematoscoeca. A gene with similarity to that from N. haematoscoeca was identified and cloned. Sequence analysis of the gene from F. oxysporum will facilitate its further study by expression in a heterologous system and by gene disruption, experiments which can determine the role of the gene in the pathogenicity of F. oxysporum.

A849
SELECTION OF PROBES SPECIFIC FOR SPECIES OR ISOLATES OF THE GENUS Pythium. Frank N. Martin, Plant Pathology Department, University of Florida, Gainesville, Fla. 32611

Selection of probes specific for species or isolates of the genus Pythium may be aided by identification of unique DNA sequences in the mitochondrial DNA (mtDNA). The circular mitochondrial genome ranges in size from 59 to 73 kb and is arranged as a large inverted repeat representing 71 to 83% of the genome, with the repeats separated by a small and a large unique region. Comparison of mtDNA restriction maps of different isolates of P. algalindum indicate that the small unique region is the most variable portion of the genome, with insertions/deletions accounting for a size variation of 0.92 to 3.92 kb. Construction of a detailed restriction map of this region and selection of the appropriate fragments provided probes that were specific for isolates sharing the same restriction map. These fragments also are useful as species-specific probes as indicated by hybridization studies with 25 other Pythium spp.

A850
DEVELOPMENT OF STEWART’S WILT ON SEQUENTIAL PLANTINGS OF SWEET CORN. P. J. Fallah-Noghadian, J. A. Lock, Plant Science, Univ. of Delaware, Newark, and J. K. Patsky, Plant Pathology, Univ. of Illinois, Urbana.

Six sweet corn hybrids partially resistant, intermediate, or susceptible to Erwinia stewartii were planted on four dates in Newark, DE; St. Louis, MO; and Urbana and Rochelle, IL. Natural occurrence of Stewart’s wilt was assessed. Incidence (%) was measured throughout the season. Severity was rated from 1 to 9 about 2 wk before harvest. Stewart’s wilt did not occur at Rochelle. At the other locations, reactions of hybrids could be determined from incidence or severity. Severity was based on 5-7.5, 3 to 5 and 1 to 3.8 for susceptible, intermediate, and resistant hybrids, respectively. Incidence varied among locations. At Urbana, final incidence was similar among plantings, ranging from 98-107, 64-109, and 22-51% for susceptible, intermediate, and resistant hybrids, respectively. Final incidence was lower at Newark and St. Louis than Urbana. Incidence decreased with each planting at Newark. Incidence was similar among plantings at St. Louis.

A851

Tomato plant samples, received in the Florida Extension Plant Disease Clinic in September 1989, exhibited leaf curl and distortion and either a bright yellow mosaic or a mottled, interveinal chlorosis. Light microscopy of various tissues (azure A-stained) revealed nuclear inclusions in phloem cells of leaved and petioles characters pathogenic of germiniviruses. Infected tissues tested positive in moderately stringent hybridization assays with DNA probes for the A components of bean golden mosaic, chino del tomatte and tomato golden mosaic viruses. The viral disease was widespread throughout the west—central and southwest tomato production areas, where incidence ranged from 5-95%, with highest prevalence occurring in early August plantings. Although affected peppers (found in one location) exhibited no foliar symptoms, fruit were misshapen and exhibited longitudinal color breaking and pod necrosis. These pepper samples tested positive for a germinivirus in cytological studies and in hybridization studies using BGMV and CdTV probes.

A852
EARLY POWDERY MILDWED OF GREENHOUSE-GROWN TOMATOES IN FRANCE. Burgeron, A., Nicot, P.C., Bertrand, F. and Blankard, D. Station de Pathologie Végétale, INRA, Domaine Saint Maurice, 84140 Montfavet, FRANCE.

A powdery mildew (tentatively Erysiphe sp.) was observed on tomatoes in France in 1988. It is now found in greenhouses in most of the tomato-growing areas of the country, where it can affect plants early in the season. In host range studies, the fungus infected and sporulated on the foliage of Lycopersicon esculentum (all varieties tested), Solanum melongena, S. tuberosum and Nicotiana tabacum "Xanthi", and on cotyledons of Cucumis sativus, Lageneria leucantha and Helianthus annuus. Single-spore clones of the pathogen are conveniently maintained in axenic culture on detached tobacco leaves or cucumber cotyledons. Isolates of the tomato powdery mildew were distinguished from cucurbit isolates of Erysiphe cichoracearum by the shape of conidia and the kinetics and abundance of sporulation. Single-spore isolates of tomato powdery mildew were unable to form perithecia when paired with complementary testers of E. cichoracearum isolated from cucurbits.

A853
EFFECT OF PRE- AND POST-PLANTING ENVIRONMENT ON POTATO SEEDPIECE DECAY. R. W. James and W. R. Stevenson, Department of Plant Pathology, University of Wisconsin-Madison, Madison, WI 53706.

Seedpiece decay, decrue in plant stand, and loss of plant vigor have been persistent, serious problems affecting Wisconsin's potato crop. These problems have often been associated with bacterial soft rot of the plant seedstock caused by Erwinia carotovora subs. atroseptica (Eca). Procedures that enhance the natural ability of potato tubers to heal, decrease the potential for bacterial seedpiece decay and the need for chemical treatments. The effects of pre-plant treatments on seedpiece decay were evaluated under growcham conditions using three soil temperatures and four soil moisture. Pre-plant treatments included combinations of pre-cutting storage temperature (4-18°C) and temperature and duration for wound healing between cutting and planting (0-5 days at 13 or 18°C). Experiments were conducted with inoculated (100 CFU/ml Eca) and unoinoculated Atlantic and Russet Burbank seedpotatoes planted in silt loam or sandy loam soils. Emergence rate (percent), severity of seedpiece decay, and mean fresh weight per shoot were compared. In general, decay was highest with moderate soil moisture and with precut, wound-healed seedpieces and was greatest when soil was saturated after planting, regardless of soil type, cultivar, pre-plant treatment, or growth temperature. There was no consistent effect of pre-cutting temperature on seedpiece decay. Additional treatment combinations are being tested in growth chamber and field experiments.

A855
IDENTIFICATION OF VIRUS DISEASES OF CUCURBITS ON GUAM. L.S. Yudin, G.C. Mall, R. Quittuq, N.R. Johnson, and R.G. Collinson, College of Agriculture & Life Sciences, University of Guam, Mangilao, GU, 96923, and College of Tropical Agriculture & Human Resources, University of Hawaii, Honolulu, HI, 96822.

Viral diseases are a common problem on cucurbits, the most important cash crops on Guam. In order to control these, it was necessary to identify them. Antisera for WMV (syn. PSSV-M), WMV, CMV, and ZYM were obtained from D. Gonsalves (Cornell University). Positive ID's were made via ELISA for WMV, CMV, and ZYM. The latter predominated in watermelon samples from the northern sector of the island, while WMV predominated in southern samples. WMV was also found on zucchini squash, cantaloupe, and in the common weeds Luffa acutangula, Momordica charantia, and Carica papaya. CMV was found on a native weed, Achyranthes canescens (Amaranthaceae). Aphid vectors found in watermelon fields were Aphis gossypii and A. craccivora.
A856

DECLINE OF SIBERIAN ELM ON THE GREAT PLAINS.


Since the drought years of the 1930s, Siberian elm (Ulmus pumila) has been widely planted on the Great Plains, both in single- and multi-row field windbreaks to control soil erosion and in farmstead shelterbelts. Many plantings are now in decline. Predisposing factors include drought, insect defoliation, herbicide injury and winter damage. Stem cankers caused by Tuberculatula ulmea and Botryodiplodia hypodermica are often the proximate cause of dieback and death. Trees in single-row field windbreaks are affected at a younger age and show more severe damage than those in larger plantings, possibly because these are more exposed to the predisposing agents.

A857

MOVEMENT OF A CYTOPLASMIC HYPOVIRULENCE AGENT IN CHESTNUT BLIGHT CANKERS. L. Shain and J. B. Miller. Department of Plant Pathology, University of Kentucky, Lexington, KY 40546.

Chestnut blight cankers initiated in the spring with a virulent (V) methionine auxotrophic (met-) strain of Cryphonectria parasitica were converted to hypovirulence by placing one or two discs of agar and mycelium of a cytoplasmic hypovirulent (CH) non-auxotrophic strain in bark wounds at the base of cankers ca. 8 wk. later. Cankers collected with increasing time after introduction of CH inoculum were monitored for movement of the CH agent by cultivating cirrhii and bark discs on met- or met+ nutrient media. Movement was confirmed by an isolate exhibiting the typical morphology of the introduced CH strain on met+ but little or no growth on the met- media. Cultures from bark showed that the CH agent moved through mycelium around the canker periphery within 3-6 wk. after its introduction at the canker margin. Conversion of the canker interior proceeded more slowly. Cirrhii, however, continued to yield V, met+ cultures up to 16 months later, even though underlying bark yielded CH, met- cultures. A reluctance of CH agents to enter the asexual sporogenic apparatus in cankers may contribute substantially to ineffective CH dissemination.

A858


Tree, site, and soil measurements taken from oak decline plots in Missouri, Kentucky, Arkansas, and Mississippi indicate that decline sites have lower site indices; greater basal area; more red oaks; slower growing, smaller trees; greater mortality; and higher incidences of insects and disease. Growth ring analysis showed the effects of previously recorded droughts, floods, and winter storms. Mean basal area increment (BAI) was also used to differentiate healthy from decline plots. Decreases in BAI in decline plots appear to be correlated with lower summer rainfall and lower mean winter temperatures. These data suggest that long term climatic trends accompanied by shorter term environmental and biological stress factors are involved in hardwood decline and mortality.

A860


In 1986 studies were initiated to determine the effects of gypsy moth (Lymantria dispar) defoliation onto bigtooth (Populus grandidentata) and quaking aspen (P. tremuloides) in Michigan. Six areas of mixed northern hardwoods of approximately 20 acres each were selected in Midland County, an area of expected high defoliation. In late June, species, DBH, & defoliation and condition were recorded for each tree (75 bigtooth and 113 quaking aspen). Average 4-year defoliation of quaking and bigtooth aspen was 37% and 18%, respectively. Mortality (1987-1988) of quaking aspen (8%/yr) was 4 times higher than that of bigtooth aspen (2%/yr). Armillaria sp. occurred on 91% of the dead trees. Only trees with 80% or more defoliation in 1986 died in 1987 or 1988. In 1989 tree mortality was not correlated with 1986 defoliation but with at least one year of defoliation greater than 70% during the preceding 4 years. Stems coded sub-dominant in 1986 died at 2 to 3 times the rate of stems coded co-dominant or dominant, although 1986 defoliation rates were similar.

A863


The health of sugar maple and associated northern hardwoods was surveyed across an air quality gradient (rainfall, pH, nitrate and sulfate) in northern Wisconsin. A habitat type classification system (Kotar, et al., 1988) was used to evaluate site quality and its possible effect on the health of sugar maple. Of the 96 ground plots evaluated, 60% were mesic forests with very rich soil nutrients, 43% mesic forests with rich soil nutrients, and 3% were dry mesic forests with medium soil nutrients. Approximately 105 sugar maple trees per acre occurred on mesic forest sites with very rich soil nutrients. Of these trees, 98% were healthy and 2% were dead or declining. The sites considered optimal habitat for sugar maple had the largest number of healthy trees and the suboptimal sites had the fewest number of sugar maple present.
A864
CHARACTERIZATION OF OAK DECLINE IN THE TENNESSEE-TOMBIGBEE RIVER BASIN. Vernon D. Ammon, Francis I. McCracken, T. Even Nebecker, Ted R. Filler, and Jim D. Solomon. Department of Plant Pathology and Weed Science, Mississippi State, MS and USDA-USFS Southern Hardwood Laboratory, Stoneville, MS.

Biological, environmental, and edaphic data collected from sites with declining oaks in the Tennessee-Tombigbee River basin were compared to data collected from an equal number of sites where oak decline was lacking. Decline sites contained primarily dominants and co-dominants, whereas trees that had poorer form were, lower in grade, smaller in diameter, and which were taller and older than trees on control plots. Declining trees were evenly distributed among the eight topographic positions evaluated. Discriminant analysis procedures are being used to develop a hazard rating system for oak decline in the South.

A865

Decline is extensively distributed on 200,000 ha of undisturbed forest throughout SE Alaska. Dating mortality, historical records, and early August that the event began about 1880. Decline is associated with poorly drained soils where mortality has continued since onset and regeneration is sparse or absent. Spread of decline to new sites is not apparent, but local encroachment (<1/m2) affects adjacent forest along a gradient from bog to better drainage. Symptoms on Chamasypepis nootkatensis, the principal victim of decline, include: root rot, mycorrhizal fungi on coarse roots and boles, slow radial growth, and thinning or yellowing of crowns. Over 50 taxa of fungi, nematodes, and bark beetles are associated with dying trees, but none can kill healthy trees. Abiotic factors are likely the primary cause of this extensive forest decline.

A866
Transmission of Leptographium procerum to eastern white pine, Pinus strobus, seedlings by the pales weevil, Hylobius pales. J. R. Nevill and S. A. Alexander, Department of Plant Pathology, Physiology & Weed Science, VPASU, Blacksburg, VA 24061-0310.

Field collected pales weevils, Hylobius pales, artificially infested with spores of Leptographium procerum were individually caged on 20 eastern white pine, Pinus strobus, seedlings and allowed to feed for 4 h. As a control, another 20 field collected weevils not artificially infested with L. procerum were individually caged and allowed to feed for 4 h on eastern white pine seedlings. To confirm transmission of fungus, white pine seedlings were inoculated with X 10 mm agar plugs containing L. procerum. Another 20 seedlings were mock inoculated with sterile agar as controls. After six months, L. procerum could be recovered from all of the seedlings fed on by weevils and from those that were not fed on by weevils. Seedlings of six families from canker-free parents were inoculated. Nettle spotting incidence was not correlated with planting cankering. Spearman's rank correlation coefficient (0.08), which permitted us to conclude that it was not significant (0.05).

A867
SCREENING LARCH IN VITRO FOR RESISTANCE TO MYCOSPHERA LARICINA. M. E. Ostrow, P. M. Plaut, and D. D. Skitting. USDA Forest Service, Western Regional Forest Experiment Station, 1992 Wofall Street, St. Paul, MN 55108.

Mycosphera laricina causes a serious needlecast disease of European larch (Larix decidua) in the north-central and northeastern United States. Resistance among seed sources varies; some trees are so susceptible that they die after repeated defoliation. In a two-year greenhouse trial, tissue culture derived clones were inoculated with mycelium of M. laricina. Diseased severity varied among seed sources, and rankings after 6 weeks correlated with results from previous field screening trials. Conidiosa with conidia of M. laricina developed on the most susceptible larch. Tissue culture and in vitro screening offer the possibility of determining relative resistance of larch selections so that resistant larch can be recommended for planting.

A868

Fusarium has been associated with an increased incidence of disease in B.C. conifer nurseries. To determine if Fusarium inoculum is introduced on conifer seed, 120 C. seed lot samples were assessed. Fusarium was found on 0% to 71% of the seeds in half of the seedlots. Incidence of Fusarium was not correlated with seedlot age or percent germination. Species isolated were F. acuminatum, aveneaeicium, lateritium, moniliforme, oxysporum, poae and sambucinum. Twelve isolates (6 seed lot samples were assessed for specificity. oxysporum isolates were the most pathogenic followed by F. moniliforme. F. lateritium caused a low incidence of disease but was ineffective when tested for biological control of F. oxysporum.

A869
FUNGI ASSOCIATED WITH NURSERY TREE SEEDLINGS IN HAITI. G. B. Ranion, W. D. Kelley, and R. K. Reid. School of Forestry, Auburn University, AL 36849 and SECID/Auburn University Haiti Agroforestry Research Project, Petion-Ville, Haiti.

Twenty-four nurseries throughout Haiti were visited and disease symptoms were observed on seedlings from 15 genera of trees. Diseased tissues were collected, cultured, and identified in moist incubation chambers and/or on agar media and identified to genus. Fusarium spp. and Phytophthora sp. were the most prevalent fungi associated with seedlings exhibiting symptoms of die-back-off. Inoculation tests revealed that many genera of trees and were most often associated with Corynebacterium spp., Alternaria spp. and Pestalotia spp. Anthracnose symptoms were consistently associated with Ophiostoma spp. Other fungi, disease levels in Haitian nurseries were low, although diseased seedlings generally exhibited symptoms of several types of disease and were associated with a large number of fungi. Further efforts on seedling diseases in Haitian nurseries will center on disease etiology.

A870
FAMILY PERFORMANCE OF WESTERN WHITE PINE IN FIELD AND BLISTER BUST INOCULATION TESTS. R. S. Hunt and W. D. Heagher, Forestry Canada, Pacific Forestry Centre, 506 West Burnside Rd., Victoria, B.C. V8N 1M5.

Eighteen open pollinated families of western white pine (Pinus monticola D. Don) were established in each of two plantations. Tallies of white pine blister rust (Cronartium ribicola Fisch.) cankerers at 12 years revealed that three of the four least-associated families came from canker-free parents. Seedlings of six families from canker-free parents were inoculated. Needle spotting incidence was not correlated with planting cankering. Spearman's rank correlation coefficient was 0.08, which permitted us to conclude that it was not significant (0.05). Although the canker incidence from inoculation of the best two field performing families was lowest (86 and 87%), the comparable incidence on the worst field performing family was not greatly different (89%). It was difficult to pick the best families from inoculation data, but such data could be used to cull some families.

A871

Dogwood anthracnose has increased dramatically in the eastern United States within the last decade. Diagnostic symptoms include small leaf lesions on infected shoots and necrotic patches in necrotic tissues. Our study reproduced these foliar symptoms in the greenhouse using techniques previously published from this laboratory. In this study leaves were inoculated with 2000 spores per milliliter of Diplodia. For microcopy. Intracellular and intercellular hyphae were abundant in the epidermis, mesophyll and leaf vascular tissues. Extensive necrosis of palisade and spongyl parenchyma cells occurred. Embedded spores were visible and stained for microcopy. Immature and intercellular hyphae were abundant in the epidermis, mesophyll and leaf vascular tissues. Extensive necrosis of palisade and spongyl parenchyma cells occurred. Embedded spores were visible and stained for microcopy.
USE OF A LEAF DISK METHOD TO DETERMINE AGGRESSIVENESS OF
SEPTORIA MUSIVA. J. M. Krupinsky. USDA, Agriculture
Research Service, Northern Great Plains Research Laboratory,
P.O. Box 459, Mandan, ND 58554

High and low aggressive isolates of Septoria musiva
obtained from Populus leaves and cankers (Phytopathology
79:413-416) were compared on leaf disks obtained from four
field grown Populus clones. One high and one low
aggressive isolate were compared in seven studies. Nine leaf
disks (three inoculated with a high aggressive isolate, three
with a low aggressive isolate, and three with distilled water)
were placed in wells in water agar in each petri dish. Clones
reacted (percentage necrosis) significantly different from one
another in all studies. Northwest was the most susceptible
clonc. Based on percentage necrosis of the leaf disks there
were significant differences among isolates in all studies.
The high aggressive isolates caused significantly more
symptoms in 5 out of 7 comparisons. In general, high
aggressive isolates can be separated from low aggressive
isolates in leaf disk inoculations of field grown leaves.

PATHOGENICITY OF XYLELLA FASTIDIOSA TO AMERICAN ELM.
JAMES L. SHERALD. Center for Urban Ecology, National Park Service,
1100 Ohio Dr. S.W., Washington, D.C. 20242.

American elm seedlings (4-mo-old, 20 cm ht) were inoculated in August
1988 with a strain of X. fastidiosa isolated from a naturally infected elm.
Ten seedlings were inoculated with 0.025 ml of a bacterial suspension
(7x10^6 cells/ml) in 3 scalpel wounds in the stem. Control seedlings were
 treated with buffer. By June 1989 all inoculated seedlings had
developed leaf scorch symptoms which progressed from older to younger
leaves and exhibited the undulating marginal necrosis typical of naturally
infected trees. Controls remained symptomless. Terminal elongation was
reduced by 32% and the stem caliper of treated and control seedlings
was 0.48 cm and 0.70 cm respectively 14 months after inoculation.
Bacteria characteristic of X. fastidiosa were isolated from 6 of the 10
inoculated seedlings but not from controls. Isolated strains gave a
positive ELISA reaction for X. fastidiosa.

SEM STUDIES OF THE DOGWOOD ANTHRACNOSE FUNGUS. S.C. Redlin,
USDA-ARS, BKRL, Beltville, MD 20705-2350.

The dogwood anthracnose fungus (Discula sp.) causes lethal
cankers on flowering dogwood, Cornus florida L., in eastern
North America. A technique of removing leaf discs (17 mm dia.)
from healthy dogwoods in the field, placing the discs in wells
made in water agar media, wounding them with a heated metal rod,
and inoculating them at the burned spot was useful for studying
condidemata. Conididemata developed as subepidermal swellings
below the two-armed trichomes. Mature conididemata containing
phialidic conidiogenous cells and conidia were observed six days
after inoculation. Oosporid conidia were released through an irregular rupture of the conidiomatal
wall. Ruptures sometimes occurred as a result of scission of the
associated trichome.

TEMPERATURE SENSITIVE MODULATION OF THE PRIMITIVE PEA CULTIVAR
IRAN BY RHIZOBIIUM LEGUMINOSARUM BV. Viciae STRAIN PF2. W.
Derrick1, D. Kluepfel1, and T.-A. Lie2, Clemson University, SC1
and Agricultural University, Wageningen, The Netherlands2.

Modulation of Iran by R. leguminosarum strain PF2 is temperature
mediated. The influence of temperature is strain specific and inherited
in pea as a single dominant gene. Time after
inoculation and region of root tissue where sensitivity to
temperature change is greatest were determined. Temperature-
switching in increments of 24 hours showed that inhibitory
effects of non-permissive (20 C) and stimulatory effects of
permissive (26 C) temperatures are greatest 3-4 days after
inoculation. Root tip marking showed that nodules formed in the
same relative position regardless of temperature treatment,
indicating that the reduction in nodule formation is not due to
delay in onset of infection. Roots grown and inoculated at 20 C
show longitudinal splitting and peeling of the epidermal cell
layers similar to HR in aerial portions of incompatibly infected
tissue. This response is absent in uninfected roots at either
temperature or in infected roots at 26 C.

1066 PHYTOPATHOLOGY
A880

Nicotiana tabacum SR1 was transformed with a vector containing both an acidic chitinase gene from petunia and a basic chitinase gene from tobacco. The genes were expressed constitutively using the 35S promoter of Cauliflower Mosaic Virus. Two of the resulting transgenic plants showed high levels of expression of both genes in Northern analysis. This elevated expression was also observed at the protein level by Western analysis. Chitinase activity in these plants was 4 and 7 times that of the nontransformed tobacco, respectively. Total protein extracts were used to measure in vitro antifungal activity against Trichoderma viride and Fusarium solani. Extracts caused lysis of hyphal tips and inhibition of growth. In these assays the transgenic plants were 5 and 10 times, respectively, more active against these fungi than the controls. These data demonstrate the expression of active chitinases in transgenic tobacco and indicate that antifungal activity correlates with the level of enzyme activity.

A881

Monensin will completely inhibit primary haustorial formation in primary barley leaf segments floating in 30 mM solution for 24 hr after inoculation. Leaf segments in water have appressoria 30% efficient at forming haustoria and segments in 10 mM calcium are 50% efficient. The optimal application for both monensin and calcium is 8-12 hr after inoculation. It is during this time period that appressoria form and attach to the host epidermal wall but prior to infection peg development. Leaf segments floating in both monensin and calcium are 70% efficient. It is suggested that high host cytoplasmic calcium prior to infection peg development favors haustorial formation. Monensin, which disrupts Na+K+ gradients, may inhibit release of stored calcium which is partially overcome by high external calcium.

A882
GERMINATION AND INFECTIVITY OF PHYTOPHthora INFESTANS IN THE PRESENCE OF FATTY ACIDS. Y. Cohen, Bar-Ilan University, Israel, U. Gisi, and E. Messinger, Sandoz Agro Research, Switzerland.

Stable, water-solubilates of five unsaturated fatty acids (Sigma) were tested for their effects on both germination of Phytophthora infestans (Isolate S49) in vitro and infection of potato (cv. Minjorje) leaf discs at 15 C. Oleic, linoleic, arachidonic and eicosapentaenoic (EP) acid did not affect zoospore discharge at concentrations of up to 16.5 mM. Zoospore germination, however, was inhibited by linolenic, EP, arachidonic and linoleic acid, with ED50 values of about 1.65, 16.5, 16.5 and 33 mM, respectively. Oleic acid stimulated zoospore germination at 16.5 mM. Arachidonic and EP acid induced a strong necrosis in potato leaf discs at ³ 16.5 mM, linoleic and linolenic acids did so at ³ 16.5 mM. Oleic acid did not induce necrosis at 16.5 mM or below. Disease development was completely inhibited by linolenic, arachidonic and EP acid at 16.5, 165 and 165 µM, respectively, and partially by linoleic acid at 1.65 mM. Oleic acid stimulated lesion development and sporulation at 16.5 mM.

A883

ACT-toxin I and II were isolated from culture filtrates of A. alternata causing leaf spot on Dancy tangerine. The structure of toxin I was identified as 8-N-2°, 3°, 4°, 5°-tetrahydroxy-4°, 6°-dimethyl-6°-octenoylviil-9,10-epoxy-9-methylene decatrienioic acid. It had two geometric isomers: (2E,4Z,6E) type of decatrieneic acid for toxin I and (2E,4Z,6Z) for toxin II. Toxin II was the 5°-deoxy derivative of I. Ib at 10 ng/ml and Ibb at 20 µg/ml induced veinal necrosis and a rapid increase in electrolyte loss from susceptible citrus leaves. Ib induced typical imagination of plasma membranes. Resistant citrus were not affected by toxins. Ibb and Ibb but Ic were released on spore germination. Infection hyphae were formed when avirulent spores were inoculated on leaves along with a small amount of Ib. ACT-toxin I plays a key role as host recognition factor in the early pathogenesis.

A887

Pectinase production in culture by high and low virulent Aspergillus flavus strains and an A. nidulans strain was compared by isolectric focusing with production in wounded-inoculated cotton bolls and on dead cottonseed. In both culture and plant tissue, the high virulent A. flavus strain...
produced three pectinase activities (P1, P2c, and P3), whereas the low virulent strain produced two (P1 and P3). The A. nidulans strain produced a pectate lyase in liquid culture containing pectin, but did not produce the enzyme in either the developing cotton bolls or dead cottonseed. Activity P2c was not catabolite repressed and its expression in cotton bolls may be related to virulence of A. flavus.

**A888**

**INCOMPATIBLE INTERACTIONS BETWEEN PHYTOPHthora MEGASPERMA F. SP. GLYCINEA AND SOYBEAN CULTIVARS WITHOUT KNOWN EFFECTIVE ERP GENES.**

R. E. Wagner and H. T. Wilkinson. Dept. of Plant Pathology, Univ. of Illinois, Urbana, IL. 61801.

Soybean cultivars without known effective Eps genes formed compatible and incompatible interactions with race 3 of Phytophthora megasperma f. sp. glycinea (Pmg) on taproots of aeroponically grown plants. The frequency of incompatible interactions depended on the cultivar's level of rate-reducing resistance, pathogen aggressiveness, inoculum concentration and temperature. The magnitude of the interaction between the cultivar Corsoy and Pmg formed a continuum from brown necrotic flecks that formed at the site of infection (incompatible) to an expanding lesion that eventually extended from the root tip to the cotyledon (compatible). The similarity between lesions that formed following incompatible interactions on cultivars with and without effective Eps genes suggest that the same biochemical mechanism(s) could be responsible for single-gene and rate-reducing resistance.

**A889**

**VARIATION IN LESION TYPE FOLLOWING INCOMPATIBLE INTERACTIONS BETWEEN PHYTOPHthora MEGASPERMA F. SP. GLYCINEA AND SOYBEAN CULTIVARS WITH EFFECTIVE ERP GENES.**

R. E. Wagner and H. T. Wilkinson, Department of Plant Pathology, University of Illinois, Urbana, Illinois 61801.

The interaction between soybean cultivars with different Eps genes and Phytophthora megasperma f. sp. glycinea (Pmg) was investigated on taproots of aeroponically grown plants. Incompatible interactions resulted in one of two lesion types, designated 1 and 2. Type 1 lesions were characterized by brown necrotic flecks that formed at the site of infection. Growth of the taproot continued unimpeded. Type 2 lesions were similar in appearance to those formed following compatible interactions, except lesion expansion was discontinuous resulting in a significantly shorter lesion. Growth of the taproot was terminated. Lesion type depended upon the cultivar, Eps gene, Pmg race, inoculum concentration, pathogen aggressiveness, and temperature. Type 2 lesions could indicate inefficient elicitation of the hypersensitive response.

**A892**

**SHOOT-TIP MUCILAGE AND DISTRIBUTION OF FUSARIUM LATERITIUM ON SWEETPOTATO.**

C. A. Clark, J. A. Wilder-Ayers, and S. W. Mathews. Dept. Plant Pathology & Crop Physiology, Louisiana Agric. Ext. Station, LSU Agricultural Center and Dept. of Botany, College of Basic Sciences, Louisiana State University, Baton Rouge, LA. 70803-1720.

Fusarium lateritium, causal agent of chlorotic leaf distortion (CLD), was isolated from NaOCl-treated parts of sweetpotato vines. It was isolated most frequently from leaf primordia, immature (folded) leaves, and axillary buds; less frequently from apical meristems, mature leaf tissue, floral parts and true seed; and infrequently from cross sections of vine nodes from CLD-affected plants. It was isolated less frequently from apical meristems and leaf primordia of plants recovered from CLD. Using light microscopy, fungal hyphae were observed on the surface of but not within sectioned symptomatic leaf or node tissue. Using scanning electron microscopy (SEM), hyphae were observed in regularly scattered clumps on the surface of unfolded leaves. On shoot tips, hyphae were present in a mucilage-like layer which covered the surface of the apical dome and leaf primordia. A similar layer was observed on healthy mericlines free of the fungus.

**A893**

**Infection of flowering dogwood (Cornus florida) by the anthracnose fungus, Discula spp.**


Discula infection of the flowering dogwood was observed with scanning electron microscopy. Detached leaves were sprayed with a Discula spore suspension (in phosphate buffer, pH 6.8) and placed in the growth chamber at 18°C. Germination was observed after 24 hrs. After 4 days, germ tubes and hyphae extended across the leaf. Neither direct penetration of the leaf surface or penetration via stomates was observed; however, germ tubes/hyphae appeared to elongate toward one of the trichome leaf surface juncture. These hyphae grew in the surface depression below these unusual leaf hairs and toward the trichome/leaf surface juncture. Hyphal penetration following this phenomenon and its role in infection is being investigated.
A896

REGENERATION OF INTERSPECIFIC FUSION HYBRIDS OF NICOTIANA TABACUM AND N. REPANDA, P. B. Nguyen, M. B. Daub, and A. E. Jennis, Dept. of Plant Pathology, North Carolina State University, Raleigh, N. C. 27695.

Nicotiana repanda has resistance to many important tobacco diseases, but will not cross with cultivated tobacco (N. tabacum) by conventional means. In order to transfer resistance genes to N. tabacum, N. repanda was first hybridized with N. sylvestris which crosses with both species. This hybrid was then hybridized with N. tabacum cv. NC2326 by protoplast fusion. Parental lines were transformed for kanamycin or hygromycin resistance using Agrobacterium vectors. Mesophyll protoplasts were isolated from the antibiotic-resistant parent lines and fused using polyethylene glycol. Hybrid calli were selected by plating on media containing both antibiotics. Shoots were regenerated from hybrid calli, and shoot hybridity was verified by analysis of isozymes of glutamate oxaloacetate transaminase. Following rooting, hybrid plants will be screened for virus and nematode resistance present in the N. repanda parent.

A900

VIRULENCE FORMS OF ASCOCHYTA RABEI ON CHICKPEA IN THE PALOUSE. Hamidullah Jan and M. V. Wiese. Plant Pathology/PSES, Univ. of Idaho, Moscow 83843.

A898


A study was conducted to determine the relationship between anthracnose stalk rot (ASR) and tissue colonization by the causal fungus, Colletotrichum graminicola, in maize hybrids Cornell 281 (susceptible) and B 37 x LB 31 (resistant). Plants were inoculated with 1ml of a 5 x 10^6 conidia/ml suspension into a wound in the internode above the brace roots and scored for ASR 21 days later. To determine ergosterol levels in tissues infected with C. graminicola, the pith tissues were homogenized in dichloromethane-MeOH (1:1), and the unspunified lipid residues extracted with hexane. Ergosterol was quantified by reverse phase HPLC at 282 nm. Ergosterol level was positively correlated with the extent of pith discoloration and recovery of the fungus from the respective internodes. Moreover, significantly less ergosterol was detected in infected B 37 x LB 31 tissues than in infected Cornell 281. Estimates of fungal colonization corroborated visual rating schemes for ASR.

A899

EVALUATION OF CAPISTUM SP. FOR RESISTANCE TO PHYTOPHthora CEPSCIL. P. L. Hartman and T. C. Wang, Asian Vegetable Research and Development Center, P.O. Box 62, Shanhua, Tainan, 741,89, Taiwan, ROC.

Evaluations of inoculations using a range of zoospore concentrations and plant ages were compared by a disease index (0 = no disease to 4 = dead plant). The disease index for 'Blue Star' (highly susceptible) was not significantly different between inoculations using 10^7, 10^3, 10^2, or 10^5 zoospores/ml at 24 days after inoculation, whereas PI 201234 (highly resistant) had no disease at concentrations up to 10^5 zoospores/ml. Three- and 7-week-old inoculated plants of 'Sheeham' (moderately susceptible) had similar disease indices, but were significantly different from younger and older inoculated plants. PI 201234 had no disease regardless of age. Ten of 1,041 accessions had no plant survival compared to PI 201234.

A901

CHARACTERIZATION OF SLOW RUSTING COMPONENTS IN MAIZE INBREDS AND SINGLE CROSSES. Z. Nnako, R. A. Frederiksen, J. Craig, and J. D. Smith, Department of Plant Pathology and Microbiology, and Department of Soil and Crop Sciences, Texas A&M University, College Station, TX 77843.

Puccinia polystroa Underw., the incitant of southern corn rust, is the most devastating among the three rusts that occur on maize worldwide. Yield losses as high as 60% were reported. Presently, use of slow rusting varieties is the most effective means to control an epidemic. Altered infection type, reduced number of pustules, small area under the disease progress curve (AUDPC), shallow disease gradient, and reduced spread/unit of time were factors used to characterize slow rusting. Consistent results were obtained using the Gompertz transformation for each cultivar, while the logistic model gave variable results. Significant correlations were found between the infection type and yield loss, the pustule number and yield loss, and between the AUDPC and yield loss.

A1003

THE ROLE OF GLUMES OF SORGHUM IN RESISTANCE TO GRAIN MOLD. S. B. Magnusius, R. A. Frederiksen, R. D. Waniska, G. N. Odvody, and J. Craig, Department of Plant Pathology and Microbiology, Texas A&M University, College Station, TX 77843.

Isolates of Fusarium moniliforme from different sorghum plant parts were virulent in causing grain mold (GM) on Rth430. Vacuum
A904
DETECTION OF SNOW MOLD RESISTANT PROPERTIES IN WINTER WHEAT PLANTS AND ANDROGENIC PLANTLETS. J. H. McBeath and F. Meh dizadegan. Agricultural and Forestry Experiment Station, University of Alaska Fairbanks, Fairbanks AK 99775-0080.

A method was developed to assess the effects of extracellular enzymes of 
Sclerotinia borealis and sclerotial low temperature ba pidiospora on winter 
wheat segments taken from the disease symptom. Froid, Roughrider and 
and from plantlets derived from the anther cultures were weighed and 
treated with various concentrations of snow mold extracellular enzymes 
(consisting mainly of cellulolytic and pectolytic enzymes). After a 7-day 
incubation at 10 C, the chlorophyll content in the leaf segments was 
extracted and measured. Gradients of responses were observed of cultivars 
and plantlets ranging from no change to chlorosis. Enhancements in resistance 
in snow mold enzymes were observed in plantlets derived from Roughrider, 
which possess moderate snow mold resistance properties.

A905
RESISTANCE OF MAIZE KERNELS TO DECAY BY ASPERGILLUS FLAVUS. J. M. Rivera and C. A. Martinson, Department of Plant Pathology, Iowa State University, Ames, IA 50011.

Maize kernels were inoculated with A. flavus spores and stored in a 92.5% RH (over 2.25 molal NaNO3) and 31.7% C environment. Gemen 
pla was ten imbred lines selled in five environments and all 
possible crosses of the lines. Kernel germination and seedling emergence 
from a sand-soil medium was the measure of resistance. Resistance 
rankeing of the imbred lines was B66 (best), B77, M18, B76, B79, M617, B73, B85, Va35, and B14A. Environmen of kernel 
production altered rankings slightly, but environment affected 
fitness and the magnitude of the resistance. Inbred line 
response to A. flavus was transmitted to the hybrid and it pred 
icted hybrid response best when used as the female parent. 
Resistance was strongly related to a maternal effect with B84 
and B77 the better female parent in hybrids. Resistance of maize 
kerne to A. flavus attack in a high humidity, high temperature 
storage environment is a genetically controlled trait that 
is influenced greatly by environment of kernel production.

A906
IMPROVEMENT IN RESISTANCE TO ASPERGILLUS FLAVUS IN AN ANTIGUA POPULATION OF MAIZE. C. A. Martinson*, J. M. Rivera*, and A. R. Hallauer*, Department of Plant Pathology* and Department of Agronomy*, Iowa State University, Ames, IA 50011.

Maize kernels were inoculated with A. flavus spores and stored in a 92% RH environment (over either 2.25 molal NaNO3 or saturated 
KNO3). Kernel germination and seedling emergence was the measure 
of resistance. A CIMMYT population from Antigua was mass select 
ed for earliness for 4 years, and then kernels from 92 selfed 
ears were assayed for resistance ranged from 0-88%, genetic coefficient of variability was 115.4%, and predicted 
heritability was 96.6%. Recurrent S selection (102 selection 
intensity) was performed for two cycles and all cycles were evalu 
at. Emergence was 40.3, 59.8, and 80.0% for cycles 0, 1, and 2, 
respectively. Seed of the base population was inoculated, 
seeded at 925 RH, and then planted in the field. Surviving 
plants were randomly harvested and equal numbers of kernels from 
each ear were mixed and recycled. Germination after A. flavus 
exposure in storage increased from 31.5% in cycle 0 to 47.1% in cycle 2. Heritability of resistance was established.

A907
INCORPORATION OF SUGARCANE MOSAIC VIRUS RESISTANCE INTO 
SUGARCANE FROM FERAL GERMLASM. M. P. Grisaham and B. D. Legendre, USDA, Agricultural Research Service, Sugarcane Research Unit, P. O. Box 470, Houma, Louisiana 70360.

A basic sugarcane breeding program was initiated at Houma, LA in 1964 with the primary objective to develop cultivars resistant to sugarcane mosaic virus (SCMV). Crosses were made 
with diverse germplasm of feral or wild Saccharum species, 
primarily S. spontaneum, and related genera. Since 1986, 
clones (F1 or BC1, progeny) selected from this program and 
assigned permanent breeding germplasm (US prefix) or candidate 
cultivar (CP prefix) designations have been examined for 
natural infection by SCMV in the field. In the pedigrees of 
the 83 US and 15 CP clones examined, only 24 of the 
recurrent parents were resistant to SCMV; however, 56% of the progeny 
were rated resistant and 44% were rated susceptible based on 
visual symptoms. The data suggest that feral germplasm may be 
used in sugarcane breeding to increase the frequency of resistance to SCMV.

A909
INVESTIGATION OF BACTERIAL FRUIT BLOTCH OF WATERMELON IN 
INDIANA. E. A. Band and R. K. Lath, Department of Botany and Plant Pathology, Purdue University, West Lafayette, IN 47907.

Bacterial fruit blotch severely affected watermelon crops on several Indiana farms in 1989. The pathogen, tentatively 
identified as Pseudomonas pseudocalligenes subsp. citrulli 
(PPc), caused rapidly expanding water-soaked lesions on the 
surface of maturing watermelon fruit. The pathogen also 
caused small necrotic lesions on foliage. Seed obtained from 
symptomatic fruit were planted in the greenhouse and more 
than 40% of the resultant seedlings developed water-soaked lesions on cotyledons. Bacterial strains recovered from 
cotyledons were similar to those collected from infected 
fruit. Seed from infected fruit was buried outdoors in soil 
and sampled periodically to determine the ability of the 
pathogen to overwinter in midwestern fields. Ipp was 
recovered 1 week after burial, but attempts to recover the 
pathogen after 3 and 4 months were not successful.

A910

A strain of X. c. pv. vesicatoria from Brazil (Xv 56) caused disease in the 
tomato breeding line Hawaii 7998 (H7998), a line resistant to fungal 
spot in Florida. It was not virulent on pepper and belongs to the tomato 
strain of groups (XcVT). Electolyte leakage patterns from susceptible 
leaves (Bonny Best) inoculated with Xv 56 and a typical strain of XcVT 
from Florida (Xv 75-3) were similar, but leakage occurred more rapidly 
after inoculation of leaves of H7998 with Xv 75-3 than with Xv 56. With 
inoculum of 10^5 cfu/ml the growth curves of Xv 56 in H7998 and 
Bonny Best were similar and populations reached almost 10^6 cfu/cm^2 in 
6 days. The growth curve of Xv 75-3 reached its maxiumum (10^6 cfu/cm^2) in 
2 days in leaves of H7998 and in 3 days (10^6 cfu/cm^2) in leaves of Bonny 
Best.

A911
OVERWINTERING OF CLAVIBACTER MICHIGANENSIS SUBSP. MICHIGANENSIS 
AND SPREAD ON ALTERNATIVE HOSTS AND NON-HOST PLANTS. W. J. 
Chang, S. M. Ries, and J. K. Pataky. Department of Plant 
Pathology, University of Illinois, Urbana, Illinois 61801.
Overwintering of Clavibacter michiganensis subsp. michiganensis (CMM) and spread of CMM on alternative hosts and non-host plants were evaluated with rifampin-resistant mutants and a selective medium. The bacterium was recovered after 196 days from tomato stems placed on the soil surface and from tomatoes buried 10, 10, and 30 cm. The survival rate was highest on the soil surface, but there were no differences in survival at the 10, 20, and 30 cm depths. Viable cells decreased about 100 to 10,000-fold from November 1988 to May 1989. No pattern of secondary spread of CMM was detected on alternative hosts or non-host plants that were grown in tomato field. Symptoms of secondary infection were not observed on all plants of nine other species, however epiphytic populations of CMM were higher on solanaceous plants than non-solanaceous species.

A912
RESPONSE TO EMMINA INOCULATION IN TISSUE CULTURE PLANTLETS OF THREE POTATO CULTIVARS. C. Skroch, D. J. Gallenberg, and P. L. Spinks, Department of Plant Science, Box 2109, South Dakota State University, Brookings, SD 57007.

Plantlets of three potato cultivars differing in their field reaction to blackleg caused by Erwinia carotovora spp. atroseptica (Eca) were grown on both standard MS and high-calcium media for 4-5 weeks prior to stem inoculation with one of two Eca strains. After one week, disease severity was rated using a 0-3 scale. Approximately 35 plantlets were inoculated in each of the 10 treatments (culture x Eca strain), and data were analyzed using SAS PROC CATMOD. Among cultivars, the field susceptible 'Norchip' had the lowest overall disease severities followed by 'Red Pontiac' (moderately resistant), with 'Russet Burbank' (resistant) showing the greatest severity. While a number of factors may contribute to this departure from field observations, it may be explained in part by tissue analyses which indicated significantly higher levels of calcium in 'Norchip' compared to 'Russet Burbank'.

A913
INFLUENCE OF GROWTH MEDIUM ON RESPONSE OF POTATO TISSUE CULTURE PLANTLETS TO EMERIA. C. Skroch, D. J. Gallenberg, and P. L. Spinks, Department of Plant Science, Box 2109, South Dakota State University, Brookings, SD 57007.

Potato tissue culture plantlets were grown on both standard MS and high-calcium media to determine the effects of medium nutrient levels on response to Emeria. After 4-5 weeks growth on the two media, plantlets were stem-inoculated in vitro with Erwinia carotovora spp. atroseptica (Eca) and observed after one week for disease severity. Across the three cultivars and two Eca strains used, disease severity was significantly lower in plantlets grown on the high-calcium medium as compared to standard medium plantlets. Tissue analyses indicated significantly greater levels of calcium in stem and leaf tissue of plantlets grown on the high-calcium medium. Increased calcium levels likely play a role in the lower disease severity. However, tissue analyses also indicated significantly greater levels of chlorides in the same tissue. The same pattern has been shown to reduce disease severity in some fungal disease systems, but its interaction with bacterial diseases is not known.

A914
USE OF POLYMERASE CHAIN REACTION TO DETECT PATHOGENIC ISOLATES OF AGROBACTERIUM. L.-C. Dong, K. L. Thies, D. S. Luthe, and C. H. Graves, Jr. 2. Department of Biochemistry and Molecular Biology and Department of Plant Pathology and Weed Science, Mississippi State University, Miss. State, MS 39762.

The polymerase chain reaction (PCR), a very sensitive tool for the identification of specific regions of DNA present in small quantities, was used to detect the presence of T-DNA in 39 Agrobacterium isolates from Vitis spp. Oligonucleotide primers were homologous to the T-DNA regions from Agrobacterium tumefaciens Ag8 and amplified a 150 base pair fragment. Twenty-three of the 39 isolates tested contained T-DNA based on the PCR results. In most cases the PCR results confirmed pathogenicity tests using detached leaves from Agrobacterium-free muscadine plants. Our results indicate that the PCR is a sensitive and suitable tool for determining potentially pathogenic isolates of Agrobacterium.

A915
USE OF RESTRICTION FRAGMENT LENGTH POLYMORPHISMS TO CHARACTERIZE STRAINS OF PSEUDOMONAS SOLANACEARUM. Elizabeth Barlow, Douglas Cook, and Luis Sequeira. Department of Plant Pathology, University of Wisconsin-Madison, Madison WI 53706.

A total of 150 strains of Pseudomonas solanacearum from a world wide range of hosts and geographical locations and representing all known biovars and races were examined by restriction fragment length polymorphism (RFLP) analysis. Double digests (EcoRI/BamHI) of total genomic DNAs were electrophoresed, blotted onto nylon membranes and hybridized with nine DNA probes. Seven of these probes encode regions required for virulence and induction of the hypersensitive reaction. In general, the RFLP patterns were as previously reported for each of the corresponding biovars, although there were many evident polymorphisms among strains. Strains were grouped according to similarity coefficients calculated on the basis of number of DNA fragments in common. The usefulness of the method was demonstrated by our ability to characterize several unknown strains from helicostomias imported to Australia as belonging to the SFR group of race 2. This group is highly pathogenic to bananas and probably originated in Venezuela.

A916
SANITATION OF CAPRIFS (MALE FIGS) REDUCES FIG ENDOSEPSIS CAUSED BY XANTHOMONAS CAMPESTRIS V. FICI IN CALIMYRNA FIGS. Thomas J. Michalides and D. P. Morgan, Dept. of Plant Pathology, Univ. of Calif., Berkeley/Kearney Ag. Center, 9240 S. Riverbend Ave., Parlier, CA 93648.

Fungicide dip or spray treatments of the mame crop (winter crop) reduced the incidence of Fusarium spp. on the mames and on the emergent, fig wasp (Dysderium psenes) feeding, but not of other organisms. The effects of a single treatment on the disinfestation of profichis (spring crop) and on the emerged wasps varied according to the fungicide treatment. In all experiments using selected symptomatic trees, the lowest percentage of contaminated profichis, emerged wasps, and Calimyra figs. Fungicide treatments of mame figs resulted in cleaning of emerged fig wasps. Recontamination of pathogen-free wasps by contaminated plant surfaces of caprifig and Smyrna fig trees could explain the high variability and the lack of effectiveness of fungicide treatments in controlling fig endoepsis in profichi and Calimyra crops.

A917

Ceratocystis fimbriata was identified as the causal agent of a limb canker of almond trees. Natural infections occur at twigs or small pruning cuts, and multiple cankers girdle and kill branches. Pruning cuts 0, 2, 4, 7, and 14 days old inoculated with a spore suspension of C. fimbriata in November, December, January, February, and March had the least incidence of X. campestris on fruit tissues and resulted in the lowest percentage of contaminated profichis, emerged wasps, and Calimyra figs. Fungicide treatments of mame figs resulted in cleaning of emerged fig wasps. Recontamination of pathogen-free wasps by contaminated plant surfaces of caprifig and Smyrna fig trees could explain the high variability and the lack of effectiveness of fungicide treatments in controlling fig endoepsis in profichi and Calimyra crops.

A918
ISOLATION AND PATHEGENICITY OF ALTERNARIA LIMIOCA ASSOCIATED WITH CITRUS LEAF SPOT IN MEXICO. N. E. Palm, USERA/AMIS and E. I. Civerolo, UNDA/AMU, BACN-West, Beltville, MD 20705-2350.

Alternaria limicola, a newly described fungus associated with a leaf spot disease of citrus in Mexico, was isolated from 15 of 16 samples of six Citrus spp. collected in 1969 and 1990 in Collins. Two bacterial strains from the Citrus pathogen Xanthomonas campestris were isolated from only one sample. Mexican lime and 'Duncan' grapefruit seedlings were artificially inoculated by spraying the terminal foliage with aqueous suspensions containing either 260-450 comidia/ml of A. limicola or 108 cfu/ml of X. campestris. Lesions similar to those observed in the field developed on citrus tissues was resistant from A. limicola inoculated plants. Neither of the xanthomus strains produced any symptoms. This confirms previous work done in Mexico that the primary cause of citrus leaf spot is A. limicola and not a bacterium.

A919
COMPARISONS OF HICKORIES INFECTED WITH CLADOPODIUM CARYGENUM USING SCANNING ELECTRON MICROSCOPY. S. V. Diehl and C. H. Graves, Dept. of Plant Pathology and Weed Science, Mississippi State University, MS State, MS 39762.
Leaves of Schley, Stuart, Success and Stevens pecan cultivars and a commercial cultivar of hickory were compared for differences in surface morphology and fungal development in scab-infected and non-infected tissues with scanning electron microscopy. Internal tissue structure was also compared by freeze-fracturing some samples. Scab lesions on both nuts and leaves of the susceptible Schley cultivar consisted of dense, compact mats of hyphae with prolific sporulation and complete breakdown of spongy and palisade parenchyma structure. In resistant Missouri hickory and 'Bartlett' hickory, sparse mycelia had sparse mycelia, no observed sporulation and minimal disruption of both surface and internal tissue structure. The reaction of other cultivars varied between these two extremes. No fungal hyphae were seen within intact or degrading tissues of leaves or nut husks. Hyphae were observed only on tissue surfaces or sub-cuticularly.

A921
ASSOCIATION OF NECTRIA RUGULOISA WITH QUICK DECLINE OF MACADAMIA TREES. W. K. Ko and R. K. Kunimoto. Department of Plant Pathology, University of Hawaii, Hilo, Hawaii 96720.

In 1986, a number of macadamia trees at Keaau on the island of Hawaii died within few months after the appearance of decline symptoms. Initial symptoms were yellowing and browning of some leaves within the tree canopy. The number of affected leaves increased rapidly and within a few months leaves on the entire tree turned brown and subsequently died. When 23 declining trees at Keaau were closely inspected, 12 had with numerous small reddish pustules of the trunk. An ascospore culture was identified by C.A.B. International Mycological Institute as Nectria cf. ruguloisa with Cylindrocarpon anamorph. When the fungus grown in a wheat- oat medium was used to inoculate branches of healthy macadamia trees, 10% of inoculated branches were infected after 3 months. The number of infected branches increased to 50% 10 months after inoculation. All of the control branches remained healthy. N. ruguloisa was reisolated from all of the diseased branches.

A922
ASSOCIATION OF XLARIA ARBUSCULA WITH QUICK DECLINE OF MACADAMIA TREES. W. K. Ko and R. K. Kunimoto. Department of Plant Pathology, University of Hawaii, Hilo, Hawaii 96720.

Fruiting bodies of several species of fungi were frequently observed on the trunks of dying or dead macadamia trees in the areas affected by quick decline at Keaau on the island of Hawaii. Club-shaped carbonaceous fruiting bodies identified by J. D. Rogers as Xylaria arbuscula were found on 50% of the declining macadamia trees. Wood tissues beneath the fruiting bodies showed extensive decay and contained black zone lines. The colony appearance of a fungus frequently isolated from diseased tissues was identical to that of X. arbuscula culture derived from an ascospore. When the fungus grown on a wheat-oat medium was used to inoculate branches of healthy macadamia trees, 40% of inoculated branches with part of the bark removed were infected after 4 months, while 80% of inoculated branches with bark gently scraped were infected during the same incubation period. All the control branches remained healthy. X. arbuscula was reisolated from all the diseased branches.

A923
ELIMINATION OF BANANA BUNCH TOP VIRUS FROM DISEASED BANANA TISSUES. R. J. Wu and H. J. Su. Development Center for Biotechnology, Chi Ching Hospital, Department of Plant Pathology and Entomology, National Taiwan University, Taipei, Taiwan.

When banana bunch top virus (BBTV)-infected tissues were cultured at 35°C, some of the buds started to produce healthy roots and developed into healthy-appearing plantlets after 3 months. A cloned 5 of 11 tissue-culture-healthy scion-top plantlets in 6 months. The crude extract from healthy appearing plantlets did not show any activity when assayed with monoclonal antibody against BBTV. When heat-treated healthy plantlets were inoculated with BBTV-infected virus, all of them developed bunch top symptoms indicating that these plantlets were not resistant to BBTV. Results suggested that uneven distribution of virus at low concentration at high temperature may give rise to BBTV-free primordial cells which in turn may develop into healthy plantlets.

A924
COLLOIDAL GOLD LOCALIZATION OF SPIROPLASMA CITRI SURFACE PROTEINS. J. Fletcher and C. Colangabe, Dept. of Plant Pathology, Oklahoma State University, Stillwater, OK 74078.

Polyclonal antibodies specific for four surface proteins (p29, p58, p77, p99) of the citrus phytoplasmas were used to characterize the prevalence and distribution of these proteins on the membrane. Electron microscopy and colloidal gold labeling techniques were applied to whole and sectioned cells for immunostaining analysis of the proteins. The random distribution of gold particles along the surface of the cells indicated that p29 (spiralin), p77, and p99 are not clustered or arranged in a random distribution. The results for the p29 agree with previous reports: p77 and p99 have not been previously studied. The p29 was more prevalent than p77 or p99. The prevalence and distribution of gold particles in p58 samples did not differ from controls treated with preimmune serum; therefore, the p58 antibodies, which were made to protein isolated from SDS gels, may not recognize the native protein.

A926
THE BEST LEAFHOPPER TRANSMITTED VIRESCENCE AGENT CAUSES A PREMATURE FLOWERING AND VIRESCENCE DISEASE OF CARROTS. M. E. Shaw, B. C. Kirkpatrick, R. W. Keis, and D. A. Colino. Department of Plant Pathology, University of California, Davis, CA 95616.

During 1988/89 a premature flowering disease of carrots was observed in several fields located in the Southern San Joaquin Valley. Roots of diseased plants were woody and unmarketable and had infected virescent symptoms. Symptomatic plants tested positively by both DNA hybridization assays using cloned fragments of BLTV extrachromosomal DNA as probes and a BLTV-specific, enzyme-linked immunosorbent assay. BLTV-MCs were transplanted three diseased carrots to herbaraceous indicator plants using Cicurcularis tenellus leafhoppers. Southern blot analyses of undigested DNA from diseased carrots showed there was tremendous diversity in the numbers and sizes of plasmids in the field-collected, BLTV-infected carrots.

A927
A SURVEY OF PLANT PATHOGENIC MOLICUTES FOR THE ABILITY TO CAUSE THE HOST INDUCTION RESPONSE. D. A. Colino*, V. Butler*, and M. Shaw. USDA-ARS*, Department of Plant Pathology, University of California, Davis, CA 95616.

The best leafhopper transmitted virescence agent (BLTV) line FC-83-13 has been demonstrated to cause flowering in plants grown under environmentally non-inductive conditions, an effect known as the host induction response (HIR). Three new lines of BLTV, three lines of aster yellows, a line of western-x and Spiroplasma citri strain -215 were screened for their ability to cause the HIR. Each pathogen was used to inoculate groups of bean, radish, celery or Chinese cabbage, all of which have been shown to exhibit the HIR. All of the BLTV lines and none of the other mollicutes were demonstrated to cause the HIR.

A929

In 1988, Tomato Spotted Wilt Virus caused diseases of begonia, eggplant, and tomato. The virus was not known to exist in Colorado prior to 1988. During 1989, the Plant Disease Clinic received 14 plant samples infected with Tomato Spotted Wilt Virus. The 1989 Colorado host list for Tomato Spotted Wilt Virus included coreopsis, eggplant, eustoma, gerbera, pepper, and tomato. The Agdia PathoScreen FTA ELISA test specific for Tomato Spotted Wilt Virus was used to confirm the presence of the virus in plant tissues. Agdia, Inc. now has PathoScreen Kits available for detection of the impatiens and lettuce isolates of Tomato Spotted Wilt Virus. These kits will be used during 1990 to screen suspected Tomato Spotted Wilt Virus-infected plants to determine if both strains are present in Colorado.

A930

Chinese dogwoods have been considered resistant to dogwood anthracnose and a source of disease resistance. However, leaves, stems, and twigs which exhibited anthracnose symptoms were obtained from Chinese dogwoods in 1989. Characteristic Discus-like acervuli
developed within 72 hrs on this diseased tissue. These acervuli were shown to contain Discula-like conidia and subcultures were taken. These isolates are similar to those from flowering dogwoods in morphology, growth rate, and sporulation. Pathogenicity of these isolates to flowering dogwoods is under investigation. These results suggest that while Chinese dogwoods may prove more anthracnose-resistant than Cornus florida cultivars, C. kousa should be screened before being incorporated in the breeding program.

A932

A POLYMERASE CHAIN REACTION FOR DETECTION OF THE TAKE-ALL FUNGUS, GAUMANNOMYCETES GRAMINIS, IN INFECTED WHEAT PLANTS. Kurt Schremer and Joan M. Henson. Dept. of Microbiology, Montana State University, Bozeman 59717

The sequence of a DNA fragment that was previously found to be specific for Gaumannomycetes was determined. Polymerase chain reaction (PCR) was used to amplify this sequence in wheat seedlings infected with Gaumannomycetes graminis var. tritici which allows detection of fungal DNA in infected tissue without culturing the fungus. Other applications using PCR for Gaumannomycetes graminis detection will be discussed.

A933

The Characterization of an Infectious cDNA Clone of Potato Virus X

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Plant Science Technology, Monsanto Company, 700 Chesterfield Village Parkway, St. Louis, MO 63198

To analyze the mechanism of coat protein mediated protection and the molecular events during PVX infection, a full-length cDNA encoding the entire genome of potato virus X (PVX) has been isolated. This cDNA was placed behind the bacteriophage T7 promoter, which allowed infectious PVX transcripts to be produced in vitro. These transcripts were inoculated onto a local lesion host, Chenopodium amaranticolor, and a systemic host, Nicotiana tabacum. Lesions appeared on these plants that were typical of PVX infection, although infectivity was lower when compared to authentic PVX RNA. Electron microscopy of lesions isolated from infected plants showed typical flexuous rods of PVX. We are currently improving the infectivity of the clone by removing extraneous DNA from the transcription vector. We are using infectious transcripts to study interactions between coat proteins in transgenic plants and viral RNA. In addition, we are studying the functions of various open reading frames encoded by the PVX genome.

A934

EVALUATION OF SOYBEAN LINES REGENERATED FROM ORGANOCULTURE CALLUS FOR THEIR REACTION TO SEPTORIA GLYCINES. H. S. Song, S. M. Lim, and J. M. Wilholm. Departments of Plant Pathology and of Agronomy and USDA-ARS, University of Illinois, IL 61801.

Soybean plants resistant to the pathototoxic culture filtrates of Septoria glycines were regenerated in vitro. In 1990, more than 700 R3 (second selfed generation) were evaluated for their reaction to S. glycines by inoculating the plants in the field. Fifteen percent of the inoculated plants did not produce brown spot symptoms until the R6 reproductive growth stage. At harvest, the severity of brown spot on these plants was less than 10%. Brown spot developed in the other inoculated plants earlier and the severity ranged from 75 to 100% at the R6 growth stage. R3 lines from all of the R2 plants will be evaluated in the field.

A935

Purification and characterization of chitinases and β-1,3-glucanases from Beta vulgaris leaves infected with Cercospora beticola


Many plants respond to infection with a pathogen by producing a number of proteins. Among these defence related proteins are the hydrolytic enzymes, e.g. chitinases and β-1,3-glucanases. We have studied the interaction between Beta vulgaris L. and the leaf pathogen Cercospora beticola. It is shown that both chitin and β-1,3-glucan are constituents of the cell wall of Cercospora. Radioactive labelled N-acetylglucosamine and glucose are specifically incorporated into the cell wall of growing hyphae and may be removed from the apex by treating the hyphae with hydrolytic enzymes. Chitinase and β-1,3-glucanase activities are strongly induced following infection with Cercospora. From infected leaves nine chitinases and five β-1,3-glucanases have been purified to homogeneity by affinity column chromatography followed by cation exchange chromatography on a FPLC system. Amino acid compositional analysis, N-terminal and partial amino acid sequencing have been carried out on the major enzymes. Antibodies have been raised against three chitinases and two β-1,3-glucanases. Two serologically different groups of chitinases are present in sugar beet.