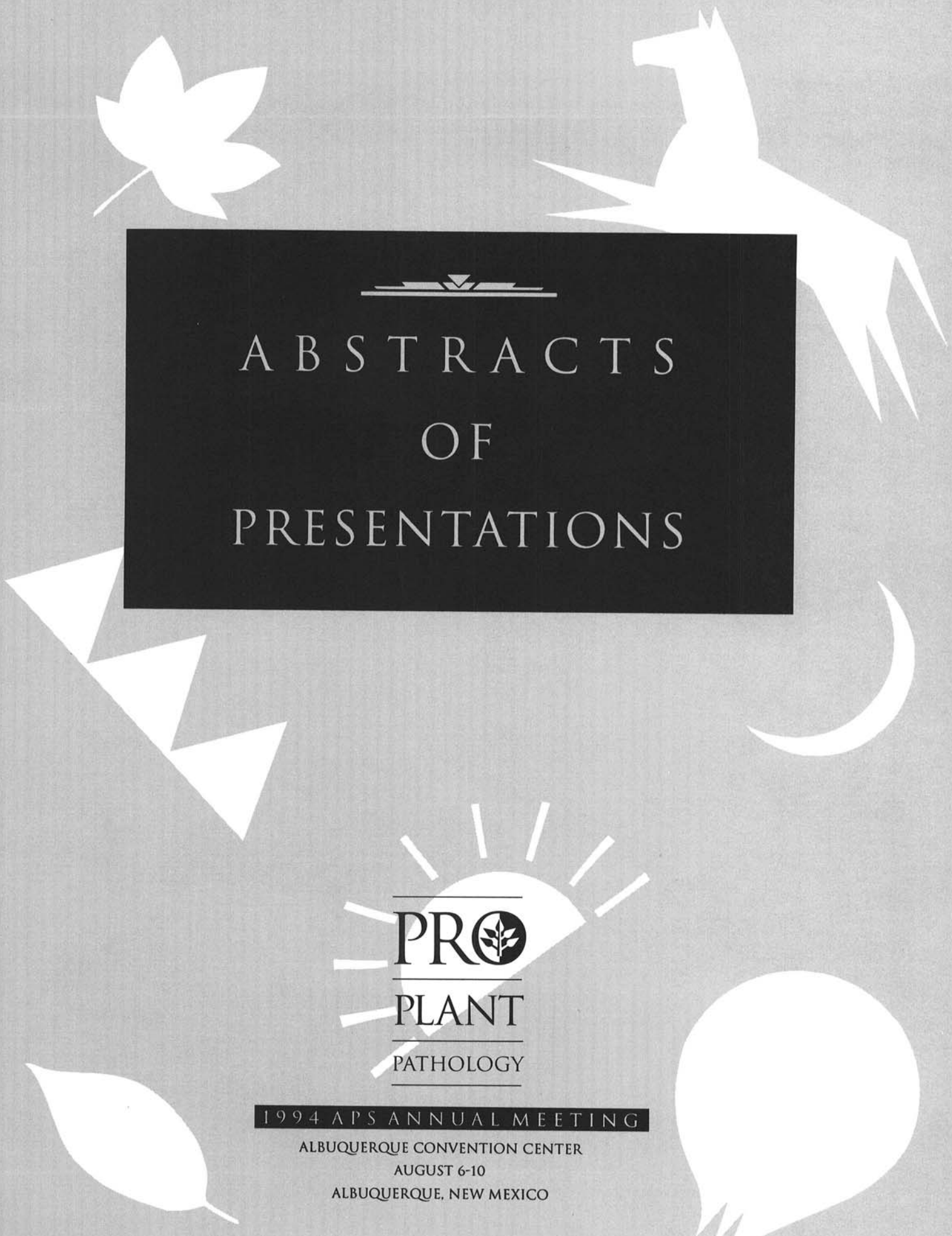

ABSTRACTS OF PRESENTATIONS

PHYTO PATHOLOGY

1994 APS ANNUAL MEETING

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APS ABSTRACTS OF PRESENTATIONS

The number above an abstract corresponds to its designation in the program of the 1994 APS annual meeting in Albuquerque, New Mexico, August 6–10.

1

A SMALL dsRNA ELEMENT OF CRY*PHONECTRIA PARASITICA*: TRANSFER AND CO-INFECTION. James J. Polashock and Bradley I. Hillman, Department of Plant Pathology, Rutgers University, New Brunswick, NJ 08903.

We have identified a small (2.7 kb) dsRNA element associated with the mitochondria of *C. parasitica* strain NB631. Like the more common dsRNAs of *C. parasitica*, which are 10-13 kb and are Potyvirus related (members of the Hypoviridae family), NB631 dsRNA can be transmitted into compatible strains by hyphal anastomosis. In contrast to the Hypoviridae, this element is also transferred to sexual progeny (ascospores). We have isolated a strain of the fungus, termed NB58F, that appears to be resistant to virus infection by members of the Hypoviridae. We have demonstrated by karyotype analysis that NB58F has a chromosomal abnormality. This lesion does not prevent infection of NB58F by the small mitochondrial dsRNA element by hyphal anastomosis or through mating. Transfer of the small dsRNA by anastomosis was accompanied by mitochondrial recombination in the recipient strain as determined by DNA fingerprint analysis. We are currently analyzing a *C. parasitica* isolate co-infected with the 2.7 kb mitochondrial dsRNA and a 12.5 kb member of the Hypoviridae (CHV2-NB58).

2

SIMULATION OF HYPOVIRULENCE TRANSMISSION IN POPULATIONS OF *CRYPHONECTRIA PARASITICA* USING SPATIALLY STRUCTURED MODELS. Y. -C. Liu, R. T. Durrett, and M. G. Milgroom, Cornell University, Ithaca, NY 14853.

A spatially explicit, interacting particle model was developed to simulate the transmission of hypovirulence viruses in a natural population of *C. parasitica*, the causal agent of chestnut blight. In this model, the field was divided into sites, each site representing a chestnut tree. The state of each site can be either vacant, an uninfected tree, a tree infected by the virulent fungus, or a tree infected by hypovirulent strains. Multiple infection on a single tree was considered such that a virulent canker can cause the loss of other cankers on the same tree. The transmission of hypovirulence was simulated by looking at different processes in the system: (i) population dynamics of chestnut trees and fungi, including tree growth, death, and reproduction of fungal populations; (ii) changes in the genetic structure of fungal populations, which involves sexual and asexual reproduction of the fungus and the dispersal of their progeny; and (iii) transmission of hypovirulence viruses which is a function of the difference in the vegetative incompatibility (*vic*) genes among strains. The model will provide insight into hypovirulence transmission in this system and provide information relevant to the biological control of chestnut blight.

3

SENESCENCE-LIKE PHENOTYPES IN dsRNA-FREE HYPOVIRULENT STRAINS OF *CRYPHONECTRIA PARASITICA*. D. H. Huber, D. W. Fulbright, H. Bertrand, C. M. Vitorello, J. Bell and B. Shaw. Michigan State University, East Lansing, MI 48824-1312

Hypovirulent strains of the chestnut blight fungus isolated from blight-recovering American chestnut trees commonly carry cytoplasmically transmissible double-stranded RNA viruses responsible for the attenuated phenotype. Recently, dsRNA-free hypovirulent strains associated with respiratory dysfunction have been isolated from recovering chestnut trees. Two of these strains, KFC9 and ARN, when subcultured from the margin of the colony on culture medium, show advanced stages of senescence as determined by the cessation of growth via subculturing to fresh medium. The phenotype of KFC9 has been cytoplasmically transferred to Ep289 a virulent strain. The phenotype has been sequentially transferred from Ep289, to two other virulent strains. Elevated levels of cyanide-resistant respiration similar to that of KFC9 were found in the converted isolate of Ep289 as well as the senescence phenotype. Conidia isolated and cultured from KFC9 showed different degrees of senescence ranging from normal growth to no growth.

4

REDUCTION OF LACCASE ACTIVITY AND OTHER HYPOVIRULENCE-ASSOCIATED TRAITS IN dsRNA-CONTAINING STRAINS OF *DIAPORTHE AMBIGUA*. W.A. Smit[1], B.D. Wingfield[2], & M.J. Wingfield[2]. [1]Infruitec, Private Bag X5013, Stellenbosch 7599, South Africa and [2]Department of Microbiology and Biochemistry, University of the Orange Free State, Bloemfontein 9300, South Africa.

A single double-stranded RNA (dsRNA) segment was detected in hypovirulent but not in virulent strains of *Diaporthe ambigua* isolated from apple rootstocks in South Africa. To test for phenol oxidase activity and gallic acid oxidation (Bavendamm's tests), the strains were grown on malt extract agar containing tannic and gallic acid respectively. Laccase, and peroxidase activity were determined with 2,6 dimethoxyphenol as substrate. Oxalic acid production of virulent and hypovirulent strains was determined by ultraviolet spectrophotometric analysis of NADH. Conversion of virulent strains was achieved by pairing hypovirulent and virulent strains on dialysis membrane on the surface of Czapek Dox agar. Pathogenicity tests were conducted on 3-year-old Merton 793 and MM25 apple rootstock cultivars. In both Bavendamm's tests, virulent strains produced a strong colour reaction, whereas hypovirulent strains showed weak or no activity. The enzyme responsible for the colour reaction on Bavendamm's medium was identified as phenol oxidase of the laccase type. DsRNA could be transmitted to strains of the same vegetative compatibility group by hyphal anastomosis. Converted strains lost virulence and showed loss of phenol oxidase activity, reduced gallic acid oxidation, diminished oxalic acid accumulation, and suppressed sporulation. From these studies we conclude that dsRNA was transferred to virulent strains via hyphal anastomosis. This resulted in hypovirulence as tested in the field, as well as reduction of laccase activity and other hypovirulence-associated traits.

5

OCCURRENCE OF HYPOVIRULENCE IN SCLEROTINIA MINOR IN OKLAHOMA. X.Li, H.A.Melouk, J.P.Damicone and K.E.Jackson. Oklahoma State University and USDA-ARS, Stillwater, OK 74078.

Colony diameters on potato dextrose agar (PDA) after two days of incubation at 25C for 62 isolates of Sclerotinia minor from peanut grown in Oklahoma were determined. The colony diameter of one isolate (9M-N) was 42mm compared to 80mm for the typical isolate (C). Isolate 9M-N formed few sclerotia after 14 days on PDA, compared to isolate C and the others which formed abundant sclerotia after five days. The virulence of the 62 isolates were compared on detached leaves of Romaine lettuce where the lesion area for isolate 9M-N was 6.28 cm^2 compared to 18.22 cm^2 for isolate C. On peanut plants (cv. 'Okrun') in the greenhouse in two experiments, isolate C killed 16 of 16 plants while 9M-N killed 0 of 16

plants after 14 days. This indicates the occurrence of hypovirulence in S.minor in Oklahoma.

6

ATTENUATION OF FUNGAL VIRULENCE BY SYNTHETIC INFECTIOUS HYPOVIRUS TRANSCRITS. Baoshan Chen, Gil H. Choi and Donald L. Nuss Roche Institute of Molecular Biology, Roche Research Center, 340 Kingsland Street, Nutley, NJ 07110-1199, U.S.A.

Non-infectious, cytoplasmically-transmissible viral double-stranded RNAs of the genus *Hypovirus* cause reduced virulence (hypovirulence) in the chestnut blight fungus *Cryphonectria parasitica*, providing the basis for virus-mediated biological control of a fungal disease. We now report that synthetic transcripts corresponding to a full-length hypovirus RNA coding strand are infectious when introduced into fungal spheroplasts via electroporation. Hypovirus infections were readily established in *C. parasitica* and in several fungal species not previously reported to harbor viruses. Viral infection of the pin oak blight fungus, *Endothia gyrosa*, resulted in reduced fungal virulence, thus extending virus-mediated virulence-attenuation to a new fungal taxonomic family. To our knowledge, this is the first example of an infectious synthetic transcript for a fungal virus and its use to expand host range thereby broadening the potential application of virus-mediated hypovirulence for understanding and controlling fungal pathogenesis.

7

INFLUENCE OF DOUBLE-STRANDED RNAs ON GROWTH, SPORULATION, PATHOGENICITY AND SURVIVAL OF CHALARA ELEGANS. Z.K. Punja, Department of Biological Sciences, Simon Fraser University, Burnaby, British Columbia, Canada.

Three strains of Chalara elegans (syn. Thielaviopsis basicola) that contained multiple (4-5) double-stranded RNA fragments were compared to spontaneously-derived cultures from these strains which were partially cured or completely free of dsRNA. In the wild-type strains, the dsRNAs was found to significantly enhance phialospore production and colony pigmentation, while radial growth and mycelial dry weight accumulation were reduced. In two partially cured strains (one 2.8 kb fragment present), pathogenicity to various plant tissues was significantly enhanced when compared to the wild- type multiple dsRNA-containing strains. However, survival in field soil was enhanced in one strain and reduced in the other. In the completely cured strain, the loss of multiple dsRNA fragments was associated with enhanced growth, reduced phialospore production and a complete loss of pathogenicity and capability for survival in soil. These results indicate that the effects of dsRNAs in C. elegans vary with the strain, and that some dsRNA fragments appear to confer an advantage to this soilborne facultative plant pathogen.

8

POSTHARVEST TREATMENT OF ORANGES WITH HOT WATER TO CONTROL STEM-END ROT CAUSED BY *DIPLODIA NATALENSIS*. G. Eldon Brown and Metwally Aly Baraka, Florida Department of Citrus, Lake Alfred, Fl 33850

Interest in non-chemical procedures, such as hot water, for control of postharvest diseases has been revived due to expense of fungicide registration and reregistration, and concerns of food safety with chemically treated fruit. Hot water may also complement biological control agents, which have poor eradicant activity and are ineffective against diseases, such as stem-end rot (SER), that develop from quiescent infections. Hot water (52C) for 5 min significantly reduced SER in some trials after 1 or 2 wk storage, but not after 3 wk. Control was significantly enhanced by the addition of ethyl alcohol or the surfactant sodium dodecylbenzene sulfonate to the hot water at concentrations of 10.0 and 0.05%, respectively. In one of three trials, the hot water-alcohol treatment was as effective as a treatment with the commercial fungicide, thiabendazole. Washing fruit before ethylene degreening reduced fruit degreening rates in some trials. Hot water treatments enhanced fruit moisture loss during degreening, but not during storage. Red pigmentation associated with minor rind injuries caused from handling was enhanced in some trials by hot water, and further aggravated by the use of alcohol.

9

MYCOFLORA AND MYCOTOXIN ASSAYS OF OVERWINTERED CORN IN CLAY COUNTY NEBRASKA; AN AREA OF ENZOOTIC AVIAN CHOLERA. Ben Doupnik Jr., University of Nebraska, Box 66, Clay Center, NE 68933

Fifty 2500-gm samples of overwintered corn were collected from each of 50 fields where waterfowl had fed in the rainwater basin area of Clay County Nebraska in March, 1992. This area is intensively used as a resting place by several million ducks and geese during their northward spring migration. This is also an area of enzootic avian cholera. The corn samples were assayed for mycoflora and mycotoxins. Predominate fungi were Fusarium spp., Penicillium spp., and Alternaria sp. Only five samples contained detectable levels of mycotoxins. Fumonisins were detected in three samples ranging from 5.3-14.1 ppm; and vomitoxin ranging from 0.3-2.0 ppm and zearalenone at a level of 0.9 ppm were detected in the other two samples. Aflatoxins were not detected in any of the samples. Mycotoxins did not appear to be major agents in either predisposing waterfowl to avian cholera or to act as immune suppressants in this study.

10

EAR ROTS AND MYCOTOXINS IN IOWA CORN. G.P. Munkvold and H.M. Stahr, Dept. of Plant Pathology and Veterinary Diagnostic Laboratory, Iowa State University, Ames 50011.

Two hundred thirty dent corn fields in Iowa were sampled in 1993 for ear rot fungi and mycotoxins. Fields were selected by a weighted sampling procedure so that each acre of corn in the state had an equal probability of selection. Ears were collected, evaluated for ear rots and shelled; kernels were ground and analyzed by TLC, HPLC or GC for seven mycotoxins. Fusarium ear rot occurred in 92.2% of fields, with mean severity of 1.42% of kernels in a 10-ear sample. Fusarium ear rot was highly correlated with insect damage. Incidence of Gibberella ear rot (*Gibberella zeae*) was 41.3% (severity 5.15%). Other ear rot pathogens included *Diplodia maydis*, *Nigrospora oryzae*, *Cladosporium* sp., and *Penicillium* sp. *F. subglutinans* was the most common species isolated from ears with Fusarium ear rot, followed by nearly equal proportions of *F. moniliforme* and *F. proliferatum*. *Fusarium* species (*F. subglutinans, F. moniliforme, F. graminearum, F. proliferatum*, and six others) were isolated from approximately 46% of asymptomatic kernels. Deoxynivalenol (DON) was detected in 67.8% of fields, but only 10.8% of samples exceeded 1 ppm. The north-central district had the highest incidence of Gibberella ear rot and DON. Fumonisins were detected in 20% of the fields, but only 2 samples exceeded 5 ppm fumonisin B1. A few samples had traces of either zearalenone or T-2 toxin.

11

EFFECTS OF RELATIVE HUMIDITY AND PREINCUBATION ON GROWTH OF *ASPERGILLUS FLAVUS* AND AFLATOXIN PRODUCTION IN MAIZE KERNELS. B.Z.Guo[1], J.S.Russin[1], R.L.Brown[2], T.E.Cleveland[2], and N.W.Widstrom[3]. [1]Dept. of Plant Path. & Crop Physio., LSU Ag. Ctr., Baton Rouge, LA; [2]USDA/ARS/SRRC, New Orleans, LA; [3]Insect Biology & Management Lab, USDA/ARS, Tifton, GA.

Growth and aflatoxin production by *A. flavus* were determined on maize kernels incubated (30°C) at constant relative humidities (RH) maintained by saturated salt solutions and water (100% RH). Kernels were pre-incubated at selected RH for 0 or 3 days, inoculated by immersing in *A. flavus* conidia suspension (10^6/ml), then incubated at these RH for 7 more days. Growth and aflatoxin production were not detected at 72.5% RH. Mycelial growth was observed at 77.5% RH on Pioneer 3154 (susceptible) but not until 80% on MAS:gk (resistant). Similarly, conidia production was observed at 84.5% RH on Pioneer 3154 but not until 91% RH on MAS:gk, although aflatoxin production was detectable on both genotypes at RH as low as 80%. When kernels were not pre-incubated prior to inoculation, aflatoxin levels were greatest at RH of 91% or 100% for each genotype. Pre-incubation for 3 d at 100% but not at 91% RH induced kernels to germinate. Germinated kernels tested at 100% RH exhibited lower aflatoxin production but greater mycelial growth than kernels of that were pre-incubated 0 d but had not germinated. Data suggest production of chemical(s) during kernel germination that inhibit toxin synthesis but allow fungal growth.

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A PUTATIVE GENE CLUSTER ASSOCIATED WITH MYCOTOXIN PRODUCTION IN *ASPERGILLUS NIDULANS*. N. P. Keller[1] and T. H. Adams[2]. [1]Dept. Plant Pathology and Microbiology, [2]Dept. of Biology, Texas A&M University, College Station, TX 77843.

Aspergillus nidulans produces the polyketide sterigmatocystin (ST), a carcinogenic mycotoxin that is the penultimate precursor in the aflatoxin pathway found in *A. flavus* and *A. parasiticus*. Polyketide biosynthetic pathways are often organized as gene clusters in microorganisms. Here we present evidence for a potential ST gene cluster located on one end of chromosome IV in *A. nidulans*. pL24B3, a cosmid mapped to this locus, contains *verA*, a gene encoding a keto-reductase necessary for ST biosynthesis. Sequence analysis of genomic DNA on either side of *verA* has revealed several open reading frames bearing homologies to proteins that likely provide enzymatic activities involved in ST biosynthesis. Northern analysis has demonstrated the presence of transcripts from pL24B3 that are present specifically when growth conditions support ST production.

13

REDUCED SUSCEPTIBILITY TO PREHARVEST AFLATOXIN ACCUMULATION IN TIFTON-8, A PEANUT GERMPLASM LINE. D. M. Wilson, W. D. Branch, B. W. Maw, R. W. Beaver,

and B. G. Mullinix. University of Georgia, Coastal Plain Station, Tifton, GA 31793.

Preharvest aflatoxin contamination in peanut (Arachis hypogaea) can be a major production problem. The objective of this study was to evaluate potential resistance by comparing aflatoxin accumulation in selected peanut genotypes. The Tifton-8 germplasm line accumulated significantly less preharvest aflatoxin over 4 years than the widely planted Florunner cultivar when peanuts were inoculated with Aspergillus flavus and A. parasiticus and grown under rainout shelters simulating late season drought. The resistance was not associated with seed infection in the field because Tifton-8 was readily invaded by members of the A. flavus group. The Tifton-8 resistance may be related to its ability to tolerate late season drought or other unidentified factors.

14

ACTIVITY OF SOME NEW FUNGICIDES AGAINST ANTHRACNOSE OF PECAN. A. J. Latham and H. L. Campbell. Department of Plant Pathology, Alabama Agricultural Experiment Station, Auburn University, Auburn, AL 36849-5409.

Fungicides with broad-spectrum activity are needed for control of late-season pecan diseases such as anthracnose (*Glomerella cingulata*). Nuts were sampled from 5 single-tree reps and sprayed at recommended rates of ICIA5504 (methoxyacrylate-chemistry), RH-7592 (fenbuconazole), and the TPTH (triphenyltin hydroxide) standard. Cheyenne nut samples of 500 g/tree were shelled; avg weight of nut meats were 3.6, 1.9, and 1.6 for ICIA5504, RH-7592, and TPTH, respectively. Effects of *G. cingulata* on nut yield and control of this pathogen were shown by analysis of culls, i.e., poorly developed nuts. Percent *G. cingulata* isolates from pecan kernels plated in PDA from well developed kernels were 0.5, 4.2, and 3.8, and from culls were 4.8, 11.4, and 21.6 from nuts of trees sprayed with ICIA5504, RH-7592, and TPTH, respectively. Thus, ICIA5504 shows strong activity for control of late-season and other pecan diseases.

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Suppression of Fairy Rings in Turf with Flutolanil. *S. B. Martin*, J. H. Blake, and G. B. Goss, Jr., Pee Dee Research and Education Center, Clemson University, Florence, SC.

Three trials were conducted to evaluate flutolanil: 1) a bentgrass 'Pennlinks' putting green, 2) a bermudagrass 'Tifdwarf' putting green and 3) a bermudagrass 'Tifway' fairway. Mushrooms were identified as *Lycoperdon pyriforme* Pers.(location 1) or *Agaricus campestris* Fr.(locations 2 and 3). Treatments included (locations 1,2): water, flutolanil at 0.402 and 0.803 g/M^2 applied as 3 or 2 sprays 30 and 45 days apart. At location 3, treatments were water, water + Triton AG-98 (1.7 ml/M^2), flutolanil (0.803 g/M^2), and flutolanil (0.803 g/M^2 + Triton AG-98. Ring symptoms were suppressed with flutolanil 42-87% at location 1 and 3. At location 2, ring symptoms were not apparent in any treatment, but mushrooms were suppressed >90%.

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EFFECT OF THREE FUNGICIDES (CHLOROTHALONIL, MANCOZEB, AND ICI5504) ON EARLY BLIGHT (ALTERNARIA SOLANI) SEVERITY AND YIELD OF TOMATO IN ARKANSAS. J.A. Duncan, J.C. Correll, J.C. Guerber, and P.E. Cooper. Department of Plant Pathology, University of Arkansas, Fayetteville, AR 72701.

The efficacy of several fungicides was evaluated in a commercial tomato field on the cultivar Mt. Delight in 1993 and 1994 in Arkansas. The fungicide treatments included chlorothalonil, mancozeb, and several rates of an experimental material, ICI5504. An unsprayed treatment served as the control. Plants were scored for early blight (Alternaria solani) severity by rating symptoms in the top, middle, and bottom portions of the canopy. Each portion of the canopy was given a score of 0 - 5, with 0 = no defoliation and 5 = > 90% defoliation. Fruit number, size (X-large, large, medium, and small), weight, and quality (Grade 1, 2, and 3) were evaluated each week. Early blight pressure was very high and was the only foliar disease observed. All fungicide treatments significantly reduced early blight severity. Mean disease ratings for the middle of the canopy at the end of the season for ICI5504, mancozeb, chlorothalonil, and the control were 2.2, 2.6, 3.2, and 4.7, respectively. The effect on yield, based on grade 1 and 2 fruit, indicated that chlorothalonil, mancozeb, and ICI5504 resulted in a 21, 36, and 48% increase in fruit number, respectively, and a 31, 54, and 60% increase in fruit weight, respectively, relative to the control.

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SENSITIVITY OF *SPHAEROTHECA FULIGINEA* TO TRIADIMEFON, BENOMYL, MYCLOBUTANIL, AND PROPICONAZOLE IN THE UNITED STATES. M. T. McGrath and H. Staniszewska, Dept of Plant Pathology, Long Island Horticultural Research Laboratory, Cornell University, Riverhead, NY 11901-1098.

Isolates of *S. fuliginea*, the causal agent of cucurbit powdery mildew, were collected between 16 Dec 1992 and 23 Sept 1993 from 11 fields treated with triadimefon (standard commercial practice) and eight non-treated fields in AZ, CA, FL, GA, MI, NC, NY, and VA. The 306 isolates exhibited a range in tolerance to the four fungicides that was related partially to fungicide usage. Only fungicide-sensitive isolates were found in two non-fungicide-treated commercial fields in MI and NY. In contrast, 64% to 100% of the isolates from fields treated at least twice with triadimefon were resistant (able to grow on disks from leaves treated with 50 ppm triadimefon). 6% and 47% of the isolates from fields treated once with triadimefon were resistant. Only benomyl-resistant isolates (tolerant of 200 ppm) were found in one of two fields examined in this study that had been treated with benomyl; almost all of the isolates collected from other fields were benomyl-sensitive. Triadimefon-resistant isolates were less sensitive to myclobutanil and to propiconazole (fungicides not registered for cucurbits in the U.S.): 92 of the 94 triadimefon-resistant isolates tolerated 2 or 20 ppm myclobutanil and 0.5 or 5 ppm propiconazole. However, growth of these isolates usually was reduced at the highest concentration, as compared with lower concentrations, of myclobutanil and of propiconazole but not of triadimefon. The highest concentration tolerated by most of the 72 triadimefon-sensitive isolates was 0.1 ppm myclobutanil (61%) and 0.1 ppm propiconazole (75%).

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EFFECTIVENESS OF HELICOPTER APPLICATIONS OF 3 DMI FUNGICIDES IN CONTROLLING MELAMPSORA LEAF RUST ON HYBRID POPLARS. G. A Chastagner, G. Newcombe and J. M. Staley, Washington State University Res. and Ext. Center, Puyallup, WA 98371

Triadimefon, propiconazole and myclobutanil were applied at 0.14, 0.28, and 0.56 kg ai/ha to *P. trichocarpa* x *P. deltoides* hybrid clone 47-174 on 25 July, 1993 in an effort to determine their effectiveness in controlling *M. medusae* f.sp. *deltoidae*. Each treatment was applied in the equivalent of 93.5 l/ha to random 21 x 115 m plots within three blocks of trees planted on 2.1 x 3.1 m spacings in 1991. Triadimefon and myclobutanil were more effective than propiconazole. Four weeks after treatment, the nonsprayed check plots had a Schreiner rust rating (SRR) of 100. After seven weeks, the SRRs of the trees sprayed with triadimefon and myclobutanil ranged from 1.7 to 10 and 1.3 to 15, respectively, while the SRRs of the trees sprayed with propiconazole ranged from 58.3 to 83.3. There was limited systemic protection of new growth by any of the fungicides.

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BICARBONATES AND *BOTRYTIS*: III. EFFECTS ON GERMINATION OF *BOTRYTIS CINEREA* CONIDIA. Palmer, C. L., R. K. Horst, H. W. Israel, and R. W. Langhans. Depts. of Flor. & Orn. Hort. and of Plant Path., Cornell University, Ithaca, NY, USA 14853.

Bicarbonates clearly inhibit *in vitro* colony growth of *Botrytis cinerea* Pers. However, effects on conidial germination and subsequent hyphal extension have not been characterized. To quantitively examine germination, conidia were placed onto dried agar/collodion coated glass microscope slides sprayed with 0.00 or 0.05 M $KHCO_3$ and with 0 or 2% potato dextrose broth (PDB). Spore production and germination as a function of culture age was also investigated. Inoculated slides were incubated for 24 h at 100% RH; then total and germinated conidia were evaluated. As culture age increased (6-, 13-, and 20-days old), total conidia increased (51.1, 498.1, 704.0, respectively) and percent of spores germinated decreased (15.5%, 5.7%, 3.4%, respectively). Addition of PDB to slides significantly ($p<0.0001$) raised germination from 2.2 to 13.1%, and $KHCO_3$ significantly ($p<0.0001$) reduced germination from 14.5 to 0.3%. Also, $KHCO_3$ affected ungerminated conidia morphology in several ways. Some spores appeared ruptured or crushed, or to have an equatorial line or constriction. Primarily, longitudinal depressions were seen, and these spores occasionally appeared plasmolyzed. We conclude that bicarbonates are detrimental to *B. cinerea* conidia. (Supported by H&I Agritech, Ithaca, NY 14850.)

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BENEFITS ASSESSMENT OF FUNGICIDE USE IN THE CONTROL OF FOLIAR DISEASES OF SEED CORN IN IOWA. S. N. Wegulo, C. A. Martinson, and F. W. Nutter, Jr. Department of Plant Pathology, Iowa State University, Ames, Iowa 50011

In 1993, eight experiments were established in Iowa commercial seed corn production fields to determine the benefits of applying chlorothalonil, mancozeb, and propiconazole to control fungal leaf diseases of seed corn. Experiments were established on land planted with corn the previous year; therefore the debris provided the initial inoculum of diseases caused by Exserohilum turcicum, Cercospora zeae-maydis, Aureobasidium zeae, and Bipolaris zeicola. Fungicide sprays were initiated at 2 to 4% disease severity about 2 weeks before detasseling, and were repeated up to 5 times at various stages of growth. The predominant disease throughout the season was common rust (Puccinia sorghi). Total yield, units

of saleable seed (especially medium and large sizes), and percent moisture at harvest were significantly higher (P=0.05) in treated plots compared to the untreated controls. Standardized AUDPC was reduced by up to 65% (P=0.01) in treated plots. Although all fungicide treatments effectively controlled disease, the best control was provided by five chlorothalonil sprays and the first 2 to 3 sprays of chlorothalonil, mancozeb, and propiconazole.

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OPTIMUM NUMBER OF APPLICATIONS OF FOLICUR FOR CONTROL OF SOUTHERN STEM ROT OF PEANUT. A. K. Hagan, K. L. Bowen, and J. R. Weeks, Auburn University, AL 36849.

On 'Florunner' peanut, control of southern stem rot and yield response to one, two, and four applications of Folicur 3.6F at 0.6 ℓ/ha was compared in nine on-farm trials conducted from 1991 to 1993. One, two or four applications were made approximately 60, 75, 90 and/or 105 days after planting as full canopy sprays. Across all sites in 1991, disease control and yield gains in all Folicur-treated plots were similar. In 1992, two and four applications of Folicur gave significantly better southern stem rot control and higher yields than than those treated only once. In 1993, two and four applications of Folicur gave equal southern stem rot control, but yield gain was higher with four applications. Disease severity and yield response to one application of Folicur and the non-treated controls did not significantly differ. Across all years, superior disease control and maximum yield was obtained only with four applications of Folicur.

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ROLE OF ASPARAGUS BEETLES IN DISPERSAL OF DOMINANT VEGETATIVE COMPATIBILITY GROUPS (VCGS) OF *FUSARIUM PROLIFERATUM* IN ASPARAGUS FIELDS. W. H. Elmer, The CT Agr Exp. Sta., Box 1106, New Haven, CT 06504.

In a previous report 88% of 110 isolates of *Fusarium proliferatum* (FP) recovered from diseased asparagus crowns were assigned to six VCGs (VCGs 1,4,5,7,8,13), and 40% of the total belonged to VCG 5. (*Phytopathology* 81:852). To determine if insects feeding on asparagus contribute in dispersal of these dominant VCGs within and among fields, over 300 asparagus beetles (*Crioceris asparagi* L. and *C. duodecimpunctata* L.) were captured in the June 1992 and 1993 from 1-6 yr-old asparagus fields. The beetles were crushed and placed on a medium selective for *Fusarium* spp. In the oldest field, recovery of FP from beetles was high (74%), but in 2-3 yr-old fields recovery of FP was lower (8-32%). Nitrate-nonutilizing (*nit*) mutants were selected from each isolate and paired with tester *nit* mutants in multiwell plates. Of 58 isolates tested so far, 40 (69%) were assigned to the six known VCGs. The VCG distribution in the oldest field reflected the previously reported pattern. In the young fields that were < 3 yr-old, all but one of the isolates assigned to known VCGs belonged to VCG 5. Asparagus beetles may vector FP into young asparagus fields.

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TRANSMISSION OF *THIELAVIOPSIS BASICOLA* AND *FUSARIUM PROLIFERATUM* BY FUNGUS GNATS. M. A. Harris, R. D. Oetting, and E. Moody. University of Georgia, Athens, GA 30602-2603.

Increased numbers of fungus gnats, *Bradysia coprophila*, are often observed in association with disease in ornamental crops. One of the pathogens with which this insect is most often associated is *Thielaviopsis basicola*, the causal agent of black root rot. This study was undertaken to determine if fungus gnats are able to transmit both *T. basicola* and *Fusarium proliferatum*, a fungus consistently isolated from the egg surface of field collected *B. coprophila*. Surface contaminated adults and larvae, adult and larval digestive tracts, and larval frass were all demonstrated to be viable means of transmission of both fungi. The incidence of infection of pansy seedlings by *T. basicola* was greatly diminished when *F. proliferatum* was present. *F. proliferatum* has previously been reported to be toxigenic (Applied and Environmental Microbiology 58:984-989) which may offer an explanation of this apparent antagonism.

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INGESTION-EGESTION AND AERIAL TRANSMISSION OF *THIELAVIOPSIS BASICOLA*, A ROOT AND STEM PATHOGEN OF CORN-SALAD, BY ADULT SHORE FLIES. M. E. Stanghellini, S.L. Rasmussen, and D.H. Kim. University of Arizona, Tucson, AZ 85721.

In 1988 *Thielaviopsis basicola* was identified as the causal agent of root and stem rot of hydroponically-grown corn-salad plants (*Valerianella locusta* L.) in a commercial production facility in Pennsylvania (Plant Disease 74:81). A wide spread reoccurrence of the disease in this facility in 1993 prompted an evaluation of the possibility that shore flies (*Scatella stagnalis*), which were in abundance on plant specimens submitted for diagnosis, could function as an aerial vector for the fungus. Endoconidia and chlamydospores of *Thielaviopsis basicola* were consistently observed in frass excreted by adult flies and larvae which were collected in the immediate vicinity of naturally infected plants. Approximately 95% of the adult flies and 85% of the larvae collected were internally infested with the fungus. Excreted spores were viable. We subsequently demonstrated that adult shore flies can, within a 4 hr acquisition time, ingest and excrete viable spores of the fungus and function as an aerial vector for transmission of this root and stem pathogen.

25

DYNAMICS OF FIG WASP (*BLASTOPHAGA PSENES*) POPULATION AND INCIDENCE OF FIG ENDOSEPSIS CAUSED BY *FUSARIUM MONILIFORME*. T. J. Michailides and D. P. Morgan, Dept. of Plant Pathology, Univ. of California, Davis/Kearney Ag. Center, Parlier 93648.

Propagules of *Fusarium moniliforme* are vectored on the body of the fig wasp pollinators and cause endosepsis disease in the syconia of male trees (caprifigs) and Calimyrna female figs (*Ficus carica*). Scanning electron microscopy showed that microconidia and mycelial fragments are attached to the wasps' bodies along with pollen grains picked up from the spring caprifig fruit crop (called profichi). None of the adult wasps artificially removed from the flower-galls had propagules of *F. moniliforme* but 91-100% of those emerged from infected caprifig fruits were infested with *F. moniliforme*. Experiments over two years showed that more than twice as many profichi caprifigs were infested by *F. moniliforme* after caprification with five or ten mamme (winter crop) than with a single mamme caprifig. Presence of more profichi caprifigs than were necessary for caprification resulted in more wasps entering the cavity of Calimyrna figs. The relationship between the number of wasps in the cavity of Calimyrna figs and disease levels was best described by a second degree polynomial (R^2 = 0.94-0.95; $P < 0.01$). These results suggest that proper management of fig wasp levels by applying the appropriate quantities of caprifigs can reduce levels of Calimyrna figs infected with *F. moniliforme*.

26

TRANSMISSION CHARACTERISTICS OF BEET YELLOWS VIRUS TO SUGAR BEETS BY *APHIS FABAE*. D. D. Limburg[1], P. A. Mauk[2], and L. D. Godfrey[1], [1]Department of Entomology, University of California, Davis 95616 and [2]University of California Coop. Extension, Sacramento 95827

Laboratory studies were conducted on transmission characteristics of beet yellows virus (BYV) to sugar beets by the black bean aphid, *Aphis fabae*. To help elucidate the potential role of *A. fabae* in the epidemiology of BYV, relative efficiency, retention, acquisition, and inoculation times were evaluated using apterous adults obtained from clonal aphid colonies maintained in the laboratory. For acquisition and inoculation, feeding time was determined by visual observation of stylet penetration, as well as by electronic monitoring. The electronic monitoring procedure recorded patterns of probing and ingestion leading to virus acquisition. Results indicated that *A. fabae* transmitted BYV at relative efficiencies of 22 to 44%, retention time of up to 24 hours, acquisition time ranged from 0.3 to 2 hours, and an inoculation period of less than 1 hour. The electronic monitoring system recorded a range of 9 to 38 minutes with an average of 19 minutes for the stylet of *A. fabae* to reach the phloem tissue.

27

ENDOSYMBIOTIC BACTERIA ASSOCIATED WITH CIRCULATIVE TRANSMISSION OF POTATO LEAFROLL VIRUS BY *MYZUS PERSICAE*. J.F.J.M. van den Heuvel, M. Verbeek & F. van der Wilk, DLO Research Institute for Plant Protection (IPO-DLO), P.O. Box 9060, 6700 GW Wageningen, the Netherlands.

In order to understand the molecular mechanisms underlying circulative transmission of potato leafroll virus (PLRV) by aphids, we have screened *Myzus persicae* proteins as putative PLRV binding molecules using a virus overlay assay of protein blots. In this way, we found that purified PLRV exhibited affinity for five aphid proteins. The one most readily detected has a molecular mass of 63 kDa, and was identified as symbionin. This is a predominant protein synthesized by the bacterial endosymbiont of the aphid and is released into the haemolymph. Since further binding studies clearly showed that PLRV also binds to native symbionin, it was envisaged that virus particles when acquired into the haemocoel of an aphid interact with symbionin. Inhibition of prokaryotic protein synthesis by feeding *M. persicae* nymphs on antibiotic-containing artificial diet prior to PLRV acquisition reduced virus transmission by more than 70%. The major coat protein of the virus was found to be degraded in the antibiotic-treated aphids which would obviously have resulted in loss of infectivity. For these reasons we conclude that endosymbiotic bacteria play a crucial role in determining the persistent nature of PLRV in the aphid haemocoel and that symbionin is probably the key protein in this interaction.

28

USE OF MONOCLONAL ANTIBODY (MAB) TO THE NONSTRUCTURAL PROTEIN (NS) OF THE S-RNA OF TOMATO SPOTTED WILT VIRUS (TSWV) TO DETECT VIRULIFEROUS THRIPS [*FRANKLINIELLA OCCIDENTALIS* (PERGANDAE)]. M.D. Bandla[1], D.E. Ullman[2], T.L. German[3], D.M. Westcot[2] and J.L. Sherwood[1]. [1]Plant Pathology Department, Oklahoma State University, Stillwater, OK 74078; [2]Entomology Department, University of Hawaii, Honolulu, HI 96822; [3]Plant Pathology Department, University of Wisconsin, Madison, WI 53706.

Identifying TSWV viruliferous thrips would be helpful in development of virus forecasting for a TSWV disease management program. Since the presence of NS

proteins is indicative of virus replication, a serological assay based on the detection of a NS protein would identify viruliferous thrips. The NSs protein encoded by the small RNA is abundant during TSWV replication in thrips. Mabs were produced against NSs and used to develop an antigen coated plate enzyme linked immunosorbent assay (ACP-ELISA). Nonspecific binding of antibody to insect tissue in ACP-ELISA resulting in high absorbance readings of non-viruliferous thrips was reduced by replacing Tween-20 with Empigen BB (E-BB) at 0.1% (AI) in the antibody dilution buffer. The sensitivity and utility of the ACP-ELISA in detecting viruliferous thrips was compared with transmission of TSWV by thrips to *Petunia grandiflora* L. A similar percentage of the thrips sampled were identified as viruliferous in both the ACP-ELISA and the transmission assay. Hence, the identification of viruliferous thrips by ACP-ELISA could be useful in developing a forecasting program for TSWV.

29

ASSOCIATION OF POTYVIRUS HELPER COMPONENT PROTEIN, VIRIONS, AND THE EPICUTICLE LINING THE MAXILLARY FOOD CANAL AND FOREGUT OF AN APHID VECTOR. E.D. Ammar, U. Järlfors, and T.P. Pirone. Dept. Plant Pathology, University of Kentucky, Lexington, KY 40546.

Transmission electron microscopy and immunogold labeling were used to study the role of the helper component protein (HC) in the transmission of potyviruses by aphids. In sections of aphids (*Myzus persicae*) that fed on a mixture of purified tobacco etch (TEV) or tobacco vein mottling (TVMV) viruses and HC, virions and HC were found associated with the epicuticle, predominantly throughout the maxillary food canal but also in the precibarium and cibarium of the foregut. No virions were found in any of these sites in sections of aphids that fed on purified virus without HC. Only aphids that fed on virions and HC transmitted TEV or TVMV. These results support the hypothesis that the HC is directly involved in binding or attachment of virions to the cuticle lining the food canal and foregut of aphid vectors. Additionally, our results, when compared with previous reports, suggest differences in retention sites between nonpersistently and semipersistently transmitted viruses in their vectors.

30

THE DEVELOPMENT OF APOTHECIA FROM STONE FRUIT MUMMIFIED AND STROMATIZED BY *MONILINIA FRUCTICOLA* IN CALIFORNIA. B.A. Holtz and T. J. Michailides, Department of Plant Pathology, University of California, Davis / Kearney Agricultural Center, Parlier, CA 93648.

Apothecia were produced in the orchard and laboratory from peach and nectarine fruit mummified by *Monilinia fructicola*. Fully stromatized and non-stromatized mummies were placed in the orchard either on the soil surface or completely buried 2 - 3 cm. Stromatized mummies consist of sclerotinized or resistant fungal tissue intertwined with decayed fruit tissue dried from 4 - 8 wk during the summer (Willetts & Harada Mycologia 76:314-325). Stromatized mummies were placed in the orchard from August 1993 to February 1994. Non-stromatized mummies, which decomposed rapidly and were soon unavailable, were only placed in the orchard in August and September. Apothecia were only found in February and early March from stromatized mummies that were placed in the field from October - December. More apothecia were produced from mummies placed in the field in November (11.0 %) than in October (3.9 %) or December (6.5 %). There was no significant difference ($P < 0.05$ Student's t test) in the development of apothecia between mummies which were buried or left on the soil surface. Stromatized mummies placed in the field in August / September 1993 and January / February 1994 did not produce apothecia, presumably because they did not have the proper conditions for apothecial initiation and differentiation. Apothecia were never produced from non-stromatized mummies. Apothecia were produced in the laboratory from stromatized mummies which were incubated in moist sand (> 95% R.H.) and in the dark for 8 wk at 2 C, and then for 2 wk at 15 C with a 12 h photoperiod.

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PREHARVEST CIGAR END ROT DISEASE COMPLEX ON *MUSA* FRUITS IN SOUTHEAST NIGERIA. C. Pasberg-Gauhl and F. Gauhl, International Institute of Tropical Agriculture, PMB 5320, Ibadan, Nigeria

Cigar end rot, caused by several fungi, is an important disease of export banana and plantain. Symptoms were observed in southeastern Nigeria on different *Musa* clones. Several pathogens were isolated from infected fingers: *Botryodiplodia theobromae, Curvularia lunata, Deightoniella torulosa, Fusarium avenaceum, F. moniliforme, F. moniliforme sp. subglutinans, F. pallidoroseum, F. solani, Glomerella cingulata, Trachysphaera fructigena* and *Verticillium theobromae*. Occasionally on bunches of the cvs. Bluggoe (ABB), Fougamou (ABB) and Calcutta-4 (AA) all fingers were 100% necrotic. On plantain (AAB) typical symptoms extended only up to 3 cm from the tip. Cigar end rot incidence and severity were evaluated weekly from February 1992 until April 1993 on the French horn plantain Obino l'Ewai at the IITA Onne Station. In both years incidence increased during the dry season (Dec.-Feb.) and was low during the rainy season (March-Nov.). In February 1992, 90% of the harvested bunches had cigar end rot symptoms. Percentage of harvested bunches attacked decreased from 93.5% in March to 58.3% in April and was only between 0 and 5.4% from May until December. In January 1993, incidence increased to 24.8% peaking at 72.8% in February. In March and April 58.4 and 56.6% of the bunches had infected fingers. Disease severity ranged between 0 and 62.2% diseased fingers per bunch. Higher temperatures combined with high relative humidity seem to favour cigar end rot development.

32

EFFECT OF DAILY FLUCTUATIONS IN TEMPERATURE ON COLONIZATION OF CITRUS ROOTS BY *PHYTOPHTHORA CITROPHTHORA* AND *P. PARASITICA*. M. E. Matheron and M. Porchas, Yuma Agricultural Center, University of Arizona, Yuma, AZ 85364

Colonization of rough lemon (*Citrus jambhiri*) roots by *Phytophthora citrophthora* or *P. parasitica* was examined when temperatures fluctuated during four 24 hr periods between values at which colonization occurred (conducive period) or did not occur (inhibitory period). Colonization of rough lemon roots by *P. citrophthora* was reduced by over 90% when a conducive incubation period of 24-27 C for 14 hr or less was followed by an inhibitory period of 28-30 C for 10 hr or more, compared to a conducive period of 24 hr where the incubation temperature range was 24-27 C. Likewise, colonization of rough lemon roots by *P. parasitica* was reduced by over 85% when the conducive incubation period of 30-33 C for 14 hr or less was followed by an inhibitory period of 34-36 C for 10 hr or more, compared to a conducive period of 24 hr at 30-33 C. Similar fluctuations in soil temperature have been recorded in young Arizona citrus groves and may limit root colonization during certain times of the year.

33

EFFECT OF HULL DEHISCENCE AND ABSCISSION OF ALMOND FRUIT AND INOCULUM CONCENTRATION ON SEVERITY OF HULL ROT DISEASE CAUSED BY *RHIZOPUS STOLONIFER, MONILINIA FRUCTICOLA* AND *M. LAXA*. Beth L. Teviotdale and Themis J. Michailides. Department of Plant Pathology, Univ. Calif. Davis, Kearney Ag. Center, Parlier, CA 93648.

Hull rot disease of almond is caused by several fungi. The most common incitants are *Rhizopus stolonifer* and *Monilinia fructicola*. The pathogens cause lesions on the mesocarp (hull) after dehiscence and apparently produce a toxin which kills nearby leaves and hoots. Although *M. laxa* is not found frequently in hull rot lesions, the fungus is the principal cause of brown rot blossom and twig blight of almond. We inoculated fruit at three stages of hull abscission and dehiscence with water suspensions of 10^4 conidia of *M. fructicola* and spores/ml of *R. stolonifer*. Percent death of leaves next to inoculated fruit decreased as hull abscission and dehiscence progressed, and *M. fructicola* caused higher average percent leaf death (47.8) than *R. stolonifer* (32.3). Inoculum concentration (10^3, 10^4, or 10^5 conidia of *M. laxa, M. fructicola* and spores of *R. stolonifer*/ml) did not affect percent leaf death near inoculated fruit. Average percent leaf death was greatest next to fruit inoculated with *M. laxa* (59.7), less with *M. fructicola* (45.8) and least with *R. stolonifer* (38.3).

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EFFECT OF CLUSTER DEBRIS REMOVAL ON BOTRYTIS BUNCH ROT OF 'CHARDONNAY' GRAPE. A. Baudoin and T.K. Wolf, Dept. of Plant Pathol. Physiol. & Weed Sci., Virginia Tech, Blacksburg, 24061, and Winchester Agric. Exp. Station, Winchester, 22601.

A field study was conducted in 1993 in two Virginia vineyards to assess the possibility of reducing Botrytis bunch rot by debris removal (DR) from individual grape clusters at either early fruit set or late fruit set, using compressed air. Differences due to DR date were not statistically significant. DR reduced Botrytis incidence (proportion of clusters with Botrytis) by 64% of the control value in early July, and by 34% at harvest in one vineyard, but did not reduce Botrytis in the second vineyard. A spreader-sticker, Nufilm 17, applied at mid-bloom, did not affect either debris retention or Botrytis bunch rot. Treatment of entire vines with a backpack leaf blower at late fruit set showed that air speeds around 50 m/s were capable of dislodging most debris from clusters. DR with a backpack blower reduced Botrytis incidence by 64% of the control in mid-July and 60% at harvest in the first vineyard, but did not reduce Botrytis incidence in the second vineyard.

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THE EFFECT OF BLOOM TIME INOCULATION WITH BOTRYTIS CINEREA ON FLORAL DEBRIS COLONIZATION AND FINAL DISEASE IN GRAPES J.C. Broome[1], J.J. Marois[1], R.A. Duncan[2], J.J. Stapleton[2], and G.W. Leavitt[3], [1]Department of Plant Pathology, University of California, Davis 95616. [2]Statewide Integrated Pest Management Project, University of California, Kearney Agricultural Center, Parlier, CA. 93648, [3]Cooperative Extension, Madera, CA 93637

Clusters of *Vitis vinifera* "Zinfandel" were inoculated with *Botrytis cinerea* or distilled water at 10%, 50%, 100% bloom and pre-close at 3 locations in the San Joaquin valley. 24 hours after inoculation iprodione was applied to half of all vines. Campbell CR10 microloggers monitored temperature, relative humidity, and leaf wetness throughout the season. Debris colonization was measured 2-3 weeks after inoculation. Fungicide application significantly reduced floral debris colonization. Bloom stage also affected debris colonization, as well as fungicide effectiveness. At two locations, the pre-close fungicide treatment did not significantly reduce colonization.

Debris colonization was reduced by high temperatures and favored by cool, moist conditions. Botrytis bunch rot incidence and severity at harvest were reduced significantly by fungicide applications. However, host bloom stage at inoculation and it's interaction with fungicide were not significant by harvest.

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AN IMPROVED METHOD FOR QUANTITATIVE ENUMERATION OF *Macrophomina phaseolina* MICROSCLEROTIA IN DRIED SOYBEAN TISSUE. O. N. Carvil and G. S. Smith, Plant Science Unit, University of Missouri-Columbia, Columbia MO 65211

Soybean taproots, dried to 7% moisture at 25 °C, were comminuted in a Wiley mill or in a Ude mill. Two-hundred-milligram tissue samples comminuted in the Ude mill were subjected to enzymatic digestion for 24 hours at 50 °C in a 0.04% solution of cellulase, either directly after grinding, or after incubation in a 0.2% acid pepsin for 3, 6, 12, or 24 hours. Additional soybean tissue comminuted in the Ude mill was also treated with three concentrations of pectinase. Tissue suspensions were plated on potato dextrose agar amended with 154 ug of Chloroneb, and 200 ug of streptomycin sulfate. Microsclerotial densities were enumerated as colony forming units/gram of dried tissue. A 56% increase in M. phaseolina populations was obtained from tissue comminuted in the Ude mill (11,783 cfu/g) compared to the Wiley mill (7,568 cfu/g). Microscopic examination of tissue comminuted in the Ude mill determined that there were no fragmented microsclerotia, and that no colonies originated from hyphal fragments. Tissue treatment with either cellulase or pectinase did not affect enumeration of microsclerotia. However, incubation of tissue in acid pepsin decreased the detectable populations by 96% after 3 hours and by 100% after 24 hours. Comminution of dried soybean taproots in a Ude mill, instead of in a Wiley mill, is proposed as a more accurate method of enumerating M. phaseolina in soybean tissue.

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A method to distinguish bias from variation in evaluating spore germination. D.S. Yohalem[1], C.M. Simmer[1], J.H. Andrews[1], and E.V. Nordheim[2], 1.Dept. of Plant Pathology; 2. Depts. of Forestry and Statistics, University of Wisconsin, Madison 53706

Spore germination assays are often used in the evaluation of fungitoxic agents. We performed an experiment in 96-well microtitre plates to determine the effect of observer fatigue on the evaluation of inhibition of *Venturia inaequalis* conidial germination by compost extracts. It was found that random outlier observations could distort the quantification of fatigue over time. We then constructed a test by which suspect observations from small samples could be evaluated for their effects on estimation of sample means. A Bonferroni prediction interval constructed as: $\bar{x} \pm \text{Student's } t_{\frac{\alpha/2}{n}, n-2 \text{ df}} \left\{ s\sqrt{1 + [1/(n-1)]} \right\}$, where $\bar{x}$ = the mean of the total sample, n = the total sample size, s = the sample standard deviation, and α = the probability of a type I error, gives a measure of the effect of the suspect observation on the sample mean. Observations outside the prediction interval are deleted. After performing this procedure on 13 running means with n=5, for four 1 h intervals, with two extracts, it was determined that inhibition of conidial germination was evaluated more liberally with time.

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QUANTIFICATION OF FOLIAR DISEASES USING TRUE COLOUR COMPUTER IMAGE ANALYSIS. L. Lamari, Dept. of Plant Science, University of Manitoba, Winnipeg, MB., Canada, R3T 2N2.

The measurement of foliar diseases has always posed a challenge to plant pathologists seeking to develop objective methods of disease assessment. Attempts made in the past using video image analysis had only limited success, due to the use of black and white video images. Software was developed to perform image acquisition, processing and analysis of true colour video images. The software uses 24 bits images (16.7 million colours) to extract information from which the background, and the areas representing healthy and diseased tissues are identified. The hardware used allows for near-real time processing of leaf samples. Leaf area measurement, disease quantification, morphometric and colour characterization of objects are discussed.

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Delivery of Environmental Horticulture Advice with the Aid of Speech Recognition Technology. David L. Clement, M. K. Malinoski, J. H. Traunfeld, and R. V. Bosmans. Regional Specialists, Cooperative Extension Service, University of Maryland, Home and Garden Information Center, 12005 Homewood Rd. Ellicott City, MD 21042.

Since 1990, Maryland residents have enjoyed a unique free service offered by the Cooperative Extension Service. Residents with questions on plant diseases, vegetable gardens, fruit trees, lawns, landscape ornamentals, house plants and pest problems have contacted the Home and Garden Information Center through a 1-800 phone number for toll free help. The original phone system that has directed the call flow and provided prerecorded information on assorted topics has relied on callers navigating through a series of touchtone directories. The recent addition of "flexword speech recognition" enables callers to access information by simply speaking the word or phrase of the message they wish to listen to. In addition, this system has shortened the length of time a caller spends listening to menus and routes calls more efficiently to their desired destination. Additional software allows conversion of typed text into machine voice. This feature enables more convenient updates and quick changes of audio material without the need for rerecording voice messages. The Center faculty have produced a series of over 250 messages on various topics that have allowed the public convenient access to unbiased information 24 hours a day.

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A PRACTICAL AND INTEGRATED APPROACH TO UNDERGRADUATE INSTRUCTION IN PLANT PATHOLOGY AT EARTH. Fritz Elango, Escuela de Agricultura de la Región Tropical Húmeda (EARTH), Apartado 4442-1000, San José, Costa Rica.

EARTH is a private 4-year international boarding university located in the humid tropics of Costa Rica. It was founded 5 years ago with the aim of training young people from the american humid tropics in sustainable agriculture and the management of this fragile ecosystem with an environmental sensibility and an enterprise mentality. EARTH uses a non-traditional intensive system of education which is unique because it includes an upside-down curriculum, a learn-by-doing philosophy, integrated curriculum, absense of departments, personalised teaching with a student:staff ratio of 10:1, a unique student recruitment system, group activities, active student participation, close contact with the neighboring communities, a work experience program, enterprise projects and an internship program etc. The plant pathology component of this curriculum which includes 15 weeks of general plant pathology, 7 weeks of work experience in aspects of integrated pest management, 36 months of enterprise projects, 15 weeks of an elective course in crop pathology and 12 months of a research project, offers the EARTH student a very practical hands-on experience in the management of plant disease problems.

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Rose Rosette Disease in Iowa - 1993

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The research on the rose rosette disease (RRD) has resulted in data which quantifies the time, manner and location for practical application of this disease agent as a biological control for multiflora rose. We have found that grafting of tissue (with buds) from naturally occurring RRD-symptomatic plants is a reliable, cost-effective method of augmentation (intensification) of the disease.

Aerial surveys have revealed multiflora rose to be present as far north as Allamakee county - roughly those counties south of a line drawn from Council Bluffs on the west to the border with Minnesota in the northeast and in Grant County, Wisconsin. RRD was found in all 47 sites thus identified and selected at random for ground-truth examination.

Strong evidence exists suggesting that the disease is endemic and its host range is limited to plants in the genus Rosa. The native species of rose appear to be highly resistant. Our data also suggests that the ornamental forms are not a favored host. An intensive risk assessment study is under way.

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BIOLOGICAL CONTROL OF WATERHYACINTH (*EICHHORNIA CRASSIPES*) BY *ALTERNARIA EICHHORNIAE*. Y.M. Shabana, R. Charudattan, and M.A. Elwakil. Plant Pathol. Dept., Univ. Florida, Gainesville and Dept. Plant Pathol., Univ. Mansoura, Egypt.

A virulent and host-specific Egyptian isolate of *Alternaria eichhorniae*, Ae5, caused severe leaf blight of waterhyacinth (*Eichhornia crassipes*) in greenhouse trials. Similar levels of disease severity (DS) occurred with both conidial and mycelial inocula. Exposure of inoculated leaves to 10 h of dew promoted disease, but the use of a hydrophilic mucilloid with mycelial inoculum eliminated the need for dew-exposure. Two alginate formulations were evaluated for possible field use. Four weeks after applying an alginate-pellet formulation of Ae5 to waterhyacinths, the plant biomass decreased by 29% from the starting fresh weight. New leaf production in the Ae5-treated plants averaged 5% compared to 81% in the fungus-free control plants. Over 6 wk, the evapo-transpirational water loss from plots containing Ae5-treated plants decreased by 31% compared to the loss from plots with fungus-free, healthy waterhyacinths. DS increased and plant biomass decreased with increasing number of applications of a powdered alginate formulation of Ae5 containing a hydrophilic polyacrylamide. Compared to the fungus-free controls, there was an 81% decrease in biomass and a 93% increase in DS in the fungus-treated plants 2 mo after four sequential applications (at 10-day intervals) of the formulation. Thus, *A. eichhorniae* was capable of curtailing waterhyacinth growth, but multiple applications of the fungal formulation may be needed to obtain similar levels of control in the field.

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DEVELOPMENT OF *Sclerotinia minor* Jagger AND *Phyllosticta* sp. FOR THE BIOLOGICAL CONTROL OF COMMON RAGWEED (*Ambrosia artemisiifolia* L.). S.C. Brière, A.K. Watson, and S.G. Hallett. Plant Science Department, Macdonald Campus of McGill University, Ste-Anne-de-Bellevue, Québec. Canada, H9X 3V9.

Sclerotinia minor causes severe infection of common ragweed under controlled environment conditions when grown and inoculated on autoclaved barley seed (Ciotola *et al.*, 1990). Effective control of mature ragweed by *S. minor* using this granular fomulation, however, is

unpredictable due to the erect nature of the plant. Control is enhanced by using spayable emulsion and invert emulsion formulations. From a collection of other fungi isolated from ragweed in 1993, the fungus *Phyllosticta* sp. has demonstrated considerable bioherbicide potential under controlled envionment conditions. This pathogen produces pycnidia on leaves and causes systemic infection spreading into flowering shoots. Within 20 days, inflorescences are killed and stem rotting is frequently observed. As a consequence, the production of pollen, the major cause of hayfever in North America, is greatly reduced. The potential of these two fungi for the effective control of ragweed in urban areas will be discussed.

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AN INNOVATIVE APPROACH FOR THE BIOLOGICAL CONTROL OF AFLATOXINS IN CORN. F. E. Vega and P. F. Dowd, Mycotoxin Research Unit, NCAUR, USDA, ARS, 1815 N. University St., Peoria, IL 61604.

An effective method to control *Aspergillus flavus* colonization and ensuing aflatoxin production in corn is lacking. Use of resistant plant varieties has met with limited success, and chemical control is not economically feasible. Use of fungal biocompetitors is a promising alternative. We have developed an autoinoculator device that uses existing traps and insect attractants, to lure the dusky sap beetle (*Carpophilus lugubris*; Coleoptera: Nitidulidae), an insect that transmits *A. flavus*. Using a blue dye in powder form as an indicator, field studies showed that sap beetles could be contaminated with dye and would carry it to 52% of damaged corn ears. In a laboratory test using *Bacillus subtilis* in powder form (marketed as Kodiak™ by Gustafson, Inc.) we found that damaged kernels in which *B. subtilis* was introduced by the sap beetles prior to *A. flavus* inoculation had 50% less *A. flavus* infection than ears where *A. flavus* was introduced first. Similarly, a field experiment using Kodiak™ showed that ears that permitted access of sap beetles before adding *A. flavus* had no *A. flavus*, in contrast to those where *A. flavus* was added first (92% infection rate).

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PATHOGENESIS IN *MYRIOPHYLLUM SPICATUM* BY *MYCOLEPTODISCUS TERRESTRIS*. J. P. Stack, M. Sabolefski, R. L. Buerkett, and H. B. Gunner. *EcoScience Corporation*, 377 Plantation Street, Worcester, MA 01605.

Mycoleptodiscus terrestris (Gerdemann) Ostazeski, a pathogen of terrestrial legumes, is a natural component of the microflora of eurasian milfoil (*Myriophyllum spicatum* L.), an exotic submersed aquatic weed in North America. Isolates of *M. terrestris* were found that varied in virulence to milfoil based on rates of disease progress. When applied to milfoil stems in a granular formulation, all pathogenic strains grew from the granule, penetrated the stem, and colonized internal tissues; disease development was optimum at 25-28 C. Necrotic lesions developed at the granule-stem contact site and progressed bidirectionally along the stem and petioles. Hyphae were observed on the surface and within stems of the host. Lacunae (internal air chambers) became extensively colonized by hyphae that breached the single cell layer between lacunae and penetrated into the outer mesophyll cells. Hyphae attached to and appeared to penetrate glandular cells within the lacunae. Lesions normally expanded to fill the internodal region, then progressed across the node into the next internodal region. However, lesion expansion occasionally ceased at a node. SEM observations of the node region revealed a contiguous layer 3-to-5 cells thick. How the nodal region inhibited disease development remains to be determined.

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DEVELOPMENTAL TEMPERATURE INFLUENCE UPON THE MORPHOLOGY AND PHYSIOLOGY OF ALTERNARIA HELIANTHI SPORES. H.K. Abbas, G.H. Egley and R.N. Paul, USDA-ARS, SWSL, Stoneville, MS 38776.

An isolate of A. helianthi was tested at various temperatures for spore production, growth and virulence on sunflower, safflower, and cocklebur. The fungus grew on sunflower leaf extract agar rapidly at 28 and 30°C, but more spores were produced at 18 to 26°C. Spores produced at 18°C were more virulent and germinated more vigorously. Histochemistry showed that the spores grown at the lower temperature stained more heavily for carbohydrates and more lightly for lipids than the high temperature spores. Spores grown at higher temperatures had many more empty segments, and exhibited much variation in segment number. This appeared to be the result of cytoplasmic degeneration at high developmental temperatures. There also appeared to be a correlation between the state of spore degeneration and the virulence of the spores on susceptible plants. This may have implications for the production of A. helianthi as a mycoherbicide.

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GENETIC DIVERSITY OF *XANTHOMONAS ORYZAE* PV. ORYZAE IN ASIA. J. Leach[1], T. Adhikari[2], S. Choi[3], C. Vera Cruz[1], Q. Zhang[4], D. Skinner[1], R. Nelson[5], and T. Mew[5]. [1]Kansas State University, Manhattan, KS 66506-5502; [2]Tribhuvan University, Nepal; [3]Rural Development Administration, Korea; [4]Chinese Academy of Agricultural Sciences, China; [5]International Rice Research Institute, Philippines.

Genomic diversity of a collection of *Xanthomonas oryzae* pv. oryzae from different countries in Asia was evaluated by analyzing for restriction fragment length polymorphism and for the presence of two DNA restriction modification (RM) systems (*Xor*I and *Xor*II). In general, genomic diversity of the collection was partitioned by country of origin. Most genetic lineages consisted of strains from a single country although some lineages were shared among countries. All possible phenotypes of the two RM systems (*XorI*$^+$/*XorII*$^+$, *XorI*$^+$/*XorII*$^-$, *XorI*$^-$/*XorII*$^+$, *XorI*$^-$/*XorII*$^-$) were detected at a ratio of 1:2:2:2. Based on their distribution, *Xor*I RM system originated in northeast Asia and *Xor*II system orignated in southeast Asia. Analysis of virulence of the strains to a set of five rice differentials indicated an association between pathotype and country of origin. Regional differentiation of virulence was most striking for strains differentiated by cultivars with the *xa-5* resistance gene. Most strains from south Asia were compatible to *xa-5*, while most strains from other countries were incompatible.

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PHENOTYPIC CHARACTERIZATION OF *XANTHOMONAS CAMPESTRIS* PV. *VESICATORIA* STRAINS FROM THE CARIBBEAN AND CENTRAL AMERICA. H. Bouzar, J. B. Jones, R. E. Stall, G. C. Somodi, R. O. Kelly, and N. Daouzli. University of Florida, 5007 60th Street East, Bradenton 34203.

Strains of *X. c.* pv. *vesicatoria* (Xcv) were isolated from tomato and pepper plants grown in production fields of the Barbados, Costa Rica, Guadeloupe, Guatemala, Nicaragua, Puerto Rico, and the U.S. Virgin Islands. Most (87%) of the 118 strains were typed to Xcv group A (i.e., 32 kDa α protein band, A serovars, *cis*-aconitate positive, nonamylolytic and nonpectolytic). All of the 103 A strains were pathogenic on pepper and 82 were also pathogenic on tomato. Four strains (1 from Costa Rica and 3 from Guatemala) were typed to Xcv group B (i.e., 27 kDa β protein band, B serovars, *cis*-aconitate negative, amylolytic and pectolytic); these strains were pathogenic on tomato but not on pepper. Eleven strains could not be assigned to either Xcv group. Four of these strains, found throughout a pepper field in the Barbados, were similar to group A strains in their antigenic make-up and their ability to utilize *cis*-aconitate, but were amylolytic and pectolytic like group B strains. The reverse was true for 7 of the 12 strains recovered from tomato fields in Costa Rica.

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SPECIFICITY OF BACTERIOPHAGES OF *PSEUDOMONAS SOLANACEARUM*. H. Abdullah. Dept. of Plant Protection, Universiti Pertanian Malaysia, UPM 43400, Serdang, Selangor, Malaysia.

Bacteriophages (phages) of *P. solanacearum* were isolated from bacterial wilt infected hosts, viz. *Solanum melongena* L., *Capsicum annuum* L., *C. grossum* L., *Lycopersicon esculentum* Mill., *Solanum tuberosum* L. and *Phaseolus vulgaris* L., from various localities, using the enrichment technique. Host range studies of these phages showed that all were specific to strains of *P. solanacearum*. Phages isolated from tomato infected with biovar 2 strains of *P. solanacearum* seemed to be specific to biovar 2 strains regardless of host. Phages isolated from potatoes infected with biovar 3 strains of *P. solanacearum* were specific to biovar 3 strains from potatoes. Thus, these phages could be used to readily differentiate biovars of potato strains of *P. solanacearum*. All phages could not infect strains of other *Pseudomonas* spp. tested i.e. *P. aeruginosa, P. fluorescens, P. putida* and several unidentified species of *Pseudomonas* commonly isolated from soil.

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DELINEATION OF A GENOMIC CLUSTER CONTAINING MYCOPLASMALIKE ORGANISMS (MLOs) ASSOCIATED WITH SWEET POTATO WITCHES' BROOM, RED BIRD CACTUS WITCHES' BROOM, AND PEANUT WITCHES' BROOM DISEASES. Robert E. Davis, Ellen L. Dally, James P. Prince, and Ing-Ming Lee. Molecular Plant Pathology Laboratory, Agricultural Research Service, USDA, Beltsville, MD 20705

MLO infections were detected in naturally diseased sweet potato and peanut and in experimentally inoculated periwinkle (*Catharanthus roseus*) through use of dot hybridizations with probes consisting of biotinylated cloned DNA fragments from sweet potato witches' broom (SPWB) MLO. All of 11 probes hybridized with DNA from SPWB MLO and red bird cactus (*Pedilanthus tithymaloides*) witches' broom (RBCWB) MLO; ten hybridized with DNA from peanut witches' broom (PnWB) MLO; and none hybridized with DNA from any of ten other MLOs from North America, Europe, and Asia. Based on these data, SPWB, RBCWB, and PnWB MLOs are recognized as closely related strains representing a single new genomic cluster. Relatedness among these MLOs was confirmed by RFLP analysis of total chromosomal DNA and of PCR-amplified 16S rDNA. The findings indicate that sweet potato, red bird cactus, and peanut may serve as sources of inoculum for cross infection.

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PRODUCTION OF CONIDIA FROM OVERWINTERED SCAB LESIONS ON PECAN SHOOTS. K. L. Reynolds, Department of Plant Pathology, University of Georgia, Athens, GA 30602-7274

Overwintered lesions on shoots of pecan (*Carya illinoensis*) are a primary source of initial inoculum for seasonal epidemics of scab caused by *Cladosporium caryigenum*. The distribution of lesions within the tree canopy and the duration of spore production were investigated in 1993. Shoot length and lesions per shoot were recorded for 1-yr-old shoots collected from the bottom, middle, and top thirds of pecan (cv Desirable) tree canopies prior to budbreak. Sporulation per lesion was determined on 1-yr-old shoots collected weekly from mid-May to mid-August. All new growth was removed, and the 1-yr-old wood was washed in deionized water amended with 0.1% Tween 20, cut into 2-cm sections, and lesions counted. Each section had 10-50 lesions, each 0.5-1.5 mm in diameter. Fifteen sections were placed in a petri dish with moist filter paper and incubated at 25 C in continuous fluorescent light. After 3 days each section was washed in 2 ml deionized water with 0.1% Tween 20 and the conidia in the wash water counted using a hemacytometer. Shoots in the top third of the tree had significantly higher lesion densities than shoots in the lower two thirds of the canopy. Sporulation per lesion was highest in May and June and decreased greatly during July, although some conidia were produced as late as August.

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FIELD TESTING OF A POWDERY MILDEW DISEASE FORECAST MODEL ON GRAPES IN CALIFORNIA. C. S. Thomas[1], W. D. Gubler[1], and George Leavitt[2]. [1]Department of Plant Pathology, University of California, Davis, CA 95616, and [2]Cooperative Extension, University of California, Madera, CA 93637.

Four vineyards were equipped with Lufft disease forecasters (model HP 100, Abbeon Cal, Inc., Santa Barbara, CA) in four different grape climate types in California: South Coast, Central Valley, Carneros, Central Napa Valley. Disease increase caused by secondary infection coincided with moderate to severe forecasts at each site except for an epidemic caused by bud perennation at one site. Grape powdery mildew ascospore infection was predicted effectively by the Mills table model for conidial apple scab, when the forecast was for severe pressure. The logarithmic increase in the epidemic occurred at each site when temperatures were first between 21 and 30 C for at least 6 hours each day for at least three consecutive days.

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COUPLING AN EXTENDED HEALTHY-AREA-ABSORPTION MODEL AND A PARAMETER ESTIMATION ROUTINE TO ADJUST YIELD EXPECTATIONS FOR MULTIPLE PEST DAMAGE ON RICE. H. O. Pinnschmidt[1], B. Hau[2], and P. S. Teng[1]. [1]Entomology and Plant Pathology Division, International Rice Research Institute, P. O. Box 933, 1099 Manila, Philippines and [2]Institut fuer Pflanzenkrankheiten und Pflanzenschutz, Universitaet Hannover, Herrenhaeuser Str. 2, 30419 Hannover, Germany.

An empirical summary model was constructed based on the healthy-area-absorption concept to estimate rice yields as affected by blast, sheath blight, other diseases, and rat- and insect pest damage. The model distinguishes between damage on vegetative plant parts and generative plant parts or whole tillers. It contains interaction terms that account for effects of crop age on crop response to pest attack. The model can be parameterized for a one- or two-layered canopy. A routine to estimate least uncertain parameter values was coupled to the model. Model parameterization was done using a multiple pest data set obtained in three irrigated lowland experiments at IRRI, Philippines. Yields estimated by the model came very close to observed yields. Pest damage on generative plant parts and whole tillers, as well as sheath blight severity displayed major effects on yields. Validation of the model indicated its potential for estimating multiple pest-affected lowland rice yields and for serving as an alternative to other methods. The model can be parameterized for other grain crops, using its parameter estimation routine.

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DEVELOPMENT AND SENSITIVITY ANALYSIS OF A RANDOM WALK MARKOVIAN MODEL FOR AIR-BORNE SPORANGIAL DISPERSAL OF PHYTOPHTHORA INFESTANS. Kiyoshi Ishiguro and W.E. Fry. Department of Plant Pathology, Cornell University, Ithaca, NY 14853.

A mechanistic and quantitative understanding of the conditions affecting release, dispersal and deposition of air-borne sporangia of *Phytophthora infestans* is essential for understanding regional epidemics of potato late blight. Vertical sporangial flux profiles 3 m downwind from a line source of the inoculum predicted by a random walk Markovian model (D.E. Aylor and F.J. Ferrandino, 1989) generally fit the profile measured in a potato field. This model predicts that both greater wind velocity and higher inoculum position (distance above ground) increase the escape of sporangia from the canopy. As a validation step, sensitivity analyses of the model were performed for several parameters. Differences in canopy structure (the vertical distribution and inclination of leaves) and in mechanisms by which sporangia are captured by plant tissues had negligible effects on the behavior of the model. The settling speed of a sporangium in still air and the Lagrangian time scale used affected the behavior of the model. All the factors had little interaction with wind velocity and inoculum height factors. More detailed data of sporangial production and release are necessary for quantitative validation of the model.

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INFECTION EFFICIENCY OF CONIDIA AND CONIDIOPHORES OF *PYRENOPHORA TRITICI-REPENTIS*. C. K. Evans, R. M. Hunger, and W. C. Siegerist, Department of Plant Pathology, Oklahoma State University, Stillwater, OK 74078-9977.

Leaves of the tan spot resistant wheat variety 'Red Chief' and the susceptible variety 'TAM-105' were quantitatively inoculated with conidia (10,000 ml^{-1}) and conidiophores (15,000 ml^{-1}) of three isolates of *Pyrenophora tritici-repentis* (PTR). Visible lesions per cm^2 leaf area was used to determine the infection efficiency (IE) of each propagule. Conidia were significantly ($P=0.001$) more efficient (11.8 lesions cm^{-2}) than conidiophores (0.4 lesions cm^{-2}) for inciting infection. Additional studies with each propagule at varying concentrations were conducted on the two wheat varieties. Regression analyses revealed that the IE of conidia was significantly higher on 'TAM-105' compared to the resistant variety 'Red Chief'. Estimates of IE for conidiophores were not significantly different from zero for 'TAM-105' and the IE for conidia indicated that one PTR isolate was significantly more virulent than the others. These results indicate that conidiophores have a negligible effect on tan spot infection, but do demonstrate the importance of quantifying inoculum of PTR and using a virulent isolate.

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RELEASE AND DISPERSAL OF ASCOSPORES OF ANISOGRAMMA ANOMALA IN EUROPEAN HAZELNUT PLANTINGS. J. N. Pinkerton[1], K. B. Johnson[2], and D. E. Aylor[3]. [1]USDA ARS Horticultural Crops Research Laboratory, Corvallis, OR 97330, [2]Department of Botany and Plant Pathology, Oregon State University, 97331-2902, [3]The Connecticut Agric. Experiment Station, New Haven, CT 06504.

For over 30 years, eastern filbert blight has spread slowly southward from Washington into Oregon. To understand processes of ascospore and disease spread, we conducted studies in diseased orchards from 1988-93. Spore release from perithecia occurred with rains from October to June. In 4 yr., >82% spore release occurred before budbreak in mid-March, when hazelnut becomes susceptible. In 1990, however, > 58% of spores were released in late April after an extended warm, dry period. In the spring of 1993, Burkard traps were placed in 2 orchards to monitor spore release. Release was correlated with branch wetness during rains, but not with RH, temperature, light, or wind. From 1988-92, 2-yr-old hazelnut trees were placed every 10 m in rows perpendicular to orchards. Disease spread to trees placed up to 150 m north, the direction of prevailing winds. Disease incidence did not differ among these trees. Few trees south of the orchard were infected. In 1993, spore traps were placed at 3 elevations on towers 0 to 60 m north of an orchard. During and immediately after rain events, airborne spore concentrations were similar at all sampled distances and elevations. Data suggest that spores are actively discharged, can disperse long distances, and weather patterns retard disease spread southward.

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TEMPORAL ANALYSIS OF ASCOSPORE RELEASE BY *GIBBERELLA ZEAE* IN ARTIFICIALLY-INOCULATED FIELD PLOTS OF WHEAT. T. Paulitz[1], and W. L. Seaman[2], [1]McGill University, Ste. Anne de Bellevue, Quebec, H9X 3V9, and [2]Agriculture Canada, Ottawa, Ontario, CANADA

A 7-day Burkard spore trap was used to collect ascospores from above the canopy of a 10 X 10-m plot of spring wheat (cv. Max) artificially infested with perithecial inoculum of *G. zeae*. Corn kernels colonized by *Fusarium graminearum* were spread in the center 4 X 4-m of the plots in the last week of May, 1992 and 1993. Perithecia formed in mid-June and ascospores were released in the first week of July. Temperature, relative humidity, leaf wetness and rainfall were measured in the plots on an hourly basis. Ascospore release was nocturnal and began around 18:00, reaching a peak between 20:00 and 24:00. Smaller peaks of release were recorded from 24:00 to 4:00. Ascospore counts ranged from 171-5143 spores/0.6 m^3/hr during release events. The beginning of ascospore release was not correlated with rainfall events, but coincided with a drop in air

temperature and a rise in relative humidity (RH) to >80% Rainfall during ascospore release reduced ascospore counts. Ascospore release was also reduced following days with rainfall or cool days with high RH and constant leaf wetness. Ascospore release by *G. zeae* may be triggered by diurnal drying and nocturnal rehydration.

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EFFECT OF CONTINUOUS AND INTERRUPTED LEAF WETNESS ON THE INFECTION PROCESS OF *PYRENOPHORA TRITICI-REPENTIS* IN CHLOROTIC AND NECROTIC WHEAT CULTIVARS. N. D. Sissons and L. Lamari, Department of Plant Science, University of Manitoba, Winnipeg, Manitoba, Canada. R3T 2N2.

Pyrenophora tritici-repentis, causal organism of tan spot of wheat, causes necrotic (nec*) or chlorotic (chl*) tan spot lesions on susceptible wheat cultivars. The effect of continuous and interrupted leaf wetness periods on the establishment of infection of *P. tritici-repentis* in chlorotic and necrotic wheat cultivars was studied. Glenlea, a necrotic wheat cultivar, and 6B365, a chlorotic wheat line were inoculated with isolate ASC1 (nec*chl*). In the continuous leaf wetness experiment, plants were incubated for 0, 2, 4, 6, 8, 12, 24, 36, 48 and 72 hours. In a second experiment, plants were incubated for 0, 2, 4, 6, 8, 10 or 12 hours of continuous wetness, dried for 6 hours, and then returned to the incubation chamber to complete the balance of 24 hours of leaf wetness. Disease severity increased when leaf wetness period increased from 0 to 12 h in both 6B365 and Glenlea. Cytological observations indicated that the infection process was similar for both cultivars. Disease severity decreased between 0 and 6 h of incubation before drying and increased between 6 and 12 h of incubation before drying. Cytological observations indicated that interrupting the infection process during appressorial formation, but prior to epidermal cell penetration, caused a significant reduction in disease severity. Under field conditions, short leaf wetness periods that are interrupted may result in lower tan spot severity.

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INFLUENCE OF TEMPERATURE AND WETNESS DURATION ON INFECTION OF CHERRY *(PRUNUS AVIUM* L.) FOLIAGE BY THE SHOTHOLE PATHOGEN, *WILSONOMYCES CARPOPHILUS*. G. G. Grove, R. J. Boal, and N. B. Roberts, Washington State University Tree Fruit Research and Extension Center, 1100 N. Western Avenue, Wenatchee, WA 98801.

The effects of temperature and wetness on infection of sweet cherry by *Wilsonomyces carpophilus* (Lev.) Adaskaveg, Ogawa & Butler were determined under controlled conditions. Cherry seedlings were inoculated with a conidial suspension of *W. carpophilus* and subjected to wetness periods of 0-24 hr at temperatures of 5-30 C. After the preassigned wetness periods, inoculated seedlings were allowed to dry and incubated for several days after which disease severity (expressed as the number of lesions per leaf) was determined by visual inspection. Infection did not occur at 5 or 30 C and required at least 12 hr wetness at 10-25 C. The optimum temperature after 12 hr was 25 C; at that wetness duration disease severity was 0, 0, 4.7, 9.7, 18, and 0 lesions/leaf at 5, 0, 15, 20, 25, and 30 C respectively. Wetness durations of 18-24 hr resulted in a downward shift of the temperature optimum to 15 C; after 24 hr of wetness disease severity ranged from 0 lesions/leaf at 5 and 30 C to 53 lesions per leaf at 15 C. A multiple regression equation using temperature and wetness duration as independent variables adequately described infection of cherry foliage.

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EFFECTS OF TEMPERATURE ON SPORULATION OF *COLLETOTRICHUM* SPECIES INFECTING IMMATURE STRAWBERRY FRUIT. W.T. King Jr., L.V. Madden, and M.A. Ellis. Department of Plant Pathology, OARDC, The Ohio State University, Wooster OH 44691.

Strawberry anthracnose is caused by *Colletotrichum acutatum, C. gloeosporioides*, and *C. fragariae*. Effects of temperature on sporulation by each species was determined by inoculating immature fruit with a conidial suspension and incubating them at temperatures from 5-35 C. Sporulation was assessed periodically after incubation times of 2-30 d, depending on the temperature. Lesion appearance and initial sporulation depended on temperature, ranging from 2-3 d at 25 C to 6-17 d at 5 C. *C. acutatum* formed lesions and sporulated before the others at the lower temperatures. Maximum sporulation occurred at 25-30 C with a 7 d incubation. Mean maximum sporulation per fruit at 25 C with 5 d of incubation was 3.7×10^7 conidia/fruit for the *C. acutatum* isolates, 7.4×10^7 for *C. fragariae*, and 4.5×10^7 for *C. gloeosporioides*. Regression analysis is being used to quantify the effects of temperature and incubation time on sporulation.

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EFFECT OF TEMPERATURE ON STRAWBERRY FRUIT INFECTION BY GEOGRAPHIC ISOLATES OF THREE *COLLETOTRICHUM* SPECIES. L.L. Wilson, L.V. Madden, M.A. Ellis, Department of Plant Pathology, OARDC, Ohio State University, Wooster, OH 44691.

In order to help determine why *Colletotrichum acutatum (Ca)* is the predominant species causing anthracnose fruit rot of strawberry, fruit infection by isolates of *Ca, C. gloeosporioides (Cg)*, and *C. fragariae (Cf)* from different regions of the U.S. were studied as a function of temperature. Attached, immature strawberry fruits were inoculated with a conidial suspension of each fungus and incubated under continual wetness for 16 h at constant temperatures of 18, 25, and 30 C. Incidence of fruit infection was recorded after 8 d. For all isolates, the lowest disease incidence was at 18 C, ranging from 4 to 80%. The incidence of infected fruit for all isolates was greatest at 25 C; *Ca* caused more fruit infection than *Cg* or *Cf* at 25 C. Incidence ranged from 23 to 100% for all isolates tested. Between 25 and 30 C, disease incidence for each isolate either increased or decreased slightly. Results indicate that *Ca* is more aggressive than either *Cg* or *Cf* at each temperature studied.

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ENVIRONMENTAL FACTORS AFFECTING CARPOGENIC GERMINATION OF SCLEROTIA OF *SCLEROTINIA SCLEROTIORUM* FROM FLORIDA. N.W. Vandervort and T.A. Kucharek. Plant Pathology Dept., Univ. of Florida, Gainesville, FL 32611.

In repeated growth chamber tests, with multiple isolates of *S. sclerotiorum*, the effects of environmental factors on carpogenic germination were measured using sclerotia placed in tap water. Significant interactions ($P = >0.01$) between isolates and treatments occurred in all tests. Alternate wetting and drying of sclerotia prior to incubation reduced germination by 53.7% compared to those kept dry. Burial of sclerotia in moist sand (20% v/w) reduced the production of apothecia by 86.2% compared to those kept on the surface, although all buried sclerotia produced stipes. The maximal depth of burial at which apothecia were produced was 2.5 cm. Carpogenic germination occurred from 5-25 C with the optimal temperature being 15 C for seven isolates. At 5 C the level of germination between isolates ranged from <10 to >90%.

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ASSOCIATION OF *Rhizoctonia* spp. WITH MORTALITY OF BIRDSFOOT TREFOIL SEEDLINGS IN OPEN AND CLOSED PLANT CANOPIES. K. M. Emery and J.T. English, Department of Plant Pathology, University of Missouri, Columbia, MO, 65211.

Throughout the 1993 growing season, the survival of seedlings of birdsfoot trefoil was evaluated in a three-year-old stand. Seeds were sown monthly in small plots which either had debris and nearby adult plants removed (open canopy), or in plots in which debris remained and surrounding plants grew over the seedlings (closed canopy). Plots were evaluated weekly to determine seedling emergence and survival. Although soil matric potential remained above -80 cbar, seedling emergence declined from 60% in both open and closed canopies in May to 17% and 27% in open and closed canopies, respectively, in September. Seedling survival declined from 55% in May to 11% in September in open canopies, and did not exceed 15% at any time in the closed canopies. *Rhizoctonia* spp. were isolated from 60% of diseased seedlings. Characterization of *Rhizoctonia* spp. and evaluation of their potential impact on seedling recruitment and stand persistence are continuing.

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DEVELOPMENT OF ANTHRACNOSE IN MIXTURES OF DRY BEAN CULTIVARS. N. Ntahimpera, H. R. Dillard, A. C. Cobb, and R. C. Seem, Cornell University, New York State Agricultural Experiment Station, Dept. of Plant Pathology. Geneva, NY 14456.

Field experiments were conducted in 1992 and 1993 to analyze the effect of cultivar mixtures on bean anthracnose caused by *Colletotrichum lindemuthianum*. Three light red kidney cultivars, Ruddy (R) race beta resistant, Redkloud (S_1) and Sacramento (S_2) beta susceptible, were mixed in different proportions to achieve the following treatments: 100R; 50R + $25S_1$ + $25S_2$; 25R + $37.5S_1$ + $37.5S_2$; 10R + $45S_1$ + $45S_2$; $50S_1$ + $50S_2$; $100S_1$; and $100S_2$. In both years, the treatments were planted in May and inoculated by introducing a spreader plant in the center of each treatment. Final disease incidence and severity were significantly reduced in the mixtures containing 25 and 50% of the resistant cultivar, while a mixture with 10%R was ineffective. Disease development was affected by rainfall amount in both seasons. Using untransformed data, incidence-severity relationships on pods and leaves were linear for all treatments but variation occurred in slope and intercept values. Disease progress for both incidence and severity were fit to four models (exponential, logistic, monomolecular, and Gompertz). The Gompertz model (with R^2 values between 0.61 and 0.96 percent) best described the disease progress in all treatments .

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FIRST REPORT OF BEAN COMMON MOSAIC VIRUS PATHOGROUP 7 ISOLATE (US-10) IN U.S. COMMERCIAL DRY BEANS. M.J. Silbernagel and G.I. Mink, USDA-ARS, WSU-IAREC, RR 2 Box 2953A, Prosser, WA 99350-9687.

Pathogroup (PG) 7 strains of bean common mosaic virus (BCMV) have been found in Europe, Latin America, and Africa, but not

in North America. PG-7 isolates overcome resistance based on recessive gene $bc2^2$, which is effective against all other BCMV strains. Seedborne BCMV isolates, which induce systemic mosaic mottle symptoms on $bc2^2$ cultivars of dry beans (*Phaseolus vulgaris* L.), were found in the seed production areas of the northwestern USA. In Washington state, the new strain (US-10) was found primarily on small red cultivars in mixtures with the Western strain (US-4), and in Idaho mixed with either the Western and/or NL-8(Id) strains. US-10 was compared to other PG-7 strains (US-6 and NL-4) biologically and serologically. It produced milder symptoms than US-6 or NL-4 on $bc2^2$ genotypes, but was very similar serologically. The three PG-7 strains can also be distinguished by western blot analysis and by coat protein peptide profiles.

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Prevalence and Partial Characterization of Citrus Exocortis and Other Citrus Viroids in Texas. *Mani Skaria*, Hongqin Miao and Nora Solis-Gracia
Texas A&M University-Kingsville Citrus Center, Weslaco, TX 78599

Sour orange, the predominant citrus rootstock in Texas may need to be replaced as part of a strategy for tristeza disease control. The presence of citrus exocortis viroid (CEV) and other citrus viroids (CV) in the scion could become a limiting factor for production when certain rootstocks tolerant to tristeza are used. Based on symptoms on 'Etrog' citron 861-S-1, the major commercial citrus cultivars showed the presence of CEV, CV II and CV III or CV IV. Viroid incidence in field surveys varied from 0-50% for CEV and 0-30% for CV II and CV III or CV IV. Twelve viroid isolates from Texas were compared with a known isolate based on reactions of tomato seedlings inoculated with the RNA extracts obtained from infected 'Etrog' citron. All but one of the Texas isolates produced symptoms on tomatoes. Three isolates and the standard viroid induced reactions such as stunting, epinasty, leaf rugosity and vein necrosis. Eight isolates did not produce vein necrosis. The efficiency of the RNA extract and crude sap as inoculum were compared. The RNA extract and crude sap induced symptoms in 100% and 27-36% tomato seedlings, respectively. The incubation period for symptom expression in tomato seedlings was 13 days with the RNA extract and 20 days with the crude sap.

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EFFECT OF DIFFERENT CITRUS TRISTEZA VIRUS (CTV) ISOLATES ON PERFORMANCE OF MARSH GRAPEFRUIT AT TWO ENVIRONMENTALLY DIFFERENT LOCATIONS. L.J. Marais[1], R.F. Lee[2], J.V. Da Graca[3] and J.M. Kotze[4]. [1]Outspan International, Nelspruit, South Africa, [2]Univ. of FL, Lake Alfred, FL 33850, [3]Univ. of Natal, Pietermaritzburg, South Africa & [4]Univ. of Pretoria, Pretoria, South Africa.

Marsh grapefruit seedlings were inoculated with 10 mild to severe CTV isolates and planted at Eastern Transvaal and Natal sites (SA). Tree performance and severity of CTV induced stem pitting were evaluated after 3 yr. Two isolates were mild and three were severe at both locations, but severity of the other five isolates depended on location. Stem pitting expression was stronger in Natal where temperatures are lower. The temperature effects on expression of symptoms were confirmed in a glasshouse study using one mild and one severe isolate.

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DETECTION OF GEMINIVIRUSES IN HAWAII. J. S. Hu, Z. C. Wu, S. Lius, R. Hamasaki, and K. Barry, Department of Plant Pathology, University of Hawaii, Honolulu, HI, 96822.

Vegetable and ornamental plant samples were tested for geminiviruses using indirect ELISA with a monoclonal antibody against a shared epitope of whitefly-transmitted geminiviruses (MAb 3F7, E. Hiebert). Samples were collected from the islands of Hawaii, Maui, and Oahu from symptomatic plants with whitefly (*Bemisia tabaci*) infestations. Five plant species, lantern 'ilima, (*Abutilon hybridum*), abutilon (*Abutilon striatum*), kabocha squash, zucchini, and papaya squash were positive in ELISA tests for geminiviruses. The lantern 'ilima, abutilon, and zucchini samples also tested positive with polymerase chain reaction (PCR) using degenerate primers (PAL1v1978 and PAR1c715, D. P. Maxwell). A ~ 1.5-kb fragment was produced and hybridized in Southern blot analysis with a DNA A-component probe of bean golden mosaic geminivirus. Sequence analysis results show that lantern 'ilima virus is closely related to published abutilon mosaic virus. Based on results from serology and molecular biology, we conclude that geminiviruses are present in Hawaii.

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TOMATO YELLOW LEAF CURL-LIKE GEMINIVIRUS DETECTED IN DOMINICAN REPUBLIC. J. E. Polston[1], D. Bois[1], C.-A. Serra[2] and S. Concepción[2], [1]Gulf Coast Res. and Educ. Center, Univ. of Florida, Bradenton, 34203, [2]ISA., Apartado 166, Santiago, Dominican Republic.

A new disease of tomato (*Lycopersicon esculentum* Mill.) was first observed in the northwestern region of the Dominican Republic in the fall of 1992. Symptoms observed in affected plants were severe stunting, flower abscission, and in leaves, extreme size reduction, curling, cupping, and marginal chlorosis. Disease incidence was high, and yields were greatly reduced. Tomato yellow leaf curl virus (TYLCV) DNA and tomato mottle virus A component DNA hybridized strongly and weakly, respectively, with Dominican Republic tomato samples in spot hybridization assays. Amplification of nucleic acid extracts of samples using the degenerate primers PAL1v1978 and PAR1c496 ("A" component specific) (Rojas, *et al.*, 1993, Plant Dis.77:340) resulted in a *ca.*1400 bp DNA fragment. Restriction analysis of this fragment gave a pattern not predicted from published TYLCV sequences and gave no evidence of multiple sequences. Whitefly transmission from tomato to tomato using the whitefly, *Bemisia argentifolii* Bellows & Perring, was confirmed in greenhouse studies. These data indicate that the disease is caused by a TYLCV-like geminivirus.

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OCCURRENCE OF THE EASTERN MEDITERRANEAN STRAIN OF TOMATO YELLOW LEAF CURL GEMINIVIRUS IN THE DOMINICAN REPUBLIC. M. K. Nakhla[1], D. P. Maxwell[1], R. T. Martinez[2], M. G. Carvalho[3], and R. L. Gilbertson[3]. [1]Univ. of Wisconsin, Madison, WI 53706, [2]Secretaria de Estado de Agricultura, San Cristóbal, the Dominican Republic, [3]Univ. of California, Davis, CA 95616.

A virus disease epidemic in 1993-1994 caused extensive losses in tomatoes in the Dominican Republic, and incidence of virus symptoms was nearly 100% in many fields. Plants were severely stunted, and leaves were curled, small and often had yellow margins--symptoms similar to those caused by tomato yellow leaf curl geminivirus (TYLCV). Squash blots of symptomatic tomato leaves hybridized weakly with a general DNA probe for Western Hemisphere whitefly (*Bemisia tabaci*)-transmitted geminiviruses (Plant Dis. 75:336-342) and strongly with a TYLCV-specific DNA probe (Phytopath. Med. 32:163-173). A 2.8-kb viral fragment was amplified from symptomatic tomato with PCR primers specific for the monopartite genome of TYLCV from Israel. The partial nucleotide sequence of the intergenic region of a cloned PCR-fragment (pTGV-DR1) was 97%, 61%, and 63% identical to homologous regions of TYLCV from Israel, TYLCV from Sardinia, and tomato mottle virus from Florida. These data indicate that tomatoes in the Dominican Republic are infected with an Eastern Mediterranean strain of TYLCV. This is the first report of a monopartite, whitefly-transmitted geminivirus in the Western Hemisphere.

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A NEW CLOSTEROVIRUS OF TOMATO IN SOUTHERN CALIFORNIA TRANSMITTED BY THE GREENHOUSE WHITEFLY (*TRIALEURODES VAPORARIORUM*). J.E. Duffus, H.Y. Liu, and G.C. Wisler, USDA-ARS, 1636 E. Alisal St., Salinas, CA 93905.

A previously undescribed virus disease of tomato was found in the Orange County area of southern California. Affected tomato plants exhibited interveinal yellowing, necrosis and severe yield losses. The disease affected virtually 100% of the crop in the Irvine hills and valley region. The outbreak was associated with the occurrence of high populations of the greenhouse whitefly, *Trialeurodes vaporariorum* (Westwood). Leaf dips showed flexuous filamentous particles of variable length similar to closteroviruses. The virus was transmitted by *T. vaporariorum* but not by either the A or B biotypes of *Bemisia tabaci* (sweetpotato whitefly). Further characterization by protein and RNA analyses and insect transmission studies will be necessary to determine

the relationship of the new tomato virus to other whitefly-transmitted closteroviruses.

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CHARACTERIZATION OF THE DISEASE CYCLE OF WHEAT SPINDLE STREAK MOSAIC IN WINTER WHEAT IN NEW YORK. J.E. Carroll[1], G.C. Bergstrom[1], and S.M. Gray[2], Dept. of Plant Pathology[1] and USDA-ARS[2], Cornell University, Ithaca, NY 14853.

Infection of winter wheat by wheat spindle streak mosaic virus (WSSMV), transmitted by *Polymyxa graminis*, is thought to occur in autumn, even though symptom expression occurs in spring. To elucidate the disease cycle, roots and leaves of two susceptible cultivars were assayed for viral coat protein by ELISA at biweekly intervals from autumn to spring. In the 1992-93 growing season, incidence of plants with WSSMV in roots remained low (4 to 18%) in autumn and winter, reaching a maximum disease incidence of 82% in May. By contrast, in the 1993-94 growing season, incidence of plants with WSSMV in roots increased rapidly during autumn, reaching 90% in December. By January of both years, as determined by ELISA, half of the plants with virus-infected roots also contained virus in leaves, but remained asymptomatic. These data indicate that maximum incidence of WSSMV infection of the autumn-sown crop can be reached either in autumn or the following spring. A factor that may be involved in promoting infection of roots in autumn is frost, which was early and recurrent in 1993, but not in 1992.

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IMPACT OF INSECTICIDES ON THE TEMPORAL AND SPATIAL DYNAMICS OF FLOWER-INHABITING THRIPS AND TOMATO SPOTTED WILT VIRUS. D.O. Chellemi, S.M. Olson, G.A. VandeKerckhove, and J.E. Funderburk. North Florida Research and Education Center, Route 3 Box 4370, Quincy, Florida, 32351, USA.

Field experiments were conducted in 1992 and 1993 at Quincy, Florida, to assess the impact of insecticide applications on flower-inhabiting thrips species and tomato spotted wilt virus (TSWV) in a tomato crop. Four replications of an unsprayed control and an insecticide treatment consisting of methamidophos and fenpropathrin were arranged in randomized complete block with each replication consisting of 600 plants. In 1992, the final incidence of TSWV was 7% in treated plots and 17% in unsprayed plots. Application of insecticides caused the species assemblage of flower-inhabiting thrips to shift in favor of *Frankliniella occidentalis*, a vector of TSWV. Analysis of the spatial pattern of TSWV and *F. occidentalis* using Morisita's index of dispersion indicated that TSWV was more aggregated in sprayed plots than in unsprayed plots while *F. occidentalis* was random in sprayed plots but aggregated in unsprayed plots.

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MOVEMENT OF BNYVV-INFESTED *POLYMYXA BETAE* FROM AN INOCULATED POINT SOURCE. R. M Harveson and C. M. Rush, Texas Agric. Exp. Stn., P.O. Drawer 10, Bushland, TX 79012.

A 3-yr study was initiated in 1992 to determine how BNYVV spreads from a known point source of inoculum as influenced by irrigation and tillage. Each year the test consisted of four 9 x 30 m plots, each containing twelve 76-cm beds. The first 3 m of each of the two outside rows of each plot were planted with HH39 sugar beet seed coated with powdered sugar beet roots infested with viruliferous *P. betae* cystosori. These areas constituted the point sources. The remainder of the test was planted with untreated seed and plots were irrigated May 14 and 17 for 1992 and 1993 studies, respectively. Half the study was watered every two weeks and the other half every four weeks. During the season, plant samples were collected at various intervals, away from the point source, and assayed by ELISA for BNYVV incidence. At the end of each season, soil samples were also collected and assayed. Plots were then mechanically harvested. After bed preparation for the 1993 season, soil samples were collected to determine virus movement by soil tillage. The plant samples showed virtually no virus movement outside inoculated areas. After the 1992 season, only five soil samples (2%) were positive. Soil samples collected for tillage effect before the 1993 season contained 29 positive samples (12%). In 1993, no plant samples proved to be infected due to irrigation.

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STUDIES ON THE ROLE OF THE ORNAMENTAL PLANT *GAZANIA* SP. IN THE EPIDEMIOLOGY OF LETTUCE MOSAIC IN THE SALINAS VALLEY. F.M. Zerbini[1], S.T. Koike[2] and R.L. Gilbertson[1]. [1]Dept. of Plant Pathology, UC Davis, Davis, CA, 95616 and [2]UC Cooperative Extension, Salinas, CA, 93901

Gazania sp. is a vegetatively propagated ornamental that is an alternate host of lettuce mosaic potyvirus (LMV), and is widely planted in the Salinas Valley. The presence of LMV in this plant was demonstrated by ELISA, aphid transmission, hybridization with a LMV coat protein (CP) probe, and cloning and sequencing of the LMV CP gene. We investigated its role in the recent mosaic outbreaks in the Salinas Valley. During 1992-1994, several surveys of gazanias in the Salinas Valley and in nurseries were carried out. LMV-infected *Gazania* was consistently detected close to the areas where LMV outbreaks ocurred and in most nurseries. Field experiments revealed that LMV could be aphid transmitted from infected *Gazania* to lettuce. The CP genes of LMV isolates from adjacent *Gazania* and lettuce plants were cloned, sequenced and compared.

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EFFECTS OF BLACK BEAN APHID INJURY AND BEET YELLOWS VIRUS ON SUGAR BEET YIELD. P. A. Mauk and L. D. Godfrey, University of California Coop. Extension, Sacramento 95827 and Department of Entomology, Davis 95616

Beet yellows virus (BYV), transmitted by *Aphis fabae* (black bean aphid) and *Myzus persicae* (green peach aphid) is a serious limitation to sugar beet production in the Lower Sacramento Valley/Upper San Joaquin Valley in California. Yield losses from BYV can be as high as 75%. In the past, the primary vector has been *M. persicae*. More recently, however, we have correlated increases in populations of *A. fabae* with this disease. To establish the effects of injury from *A. fabae*, BYV, or *A. fabae* + BYV the following treatments were established in field plots: 1) nonviruliferous *A. fabae* for full season, 2) BYV only, 3) *A. fabae* + BYV, and 4) check (aphids and virus excluded). Treatments were initiated at 4 wk after emergence in 1992 and at 3, 6, or 9 wk after emergence in 1993. Plants were infested with aphids beneath floating row cover material to maintain the integrity of the treatments. In the 1992 trial, sugar beet yield was 18.3 tons/A in the check where aphids and virus were excluded. Yield for the *A. fabae* treatment was reduced 28.4%; whereas, yield was reduced by 68.3% in the *A. fabae* + BYV treatment. In the 1993 trial, yield in the 3 week inoculation time was reduced by about 50% for all aphid or virus treatments as compared to the check. Inoculations at 6 or 9 wk postemergence had only a slight reduction in yield as compared with the check. Percent sugar was not significantly different in any of the treatments and averaged 13.1% in 1992 and 10.9% in 1993.

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ISOLATES OF *STAGONOSPORA NODORUM* FROM ALTERNATIVE HOSTS AFTER PASSAGE THROUGH WHEAT. J.M. Krupinsky, USDA, Agriculture Research Service, Northern Great Plains Research Lab, PO Box 459, Mandan, ND 58554.

Fourteen low aggressive isolates of *Stagonospora nodorum* (= *Septoria nodorum*) from smooth bromegrass, barley, western wheatgrass, intermediate wheatgrass, Altai wild-rye, basin wild-rye, and *A. repens* X *A. desertorum* were passed through wheat five times. When comparing the reisolated cultures with original isolates in detached leaf inoculations, lesion length which was used as a measure of aggressiveness, was not increased. Nonsignificant cultivar X isolate interactions indicated a lack of specificity among the original isolates and cultures reisolated from wheat. Inoculations of seedlings confirmed the results obtained with the detached leaf inoculations. The isolates tested did not change their aggressiveness to wheat. This indicates that these isolates were stable for low aggressiveness and would not pose a threat to wheat.

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A GENETIC STUDY OF THE VEGETATIVE INTERACTION GROUPS OF *SCLEROTIUM ROLFSII*. F. A. Nalim[1], N. P. Keller[1], J. L. Starr[1], K. Woodard[2]. [1]Dept. of Plant Pathology and Microbiology, Texas A&M University, College Station, TX 77843; [2]Texas Exp. Stn, Stephenville, TX.

Sclerotium rolfsii causes southern blight of peanuts; 209 isolates of *S. rolfsii* collected from symptomatic plants in four central Texas counties were examined for interaction groups (i-groups) based on the presence or absence of an antagonism zone (a clearing of mycelia) between paired colonies. All isolates could be placed in one of 11 i-groups. I-group 6 was detected most frequently and was identified among isolates obtained from three widely separated fields. The ITS region of the rDNA from several isolates from different i-groups was amplified by PCR and the amplification product digested with the restriction endonuclease MboI. Preliminary data shows that a pattern can be shared by several i-groups, and that isolates within an i-group have the same pattern. Therefore, i-groups and DNA fingerprint patterns were not mutually exclusive. When a 16-base pair oligonucleotide primer was used in PCR, two distinct fingerprint patterns were observed. I-group 6 isolates of *S. rolfsii* had a distinct pattern in these tests.

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GENETIC CONTROL OF SPORULATION IN *MAGNAPORTHE GRISEA*. Hei Leung and Zhixin Shi. Department of Plant Pathology, Washington State University, Pullman WA 99164.

Using chemical and plasmid-insertional mutagenesis, we have identified five genes controlling conidial formation in *M. grisea*. Three genes, *con1*, *con2*, and *con5*, block the central pathway of sporulation whereas two genes, *con3* and *con4*, modify spore development. The *con5* mutation blocks the initiation of conidiophores and results in no spore formation. The *con1* and *con2* mutations cause abnormal spore morphology and reduce sporulation. The *con1*, *con2*, *con3*, and *con4* mutants are defective in appressorial formation. Three mutations, *con1*, *con4*, and *con5* were obtained by integrative transformation with plasmid pAN7-2. All mutant phenotypes cosegregated perfectly with hygromycin B resistance in ascospore progeny, indicating that the mutations were caused by insertional inactivation. Genomic DNAs flanking the inserted vectors in *con1* and *con4* mutants were recovered. Gene replacement experiments confirmed that the genomic sequence recovered from the *con4* mutant contained the inactivated gene.

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dsRNA TRANSFER *VIA* HYPHAL ANASTOMOSIS IS ASSOCIATED WITH A CHANGE OF PHENOTYPE OF *MONOSPORASCUS CANNONBALLUS*. B. R. Lovic, J. L. Newsum, R. D. Martyn, and M. E. Miller. Department of Plant Pathology and Microbiology, Texas A&M University, College Station 77843-2132, and Weslaco 78596.

dsRNA has been associated with the variability in aggressiveness and culture degeneration (reduced growth rate, number of perithecia, pigment production) in *Monosporascus cannonballus*, causal agent of a vine decline/root rot disease of cucurbits. Of the 300 isolates originating from the Lower Rio Grande Valley, 65% harbored dsRNA fragments. Four isolates characterized by having different dsRNA length polymorphisms were paired in all possible combinations on water-agar-coated microscope slides. Areas of anastomosis were excised and hyphal-tipped twice on V8 agar plates. The products of pairings were analyzed for production of perithecia on solid medium and for the type of dsRNA length polymorphisms. Hybrid dsRNA length polymorphisms could be observed in the products of pairings involving all parents that exhibited dsRNA fragments discernable by size. A proportion of those cultures produced significantly fewer perithecia then either of the parents. Cultures that were products of parent self-pairing exhibited no variation in dsRNA type and produced similar numbers of perithecia. Transfer of dsRNA *via* hyphal anastomosis appears to be associated with phenotypic change in *Monosporascus cannonballus*.

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OUTCROSSING RATES OF *CRYPHONECTRIA PARASITICA* IN EUROPEAN CHESTNUT (*CASTANEA SATIVA*) FORESTS. M. Bissegger and M. G. Milgroom, Cornell University, Ithaca, NY 14853-5908

Vegetative compatibility (vc) and DNA fingerprinting were used to estimate the outcrossing rates of the chestnut blight fungus (*Cryphonectria parasitica*) in two Swiss forests (Lumino and Gnosca). In 1993 a total of 57 perithecia (Lumino 30, Gnosca 27) were collected in the two plots that each measured 50 x 50 m. Vc tests were performed by pairing 12 single ascospore isolates of each perithecium on potato dextrose agar. Ascospore isolates from 34 (Lumino 18, Gnosca 16) of the 57 perithecia segregated into more than one vc group, indicating outcrossing. From each of the remaining 23 perithecia, 8 ascospore isolates were assayed by DNA fingerprinting. Ascospore isolates of 19 perithecia (Lumino 11, Gnosca 8) segregated for at least one fingerprint band, while all progeny of 4 perithecia (Lumino 1, Gnosca 3) yielded identical DNA fingerprints each. The outcrossing rates were not significantly different in the two plots. These results indicate an outcrossing rate of 93% for Swiss *C. parasitica* populations which is significantly higher than the 73% observed in the US (Milgroom et al. 1993).

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SELFING AND OUTCROSSING IN THE CHESTNUT BLIGHT FUNGUS (*CRYPHONECTRIA PARASITICA*) IN NATURAL POPULATIONS. R. E. Marra and M. G. Milgroom, Cornell University, Ithaca, NY 14853-5908

Populations of *C. parasitica* representing diverse ecological areas in the central Appalachians of West Virginia have been sampled and assayed for outcrossing rates. We developed a technique in which "diploid" genotypes of bulked random collections of ascospores from single perithecia are compared to maternal genotypes using a DNA fingerprinting probe to determine outcrossing or selfing. In a hierarchical sampling scheme, random collections of cankers were intensively sampled to estimate variability in outcrossing for perithecia within stromata, stromata within cankers, and cankers within clusters. Inferred paternal genotypes for outcrossed perithecia within stromata, and within cankers, allow for a determination of the degree to which matings within stromata and within cankers are correlated. Mating type assays are also being conducted to elucidate the genetics of selfing in the mixed mating system of this fungus. The first stages of our work have allowed us to determine to what degree variability in the mating system of *C. parasitica* is determined by genotype and/or environment.

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NEW VIRULENCE PHENOTYPES FOUND AMONG SINGLE-OOSPORE ISOLATES FROM ESTABLISHED *PHYTOPHTHORA SOJAE* RACES. R. G. Bhat and A. F. Schmitthenner, Deptartment of Plant Pathology, OARDC, The Ohio State University, Wooster, OH. 44691.

Partial collections of the 27 reported races of *Phytophthora sojae* were evaluated for virulence on differential soybean cultivars with *Rps*1a, 1b, 1c, 1d, 1k, 3, 6 and 7 resistance genes. 56% of the cultures differed from the described reactions to one or more differentials. Virulence of 100 single-oospore cultures from each of 10 known races was evaluated. Ninety-seven new virulence phenotypes were found; 3 from Race 1, 17 from Race 3, 14 from Race 4, 7 from Race 7, 4 from Race 9, 5 from race 10, 5 from race 12, 9 from race 14, 10 from race 16, and 23 from race 25. Seven of these new virulence phenotypes were obtained from plants and soil. Ten virulence phenotypes were avirulent on the universal suscept, but virulent on various other *Rps* genes. Potential for many races exists in *P. sojae* which can be expressed following recombination of virulence genes during oospore formation.

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HOMOTHALLISM AND GENETIC VARIATION IN THE SELFED PROGENY OF THE HETEROTHALLIC *PYTHIUM SYLVATICUM*. Pia D. Gavino and Frank N. Martin, Plant Pathology Dept., University of Florida, Gainesville, FL 23611.

Pythium sylvaticum is normally a heterothallic species; however, some isolates are self-fertile and are capable of forming gametangia even in the absence of an opposite mating type. Depending on the isolate, different degrees of homothallic behavior were observed in isolates from the field or culture collections. Crosses involving strictly heterothallic parental isolates also have generated self-fertile isolates in 10% of the F1 and 7% of the backcross progenies. In addition to their ability to form sexual structures in single culture, these homothallic isolates also behaved as defined mating types in crosses. Self-fertile isolates may vary in the number of fertilized oogonia that become aborted and fail to mature into oospores during inbreeding. Despite the low frequency of oospore production and germination, selfed progenies were isolated and observed to vary in colony morphology, growth rate, and degree of homothallism, but acquired the same antheridial mating type from the parental isolate. Selfing also has generated polymorphisms as determined by random amplified polymorphic DNA markers and chromosomal karyotypes in the progeny. The potential for mutational events such as translocations in the chromosomes was apparent as the progeny contained parental and non-parental sizes of the chromosomes identified by rDNA and cDNA probes. Thus, selfing may generate phenotypic and genetic variations in progenies through segregation of traits and recombination events following meiosis.

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GENETIC VARIATION WITHIN A POPULATION OF *PHYTOPHTHORA CAPSICI* FROM SOUTHERN NEW MEXICO BASED ON RAPD ANALYSIS. D.L. Adorada, C.M.

Liddell and C.L. Biles, New Mexico State University, Department of Entomology, Plant Pathology and Weed Science, Box 3BE, Las Cruces, NM 88003, U.S.A.

Genetic variation within a population of *Phytophthora capsici* from southern New Mexico was studied using random amplified polymorphic DNA (RAPD). Thirty isolates from different pepper fields of Dona Ana county in southern New Mexico of both the A1 and A2 mating types were examined and compared with isolates from the California worldwide collection representing the three *P. capsici* subspecific CAP groups (Oudemans and Coffey, *Mycological Research* 95:1025-1046). Several primers (random decamers) amplified DNA segments (0.3-1.5 KB); some were polymorphic and some were shared among isolates. RAPD analysis confirmed the delineation of groups CAP1, CAP2 and CAP3 and further confirmed that all southern New Mexico isolates are in the CAP1 group. Within CAP group variation was smaller than among CAP group variation. No correlation was found between RAPDs and mating type or site of collection in southern New Mexico. Measuring genetic diversity in southern New Mexico populations of *P. capsici* using RAPDs may help select isolates for use in pepper breeding programs. RAPDs also provide a simple DNA-based method to better understand the population dynamics and mating behavior of *P. capsici* in the field.

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DEVELOPMENT OF RFLP MARKERS TO ELUCIDATE THE POPULATION GENETICS OF COLLETOTRICHUM GRAMINICOLA. U.L. Rosewich, R.A. Frederiksen and B.A. McDonald, Department of Plant Pathology and Microbiology, Texas A&M University, College Station, Tx 77843-2132.

Colletotrichum graminicola is generally considered as a variable pathogen, especially in sorghum pathology. Studies have been initiated to quantify the genetic variability in populations of this important pathogen. Initial work concentrated on developing a set of DNA probes which are useful to examine the population genetic structure of *C. graminicola*. Two pUC18 plasmid libraries containing anonymous fragments of *C. graminicola* DNA were constructed. A screen for RFLP variation was conducted using nine single-spored isolates, originating from North- and South America and Africa. Of the 269 enzyme-probe combinations which were evaluated, 86% detected RFLP in at least one of the nine isolates. Most of the tested probes hybridized to single- or low copy fragments, though 19% hybridized to more than 10 fragments or produced a smear, suggesting a very high copy number. The restriction enzymes *HindIII* and *PstI* detected polymorphisms most efficiently.

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ASSESSING THE VEGETATIVE COMPATIBILITY GROUPS IN *VERTICILLIUM DAHLIAE* USING RANDOM AMPLIFIED POLYMORPHIC DNA. K.-N. Li, T. L. German, and D. I. Rouse. Department of Plant Pathology, University of Wisconsin, Madison, WI 53706

Differentiating *Verticillium dahliae* isolates by Vegetative Compatibility Groups (VCG) varies depending on different complementary mutants used. RAPD analysis was performed on thirty-two isolates of *V. dahliae*, representing the four VCGs *sensu* Joaquim and Rowe, and on one isolate each of *V. albo-atrum* and *V. tricorpus*, which served as out-groups in data analysis. The data were analyzed with the unweighted pair group method using arithmetic average (UPGMA) and parsimony methods to construct dendrograms representing the relationship among the isolates. Isolates originally assigned to VCG 1 formed one group, while the rest formed another, which could be divided into subgroups. No correlation between the host and/or geographical origins, and the RAPD groups could be found. Our data indicated that the genetic complexity in *V. dahliae* may be less extensive than originally thought. DNA probes specific for the RAPD groups were also identified and may aid in future identification and genetic study of VCGs in *V. dahliae*.

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VARIATION IN VIRULENCE, PHYTOALEXIN TOLERANCE AND POLYMORPHISM IN DNA MARKERS AMONG ISOLATES OF *FUSARIUM OXYSPORUM* F.SP. *LYCOPERSICI* Patrice Suleman, David C. Straney. Department of Botany and MAES, University of Maryland, College Park MD 20742. Amgad Saleh, Abdel Mohsen Tohamy, and Magdy Madkour, Agricultural Genetic Engineering Research Institute, Agricultural Research Center, 9 Gamaa st., Giza Egypt

Fusarium oxysporum f.sp. *lycopersici* (*F.o.l*) causes wilt of tomato. Thirteen *F.o.l.* field isolates from Egypt and the U.S. (Maryland) were evaluated to determine if the variation in virulence observed between isolates may be correlated to a variation in tolerance to tomato phytoalexins or unknown genetic factors linked to DNA polymorphisms. Isolates from both Egypt and the U.S. separated into highly virulent and less virulent groups, based upon overall symptoms (Wellman's scoring), xylem function, and stem colonization. Rishitin and tomatine, two phytoalexins of tomato, did not significantly inhibit germination of macroconidia in PDB media, but did inhibit germ tube elongation in PDB and mycelial growth on PDA at 100 µg/ml. However, a lack of wide variation in tolerance among the isolates indicates that this biochemical trait may not explain the variation in virulence. Characterization of DNA polymorphisms using RAPD markers indicates significant genetic differences between isolates and indicate groupings of genetic similarity.

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VIRUS SUPPRESSION OF FUNGAL MATING-TYPE-SPECIFIC GENE EXPRESSION. Lei Zhang and Neal K. Van Alfen. Dept. of Plant Pathology and Microbiology, Texas A&M University, College Station, TX 77843

The dsRNA virus (CHV1) responsible for the hypovirulent phenotype of the chestnut blight pathogen, *Cryphonectria parasitica* suppresses the expression of specific fungal genes. We have previously shown that deletion of one of these genes, *Vir2*, results in a phenotype that mimics a portion of the symptoms of virus infection. We now report that *Vir2* encodes the sex pheromone produced by one of the two mating types of this fungus (*MatA*). Deletion of *Vir2* resulted in a disruption of sexual mating; perithecia were produced but they were barren. A second copy of this pheromone gene is encoded by *Vir1*, a gene in which the pheromone coding sequence is conserved in a short ORF, but otherwise is significantly different from the *Vir2* sequence. Silent copies of both genes are found in *Mata* strains. The effect of the virus on expression of mating specific genes was confirmed by the cloning of a gene expressed only by *Mata* strains. Expression of this gene was also suppressed by the virus. By suppressing fungal mating the virus stops the reassortment of fungal vegetative compatibility genes, the only known defense of the fungus against the virus.

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Genetics of Resistance to Oat Mosaic Virus and Oat Golden Stripe Virus in Oats. S.L. Walker, J.P. Murphy, S. Leath, S.A. Lommel, Dept. of Plant Pathology, North Carolina State University, Raleigh, NC 27695-7616.

Coker 716, a hexaploid winter oat cultivar resistant to oat mosaic virus (OMV) and oat golden stripe virus (OGSV) was crossed to three susceptible oat cultivars: Brooks, Madison, and Tech. A total of 190 $F_{2,3}$ derived lines were developed from the three crosses and will be evaluated for disease resistance and yield response in field plots located in four environments. A screen of 90 randomly amplified polymorphic DNA (RAPD) primers on each of the four parental cultivars revealed that 31 primers generate 194 polymorphisms which occur only in the resistant Coker parent. Disease intensity for each line will be compared to RAPD results. The segregation of the 194 polymorphisms in the $F_{2,3}$ lines and level of disease resistance in the field for each line should establish specific markers for resistance to OMV and OGSV which could be used for early generation screening of oat breeding lines.

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INTERACTION OF SOYBEAN MOSAIC POTYVIRUS WITH THE *Rsv*1 RESISTANCE GENE OF SOYBEAN. Alan L. Eggenberger[1], Roger N. Beachy[2], and John H. Hill[1]. [1]Department of Plant Pathology, Iowa State University, Ames, Iowa. [2]Division of Plant Biology, The Scripps Research Institute, La Jolla, California.

*Rsv*1 acts as a single dominant gene conferring resistance to many strains of soybean mosaic virus (SMV). Current studies involve the N strain of SMV, which cannot overcome the *Rsv*1 resistance, and the G7 strain of SMV, which can. A full length cDNA of SMV N behind the T7 RNA polymerase promoter has been constructed from which infectious transcripts have been synthesized *in vitro*. Chimeric SMV N/SMV G7 cDNAs have also been made by replacing portions of the SMV N genome with homologous segments of the SMV G7 genome, and virus derived from these clones is being assayed for the ability to infect soybean cultivars containing the *Rsv*1 gene. Using this system a region of the SMV G7 genome has been identified which is involved in overcoming the *Rsv*1 resistance. Work is in progress to further characterize the resistance-breaking determinant(s) of SMV G7.

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RESISTANCE TO PVY INFECTION IN TOBACCO USING PVY NONSTRUCTURAL GENES. P.J. Shiel and P.H. Berger, Dept. of Plant, Soil and Entomological Sciences, University of Idaho, Moscow, ID 83844-2339.

In 1993, Vardi and coworkers[1] engineered a gene from potato virus Y (PVY) containing portions of the NIa and NIb and demonstrated virus resistance. Using polymerase chain reaction, we have generated several genes from PVY and separated them at the protease cleavage site and according to function. These include the VPg coding region, the protease domain, NIa, NIb, and a NIa/NIb fusion. Several of the primary transformants containing the VPg coding region, and NIa showed little or no virus accumulation after 31 days. Several of the plants showed virus symptoms, but appeared to accumulate virus in an aberrant manner. Plants from two of the constructs accumulated virus at the apex normally, but did not accumulate virus in the lower leaves at day 31. Two plants accumulated virus in the lower leaves, but failed to accumulate virus in the apex. These results suggest a complex interaction between the plant with the resistance gene, and the virus.

[1]Vardi, E., Sela, I., Edelbaum, O., Livneh, O., Kuznetsova, L., and Stram, Y. 1993. Plants transformed with a cistron of a potato virus Y protease (NIa) are resistant to virus infection. Proc. Natl. Acad. Sci. USA 90:7513-7517.

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TRANSFORMATION OF 'NOVA' TANGELO WITH THE COAT PROTEIN GENE OF CITRUS TRISTEZA CLOSTEROVIRUS. J.L. Schell[1], H.R. Pappu[2], S.S. Pappu[2], K.S. Derrick[1], R.F. Lee[1], C.L. Niblett[2], and J.W. Grosser[1]. [1]Citrus Research and Education Center, Univ. of Florida, Lake Alfred FL 33850 and [2]Dept. of Plant Pathology, Univ. of Florida Gainesville, FL 32611.

Citrus tristeza closterovirus (CTV) causes major disease problems worldwide in citrus. As an approach to develop transgenic *Citrus* resistant to CTV, protoplasts isolated from a nucellar-derived suspension culture of 'Nova' tangelo ('Clementine' mandarin (*Citrus reticulata* Blanco) X 'Orlando' tangelo (*C. reticulata* X *C. paradisi* Macf.)) were transformed by PEG-mediated direct DNA uptake. Plasmid pMON10098 containing the coat protein (CP) gene from CTV isolate T36 (a Florida severe strain) was introduced using a PEG/high calcium/high pH procedure. Plants were allowed to regenerate from the protoplasts, and were screened by polymerase chain reaction (PCR) analysis using primers to the CP gene. Of the 36 plants regenerated, 3 were identified that appeared to be transformed based on PCR and western blot analysis. Confirmation of transformation is underway using Southern hybridization.

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USE OF CULTURE FILTRATES OF *MACROPHOMINA PHASEOLINA* AND *LASIODIPLODIA THEOBROMAE* TO SCREEN GUAYULE GERMPLASM FOR RESISTANCE. R. L. Schading,[1] J. O. Kuti[1], B. A. Mullin-Schading[2] and J. O. Bradford[2]. [1]Dept. Of Agronomy and Resource Science, Texas, A&M University-Kingsville, Kingsville, TX 78363, and [2] USDA-ARS, CPSR, Weslaco, TX 78596.

Charcoal rot caused by *Macrophomina phaseolina* and seedling dieback caused by *Lasiodiplodia theobromae*, constitute major disease problems in the cultivation of dryland guayule (*Parthenium argentatum*) in south Texas. A method was developed to screen guayule germplasm for resistance to the two pathogens using cell-free culture filtrates (CFCF). Cultures of the pathogens were grown in broth medium for one wk, filter-sterilized and incorporated into Gamborgs medium. The medium was amended with 0, 5, 10, 25, and 50% CFCF (v/v) before solidifying with 0.8% agar. One-week-old seedlings were placed in the medium and incubated at 27 $\pm 2^{\circ}$C. Data on growth, lesion development and severity were collected. There was a significant ($P = 0.05$) growth reduction at with, 25 and 50% CFCF. Lesion severity was highest at 25 and 50% CFCF. Eight guayule genotypes were screened using a 20% CFCF / nutrient medium combination. Results will be compared to 6-wk-old plants inoculated in soil with these pathogens using standard techniques.

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INHERITANCE OF STRAWBERRY POWDERY MILDEW (*S. FRAGARIAE*) RESISTANCE IN GREENHOUSE- VS. FIELD-GROWN CALIFRONIA PROGENIES. M. D. Nelson[1], D. V. Shaw[2], and W. D. Gubler[1]. [1]Dept. Plant Pathology and [2]Dept. Pomology, Univ. CA, Davis, 95616.

Seventeen unique full-sib families were evaluated for powdery mildew resistance in a Davis, CA greenhouse (GH) and at two field sites - Watsonville (Wat) and Santa Maria (SM), CA. Mean disease incidence (DI) and severity (DS) ratings were relatively high at the GH and SM trials, but relatively low at the Wat trial. Genetic variance components for powdery mildew resistance inheritance were estimated using partial diallel analyses. General Combining Ability was found to be highly significant ($P=0.01$) for both the GH and SM trials, while Specific Combining Ability was found to be highly significant for the Wat trial. Narrow-sense and broad-sense heritabilities for DS ratings were 0.12 and 0.70 for the Wat trial, 0.66 and 0.72 for the SM trial, and 0.90 and 0.94 for the GH trial. Genetic correlations and parent breeding value correlations between field and GH DS ratings increased from 0.43 to 0.97 and from 0.52 to 0.93 when relatively low and high (Wat and GH) vs. uniformly high (SM and GH) infection levels were compared.

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RESISTANCE OF *ARABIDOPSIS THALIANA* TO INFECTION BY BEET CURLY TOP VIRUS Keith R. Davis[1,2], Sukchan Lee[2], and David M. Bisaro[1,3] Biotechnology Center[1], Depts. of Plant Biology[2] and Molecular Genetics[3], The Ohio State University, Columbus, OH.

We have found that several ecotypes of *Arabidopsis thaliana* are differentially resistant to two strains of the geminivirus, beet curly top virus (BCTV). Analysis of viral DNA accumulation indicated that symptom development and severity were correlated with the amount of viral DNA present in the plants. Viral DNA was undetectable in two ecotypes that were phenotypically resistant to BCTV-Logan. Studies of viral DNA replication in excised inflorescence pieces demonstrated that BCTV-Logan could replicate in tissues from these resistant ecotypes, suggesting that resistance was due to a block in viral movement. Genetic studies of these two ecotypes indicate that resistance is due to a single, recessive locus. Preliminary allelism tests of the two resistant ecotypes indicate that they contain distinct resistance loci. This is the first example of a single resistance locus for any geminivirus. The identification of ecotypes resistant to specific BCTV strains provides an excellent model system for the genetic and molecular analysis of the interaction of a plant host with geminiviruses.

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Genetic analysis of resistance/susceptibility in individual F3 families of rice against *Magnaporthe grisea* isolates that contain different combinations of avirulence genes and suppressor genes. Chih-Cheng T. Chao[1], Karen A. K. Moldenhauer[2], and Albert H. Ellingboe[1]. 1. Plant Pathology Department, University of Wisconsin, Madison WI; 2. University of Arkansas, Rice Research and Extension Center, Stuttgart AR.

Magnaporthe grisea is the causal agent of the rice blast disease. We have previously identified two avirulence genes (P11 and P12) and their corresponding suppressors (S11 and S12) that control avirulence/virulence on rice cultivar Katy (Phytopathology 83:375-382). The F2 plants from a cross between resistant cultivar Katy and a suspectible cultivar Lemont segregated 3 resistant:1 susceptible when tested with an isolate of genotype p11 P12 s11 s12. Individual F3 families have been inoculated with a series of isolates that have different genes controlling interactions with Katy. Different segregation ratios have been observed when the F3 families are inoculated with isolates that are avirulent based on different genes. The relationship between the presence of P and S genes in a pathogen isolate and the resistant:susceptible segregation ratios observed among progenies of crosses involving Katy as one parent will be discussed.

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TYPHULA SNOW MOLD TOLERANCE IN WINTER WHEAT. F. Mohammad, J.M. Windes, and E.J. Souza. University of Idaho. P.O. Box AA, Aberdeen, ID, 83210-0530.

Typhula idahoensis Remsb., a causal organism of snow mold, infects winter wheat when snow covers unfrozen soil for long periods of time. Wheat cultivars capable of storing large amounts of total nonstructural carbohydrates (TNC) survive infection in high elevation areas of the Pacific Northwest where snow mold is prevalent. F_6 progeny lines of two crosses (Blizzard*2/Sumner and Manning*2/Survivor) varying for TNC content were used to predict snow mold tolerance in an environment suitable for snow mold infection. Three experiments using a 5x5 balanced square lattice design with six replications were planted in Tetonia, Idaho, in Fall 1992 and 1993. Plant samples were collected before snow fall from all three experiments to determine TNC. Heritability estimates for TNC levels were 84% and 52% in Blizzard populations and Manning populations, respectively, and 49% and 33% for survival. Sampling for TNC content is a more efficient method of estimating snow mold tolerance than field survival, allowing the simultaneous screening of many lines without depending upon the irregular occurrence of snow mold in the field.

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BREEDING FOR RESISTANCE TO KARNAL BUNT (*Tilletia indica*): DEVELOPMENT OF BREAD WHEAT ADVANCED LINES. Sanjaya Rajaram and Guillermo Fuentes-Davila, International Maize and Wheat Improvement Center (CIMMYT), Apdo. Postal 6-641, 06600 Mexico, D.F.

Through hybridization between Karnal bunt resistant lines and high yielding genotypes of bread wheat, more than 15,000 segregating populations (F2-F7) have been evaluated for Karnal bunt resistance over a five year period. The principal sources of resistance identified at CIMMYT have originated from China, India and Brazil. The segregating progenies are artificially field tested in northwestern Mexico by syringe-inoculation during the boot stage with a sporidial suspension of 10,000/ml, injecting 1 ml/spike. During the wheat cycle 1990-91, CIMMYT had 1229 lines from F3-F7, including the first group of 266 advanced lines of which sixty eight had an infection level range of 0-2.5%. This is comparable to resistance durum cultivar Altar C84, while the bread wheat cultivars tested had a mean of 32% infection. Three sister lines out of the 68, and with a Chinese parentage were used for further testing. Based on yield, quality and reaction to Karnal bunt, one of the lines was released by INIFAP (Mexico' National Institute of Agriculture, Forestry and Livestock Research) for commercial cultivation in southern Sonora, under the name "Arivechi M92", the first bread wheat cultivar with resistance to *Tilletia indica* in that area.

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PARTIAL RUST RESISTANCE IN THREE PEARL MILLET INBREDS. J. P. Wilson, USDA-ARS Forage and Turf Research Unit, University of Georgia, Coastal Plain Experiment Station, Tifton, GA 31793.

Pearl millet inbreds Tift 383, 700481-21-8, and ICMP 501 were evaluated for partial resistance to rust (*Puccinia substriata* var. *indica*) compared to susceptible inbred Tift 23DB in the greenhouse and field. Seedlings of the inbreds inoculated with a bulk culture and four single-uredinium isolates expressed susceptible infection types (IT) 3 or 4, except ICMP 501 which had a moderately resistant IT 2 to isolate PS89-775. Uredinium lengths, widths, and areas on Tift 383, 700481-21-8, and ICMP 501 seedlings were smaller than those of Tift 23DB. Whole-plant latent periods for adult plants of the three inbreds were longer than for Tift 23DB when inoculated with a bulk culture, but longer only for ICMP 501 when inoculated with isolate PS92-1. In 1992, only ICMP 501 had rust severities lower than Tift 23DB. When evaluated in three dates of planting in 1993, results were variable, however, the three inbreds generally had lower rust severities than did Tift 23DB. Although these inbreds exhibit many characteristics of slow-rusting resistance, variables

such as pathogen race, the presence of other diseases, or environmental conditions affected the expression of resistance. Urediniurn dimensions on hybrids Tift 383 x 700481-21-8 and Tift 383 x ICMP 501 inoculated as seedlings with PS92-1 were intermediate to those on the parents suggesting that the resistance gene(s) in the inbreds are partially recessive, and those of Tift 383 differ from those of 700481-21-8 and ICMP 501.

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YIELDS OF *APHANOMYCES*-RESISTANT ALFALFA VARIETIES IN NATURALLY INFESTED AND UNINFESTED SOILS. P. Vincelli, L. M. Lauriault*, and J. C. Henning*, Depts. of Plant Pathology & *Agronomy, Univ. of Kentucky, Lexington, 40546.

Field tests were conducted over 4 yr to test whether *Aphanomyces*-resistant (MR rating or higher) alfalfa varieties and experimental lines (RV) would collectively outyield susceptible varieties (SV) in sites infested with *Aphanomyces euteiches*. All entries had an R or HR rating to Phytophthora root rot. At the end of the tests, the mean dry matter yield of RV was higher (4.2% increase, P=0.069) than that of SV in a site naturally infested with *A. euteiches* and *Phytophthora medicaginis*, although total yields of certain SV equalled or even exceeded (*P*=0.10) those of certain RV. No significant difference was observed between SV and RV in two sites infested with *A. euteiches* alone nor in four sites not infested with either pathogen. Our data are consistent with the hypothesis that selection for *Aphanomyces* resistance contributes to an overall improvement of elite alfalfa germplasm for cultivation in poorly drained soils infested with both *A. euteiches* and *P. medicaginis*. However, considered alone, the *Aphanomyces* resistance rating of an individual variety or line had limited value for predicting performance in sites infested with *A. euteiches*.

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RESISTANCE OF SOYBEAN CULTIVARS GROWN IN IOWA TO *PYTHIUM IRREGULARE*, *P. ULTIMUM*, AND *RHIZOCTONIA SOLANI*. S.S.A. Rizvi, X.B. Yang, G.L. Tylka, and D.C. McGee. Dept. of Plant Pathology, Iowa State Univ., Ames, IA 50011.

Soybean cultivars grown in Iowa were evaluated for resistance to post-emergence damping-off caused by *Pythium irregulare*, *P. ultimum*, and *Rhizoctonia solani*. Hypocotyls (10 days old) were inoculated with mycelium. Of the 63 cultivars tested, six were resistant to all three fungal pathogens. Twenty four were resistant only to *P. irregulare*, 35 were resistant only to *P. ultimum*, and 40 were resistant only to *R. solani*. Nine, six, and three cultivars were highly susceptible to *P. irregulare*, *P. ultimum*, and *R. solani* respectively. Rest of the cultivars differed in their resistance to the individual pathogens. There was a negative correlation between resistance to *P.irregulare* and *R. solani*. No significant correlation was found either between *P. ultimum* and *P. irregulare* or between *P. ultimum* and *R. solani*.

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EVALUATING RESISTANCE IN SWEETPOTATO TO RHIZOPUS SOFT ROT. C. A. Clark and M. W. Hoy, Department of Plant Pathology & Crop Physiology, Louisiana Agricultural Experiment Station, LSU Agricultural Center, Baton Rouge 70803-1720.

Two methods were developed and evaluated for screening sweetpotato storage roots for resistance to Rhizopus soft rot. For both methods, roots that had been cured and stored at 16-18°C for at least 3 wk were first washed. In the dip method, roots were wounded by allowing them to drop about 1 m off the washer into crates and then were dipped in a suspension of sporangiospores. In the screw method, deep-threaded wood screws were dipped in a spore suspension and then hammered about 1 cm deep into the median of the root. Rankings of cultivars were similar by both methods and there was an overall correlation in soft rot incidence (R^2=0.22) between the methods, but differences among genotypes were greater with the screw method. In the first test, most lines were more susceptible by the screw method to Rhizopus stolonifer than to R. arrhizus, and thus, only R. stolonifer was used subsequently. Genotypes with white-fleshed storage roots were uniformly susceptible while orange-fleshed genotypes varied. Linear relationships were found in infectivity titrations between inoculum concentration and percent soft rot with the screw method after 3 days incubation, but with the dip method, soft rot incidence was similar at concentrations of 3.5×10^2 to 3.5×10^4 sporangiospores/ml. Of the cultivars evaluated, Beauregard was most resistant, Jewel and Hernandez were intermediate, and HiDry and Sumor, both white-fleshed, were among the most susceptible.

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THE EFFECTS OF SOME POTENTIAL BACTERICIDES ON *ERWINIA AMYLOVORA*. E. Hacioglu, and M. T. Momol[1], Dept. of Plant Pathology, Akdeniz Univ., Antalya, Turkey and [1]Dept. of Plant Pathology, Cornell Univ., Geneva, NY 14456.

Experiments were conducted to evaluate the effects of some chemicals and essential oils of origanum (*Thymbra spicata*) toward *Erwinia amylovora*, compared with copper oxychloride/maneb mixtures which are known to have bactericidal effects toward fire blight under orchard conditions and toward some bacterial diseases of tomato. In this study copper oxychloride plus maneb and copper oxychloride plus mancozeb were found to be effective bactericides against *E. amylovora* in agar diffusion and agar dilution tests, and in Norelli and Gilpatrick's immature pear fruit test. The addition of dithiocarbamate to copper oxychloride enhanced the effectiveness of copper to *E. amylovora*. The volatile phase of the essential oil of origanum was found to be effective as a bactericide against *Erwinia amylovora* in the agar dilution test and in the immature pear fruit test.

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UTILIZATION OF A BACTERIAL METABOLITE FOR THE MANAGEMENT OF DOLLAR SPOT DISEASE OF CREEPING BENTGRASS (*AGROSTIS PALUSTRIS*). J. F. Powell[1], M. G. Nair[2], and J. M. Vargas, Jr[1], [1]Department of Botany and Plant Pathology and [2]Department of Horticulture, Michigan State University, East Lansing, MI 48824.

Two *Pseudomonas* strains were isolated from soil and exhibited strong antifungal activity toward many fungal turfgrass pathogens in *in vitro* bioassays. A single antibiotic was isolated and purified which proved inhibitory to turfgrass fungal pathogens in laboratory bioassays. Characterization of the antibiotic indicated that it is structurally different from other antibiotics reported from Pseudomonas. Minimum inhibitory concentrations of this antibiotic to many pathogenic turfgrass pathogens were 10 to 25 µg/ml. Greenhouse evaluation showed the antibiotic to be a potential chemical treatment for the management of dollar spot. Field evaluation of the antibiotic resulted in significant reductions in the incidence of dollar spot.

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BOTANICAL OILS ALONE AND IN COMBINATION WITH FUNGICIDE FOR CONTROL OF ROSE BLACK SPOT. M. R. Carter and J. C. Locke. 1994. USDA, ARS, USNA, FNPRU BARC-West, Beltsville, MD 20705-2350.

Botanical oils were evaluated for control of black spot disease of rose, caused by the fungus *Diplocarpon rosae*. Experiments were conducted on two susceptible cv. of *Rosa* sp. both *in vitro* using a detached leaf assay and in the field using whole plants. *In vitro* assays were performed for 18 days measuring mean days until lesion development (LD) and sporulation (SP), and percent control by day 10. Botanical oils alone or in combination with $NaHCO_3$ w/v delayed LD for 6-10 days and neem seed oil (NSO) plus Funginex® at lower than labelled rates inhibited LD. A significant delay in SP was not detected, however, complete control by day 10 was achieved with NSO plus Funginex® or Domain. In the field, treatments were applied at 7 or 14 day intervals and disease severity (0-3) and percent plant defoliated (0-5) were assessed. NSO alone significantly reduced disease severity and percent defoliation. Better control was achieved with an NSO and Funginex® combination resulting in low disease severity (0-1) and approx. 0-5% defoliation. Synergism of NSO plus Funginex® was significant.

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EFFICACY OF BLOOM SPRAYS FOR CONTROLLING BUNCH ROTS OF WINE GRAPES IN THE SAN JOAQUIN VALLEY. R.A. Duncan[1], J.J. Stapleton[1], J.C. Broome[2], J.J. Marois[2], G.W. Leavitt[3], K.M. Kelley[4], and T. Martin-Duvall[3], [1]Statewide Integrated Pest Management Project, University of California, Kearney Agricultural Center, Parlier, CA 93648, [2]Department of Plant Pathology, University of California, Davis, CA, 95616, [3]Cooperative Extension, Madera, CA 93637, and [4]Modesto, CA 95355.

Clusters of *Vitis vinifera* cv. Zinfandel in Sacramento County (Northern San Joaquin Valley), Stanislaus County (Central) and Madera County (Southern) vineyards were sprayed with water or conidial suspensions of the fungal pathogens *Botrytis cinerea*, *Aspergillus niger*, or *Penicillium glabrum*. Iprodione fungicide (2.4 g a.i./L) was applied 24 hours after inoculation. Treatments were applied at 10%, 50%, 100% bloom, or 2-3 weeks post bloom. Portions of clusters were sampled ca. 2 weeks after treatment and examined for colonization of senescent floral parts by inoculated fungi and for epiphytic fungal populations on berries. Remaining clusters were evaluated for bunch rots at harvest. Fungicide applications reduced debris colonization and epiphytic populations of *B. cinerea* at all three locations. Floral colonization and epiphytic populations of *A. niger* and *P. glabrum* were not consistently affected by the fungicide. Although bunch rot caused by *B. cinerea* was reduced at all locations, total incidence of all bunch rots was not reduced in any trial. In the arid south valley, total rot and rot caused by *A. niger* were significantly higher in the fungicide treatments.

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Control of scab (*Venturia inaequalis*) and European red mite (ERM, *Panonychus ulmi*) on Empire apples with fluazinam. R.P. Kaiser, and T. White, ISK Biotech Corp. Mentor, OH 44061, and Crop Management Strategies, Inc., Hereford, PA 18056.

Fluazinam 500F, a broad spectrum protectant fungicide, was tested at 1.0 L /1000 L water following a i) 12 spray conventional scab schedule, ii) a 6 spray extended schedule, iii) a

fluazinam plus Nova followed by fluazinam alone for the summer covers, iv) a "standard" treatment of Captan + Nova + Benlate until ERM reached threshold, then switching to fluazinam. All treatments gave good to excellent control of foliar scab, fruit scab, sooty blotch and flyspeck under moderate disease pressure. Six sprays were slightly less effective against late season terminal infections. ERM populations remained low on all trees treated with fluazinam. In trees treated with the standard fungicides, mites were reduced from threshold levels to levels equal to the Omite standard after two applications of fluazinam. Fluazinam promises to be an excellent apple fungicide. It will be useful in managing resistance to systemic fungicides and will reduce the need for a miticide.

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LONG-TERM FUNGICIDE CONTROL OF BLACK KNOT OF PLUM. A. J. Latham and H. L. Campbell. Department of Plant Pathology, Alabama Agricultural Experiment Station, Auburn University, Auburn, AL 36849-5409.

Black knot of plum (*Apiosporina morbosa*) is a severe disease of trees in Alabama. In 1988, tests were initiated on AU Amber plums (Methley 12-10) to evaluate fungicide control of *A. morbosa*. During the bloom and fruiting seasons, fungicides applied every 10-14 days were: 1) captan 1.1. kg a.i./ha + benomyl 0.070 kg a.i./ha; 2) captan 1.1 kg a.i./ha + benomyl 0.140 kg a.i./ha; 3) propiconazole 0.292 L a.i./ha; 4) propiconazole 0.438 L a.i./ha; 5) propiconazole 0.584 L a.i./ha; 6) nontreated control. Prior to bloom, black knots were counted on each tree in the orchard to determine control by the previous year's treatments. The six year study showed significant control of black knot with each of the fungicides and no significance among fungicides. Mean number of knots in the control was nearly 4 times greater than with any fungicide treatment. Applications of fungicides over several years can significantly suppress *A. morbosa* on plums.

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LACK OF CORRELATION BETWEEN FITNESS AND RESISTANCE TO STEROL BIOSYNTHESIS-INHIBITING FUNGICIDES IN *PYRENOPHORA TERES* T.L. Peever and M.G. Milgroom, Department of Plant Pathology, Cornell University, Ithaca, NY.

Fitness costs associated with resistance to sterol biosynthesis-inhibiting fungicides (SBIs) were investigated by calculating correlations between SBI-resistance phenotypes and fitness phenotypes in *Pyrenophora teres*. Correlations between resistance to two triazole SBIs (triadimenol and propiconazole) and two components of fitness (latent period and sporulation) were estimated using *P. teres* isolates randomly sampled from populations in North Dakota, U.S.A. and Bavaria, FRG. Significant genetic variation ($P < 0.05$) in fitness components was detected among *P. teres* isolates from both populations in three of four experiments. Significant genetic variation in resistance to triadimenol and propiconazole ($P < 0.001$) was detected among *P. teres* isolates from both populations. However, no significant correlations between fitness and resistance were observed in these experiments and we conclude that strategies to manage SBI resistance cannot depend upon the existence of fitness costs.

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CONTROL OF PUCCINIA HORIANA, CAUSE OF CHRYSANTHEMUM WHITE RUST, WITH MYCLOBUTANIL. M. R. Bonde[1], G. L. Peterson[1], and S. A. Rizvi[2], [1]USDA, ARS, Frederick, MD 21702; [2]APHIS, PPQ, U. S. Customs House, Baltimore, MD 21202.

In December 1991, chrysanthemum white rust, a disease foreign to the United States, was discovered in Santa Barbara County, California, in commercial nurseries. As a result, a joint ARS/APHIS research project was initiated to evaluate the efficacy of the fungicide myclobutanil as a means of preventing further spread and recurrence of the disease. Five varieties of chrysanthemum were used in tests. Spraying plants with myclobutanil (100 mg a.i./liter) 5 days prior to inoculation with P. horiana was not highly effective as a prophylactic treatment. Spraying plants 5 days after inoculation was extremely effective in preventing disease development. Furthermore, cuttings taken 5 days after plants were inoculated with P. horiana produced disease-free plants when cuttings were dipped in myclobutanil (100 mg a.i./liter) before rooting.

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RESPONSE OF PEANUT CULTIVARS DIFFERING IN SUSCEPTIBILITY TO SCLEROTINIA BLIGHT TO FUNGICIDE TREATMENTS. J.P. Damicone and K.E. Jackson, Dept. of Plant Pathology, Oklahoma State Univ., Stillwater 74078-9947.

In two trials, two applications of the fungicide iprodione at 1.12 kg/ha reduced the incidence of Sclerotinia blight in the susceptible cultivar Okrun from 63% to 28% while three were required to increase yield (from 2034 to 2835 kg/ha). Iprodione did not affect disease incidence or yields of the moderately resistant cultivar Spanco or the resistant cultivar Tamspan 90 which averaged 9% and 2475 kg/ha, and 3% and 2903 kg/ha, respectively. In three other trials, the experimental fungicide fluazinam reduced disease incidence in one or more of the trials and increased yields across trials for all cultivars. Two applications at 0.56 kg/ha reduced disease incidence in Okrun for all trials from 77 to 22%, but one or two applications increased yield from 2153 to 3377 kg/ha. Reductions in disease incidence with fluazinam for Spanco and Tamspan 90 were variable and yields were increased to a lesser degree than for Okrun. One application to Tamspan 90 reduced disease incidence from 10 to 5% and increased yield from 3348 to 3891 kg/ha. Two applications were needed for Spanco to reduce disease incidence from 17 to 7% and increase yield from 2900 to 3484 kg/ha. Yields of Tamspan 90 were the highest in all trials for any level of fungicide treatment.

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EVALUATION OF SELECTED SEED TREATMENTS FOR SHRUNKEN-2 SWEET CORN. R. E. Baird[1], C. Nankam[2], P. F. Moghaddam[2], and J. K. Pataky[2], Dept. of Plant Pathology, University of Georgia, RDC, Tifton, Georgia 31794[1]; Dept. of Plant Pathology, University of Illinois, Urbana, Illinois 61801[2].

Field trials were conducted at Vincennes, IN and Urbana, IL in 1992 and 1993 to evaluate seed treatments to enhanced stands and seedling growth of *shrunken-2 (sh2)* sweet corn and to determine fungi most prevalent in seed 5 and 10 days after planting. In 1992, the most common fungi were *Pythium ultimum, Rhizopus stolonifer, R. arrhizus,* and *Trichoderma*. In 1993, *P. ultimum* was not isolated as frequently as *Trichoderma* spp., *Fusarium moniliforme,* and *Penicillium oxalicum*. Treatments which enhanced emergence and growth the most included several components. Ongard (polymer seed coating)/standard fungicide/bioprimed was the only treatment in twenty for which stands were significantly greater than the untreated, primed control in all four trials. Solid matrix priming of seed increased stands for fungicide treatments which had relatively poor stands in the absence of priming. The beneficial effect of priming on plant height was much more evident than the effect on stand. Unless adverse environments can be predicted, multi-component seed treatments provide the best protection against poor stands and seedling diseases of *sh2* sweet corn.

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BASE-LINE SENSITIVITY OF MONILINIA FRUCTICOLA TO SIX DMI FUNGICIDES. W. F. Wilcox and J. A. Burr, Department of Plant Pathology, Cornell University, New York State Agr. Expt. Sta., Geneva, NY 14456.

Single-conidia isolates of *Monilinia fructicola* obtained from six wild-type populations (n=13, 17, 43, 50, 50, and 50, repsectively) were evaluted for their sensitivities to six DMI fungicides, based on ED_{50} values for mycelial growth on fungicide-amended PDA. From most to least active, these fungicides, their mean ED_{50} values (and ranges) in ug/ml were: fenbuconazole, 0.003 (0.001-0.014); tebuconazole, 0.007 (0.003-0.034); propiconazole, 0.009 (0.004-0.080); fenarimol, 0.070 (0.017-0.457); myclobutanil, 0.280 (0.080-1.7); and triforine (one population only), 2.37 (0.850-6.5). In field and greenhouse trials, myclobutanil applied at a rate of 12.0 g (a.i.)/100 L was compared with fenbuconazole at a rate of 0.16 g/100 L for control of fruit and blossom infections of sour cherry caused by *M. fructicola*. Both treatments, comparable in terms of the above *in vitro* activities, also provided comparable levels of disease control. These results indicate a great variation in the intrinsic activities of DMI fungicides against *M. fructicola*, and provide a basis for future resistance monitoring.

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

The incidence of thiabendazole (TBZ) resistance in *Fusarium* spp. associated with dry rot of potato tubers was estimated during 1992 and 1993. We isolated from preexisting lesions or wounds on

randomly-collected seed, table stock, and processing tuber samples from the northeastern United States. Of 154 samples, 99 yielded one or more *Fusarium* isolates, >95% of which were pathogenic. The most frequently recovered species were *F. solani, F. oxysporum,* and *F. sambucinum,* but *F. avenaceum, F. culmorum,* and *F. equiseti* were also isolated. Of the 198 *Fusarium* isolates, 82 were resistant to TBZ (5 µg/ml). The resistant isolates identified to date include 58 *F. sambucinum,* five *F. solani,* three *F. oxysporum* and one isolate each of *F. culmorum* and *F. equiseti* .

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METALAXYL-RESISTANT CLONAL GENOTYPES OF *PHYTOPHTHORA INFESTANS* IN THE UNITED STATES AND CANADA WERE PROBABLY INTRODUCED FROM NORTHWESTERN MEXICO. S. B. Goodwin, L. S. Sujkowski and W. E. Fry. Department of Plant Pathology, 334 Plant Science, Cornell University, Ithaca, NY 14853.

Clonal genotypes of *Phytophthora infestans* from the USA and Canada were identified by analyses of genetic variation at the mating type, two allozyme and 25 DNA fingerprint loci. Isolates that were identical for these markers were assumed to be the same clone. Among 230 isolates analyzed for sensitivity to the fungicide metalaxyl, 76% were highly resistant. Most (> 90%) of the sensitive isolates had a single clonal genotype, US-1. The US-1 genotype has been present in the USA and Canada for many years, where it is still effectively controlled by metalaxyl. For three other clonal genotypes (US-6, US-7 and US-8), all isolates tested were highly metalaxyl resistant, including those from home gardens that were never sprayed. In a worldwide database of over 1500 isolates, the only other location where the US-6, US-7 and US-8 genotypes occurred was in northwestern Mexico. In that part of Mexico the frequency of metalaxyl-resistant isolates is near 100%. Thus, recent migration from northwestern Mexico was the likely source for the highly metalaxyl-resistant genotypes of *P. infestans* in the USA and Canada.

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EFFECT OF PRIMARY INFECTION LEVEL OF *COLLETOTRICHUM GLOEOSPORIOIDES* ON ANTHRACNOSE DEVELOPMENT OF NORTHERN JOINTVETCH AND WINGED WATERPRIMROSE IN THE FIELD. Wei Zhang and D. TeBeest. Department of Plant Pathology, University of Arkansas, Fayetteville, AR 72701.

Northern jointvetch (NJV) and winged waterprimrose (WWP) were inoculated with *C. gloeosporioides* f.sp. *aeschynomene* (CGA) and *C. gloeosporioides* f.sp. *jussiaeae* (CGJ) respectively at three inoculation levels in the field. CGA produced 4.3 or 18.8 primary lesions per plant 7 days after toothpick inoculation at 1 or 10 locations on the main stem. The rate of increase in disease severity for the two respective treatments were 0.6% and 1.2% per day during the early part of the season before the disease severity leveled off. An aerial application of $2x10^7$ spores/m^2 had an intermediate response with 15.1 primary lesions per plant and 0.7% percent increase in severity per day. WWP inoculated with drops of CGJ conidial suspension on 1 or 10 leaves per plant or sprayed at a similar rate as for CGA had 3%, 6%, or 25% primary disease severity and a rate of increase at 0.7%, 1.4%, or 2.0% per day during the early season, respectively.

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USING AN ASYMPTOTIC PARAMETER TO REVISE WEIBULL MODEL. Chang-Lin Xiao, K.V. Subbarao, Dept. of Plant Path., Univ. of Calif., Davis, c/o U.S. Agric. Res. Stn, Salinas, CA 93905, and Shi-Mai Zeng, Dept. of Plant Prot., Beijing Agric. Univ., Beijing 100094, P.R. China.

Traditional growth curve models used in the analysis of plant disease epidemics assume an asymptote and a maximum disease severity (K_{max}) of 1.0. It often is inappropriate to assume K_{max}=1.0, especially for disease progress curves that have low asymptotic values in which rates of disease increase are underestimated (Phytopath. 75:786-791; Phytopath. 82:811-814). Neher and Campbell (1992) discussed a K_{max} parameter used with Monomolecular, Gompertz and Logistic growth models in describing epidemics, but did not include Weibull model in their studies. Weibull is a flexible growth model that describes both the general population growth and plant disease progress. But lack of an asymptotic parameter has limited its wider applications, especially those using the absolute numerical data to describe organism growth (eg. lesion expansion). For this reason, we incorporated an asymptotic parameter K to the Weibull model, written as: $y = K\{1-\exp[-((t-a)/b)^c]\}$ in which a, b, c and K are location, scale, shape, and asymptotic parameters, respectively. Describing disease progress using the above model allows retention of flexibility in the original Weibull model because the value of parameter K may fluctuate with different epidemics (each season with one value). However, we propose that there is a K_{max} representing the maximum level of disease that is an intrinsic constant for a given pathosystem. The computational method, Marquardt was used in the nonlinear regression procedure to estimate the four parameters simultaneously. The disease progress and lesion expansion data from tomato late blight (*Phytophthora infestans*) epidemics fitted to the model confirmed both the appropriateness and flexibility of Weibull model with four parameters.

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DEVELOPMENT OF PREDICTIVE MODELS FOR RICE BLAST BASED ON FAVORABLE ENVIRONMENTS. S.B. Calvero, S.M. Coakley, Department of Botany and Plant Pathology, Oregon State University, Corvallis, OR 97331; and P.S. Teng, International Rice Research Institute, Manila, Philippines.

Predictive models of rice blast on cultivar Jin heung at Icheon, South Korea and on IR50 and C22 at Cavinti, Philippines were generated using as predictors, weather factors identified by the WINDOW PANE program to be highly correlated with disease. Relative humidity and rainfall factors were important predictors of disease at Icheon; these factors together with temperature and wind speed were predictors in the models at Cavinti. Path analysis was used to further analyze weather factors important to disease predictions. Consecutive days with humidity above 79% had a large positive effect on leaf blast at Icheon, but a negative effect on panicle blast at the same site. At Cavinti, minimum temperature and consecutive days without rain had the largest positive influences on leaf and panicle blast on IR50, respectively. On C22, total precipitation and maximum temperature had the largest direct effects on leaf and panicle blast, respectively. The models were validated with observations not included in model development.

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GEOSTATISTICAL ANALYSIS OF SOIL WATER CONTENT AND POPULATION LEVELS OF *PHYTOPHTHORA CAPSICI* IN RELATION TO DISEASE SEVERITY IN BELL PEPPER FIELDS. Robert P. Larkin[1], Marcia L. Gumpertz[2], and Jean B. Ristaino[1], [1]Dept. of Plant Pathology and [2]Dept. of Statistics, North Carolina State University, Raleigh NC 27695-7616.

Spatial and temporal dynamics of disease, soil population levels of *P. capsici*, and soil water content in three commercial bell pepper fields were analyzed using geostatistical techniques. Semivariograms constructed over four directions of orientation (0, 45, 90, and 135° azimuth) demonstrated strong spatial dependence and anisotropy for soil water content and disease severity in all fields. Ranges of spatial dependence averaged 13 to > 16 m in multiple directions for these factors. Soil water content early in the development of the epidemic was spatially correlated with disease severity throughout the season in two fields (r=0.53 to 0.67). Cross-correlograms also indicated a close relationship beween soil water content and disease development. Inoculum levels of *P. capsici* were spatially dependent within rows early in the season in two fields, but were not strongly correlated with disease severity at any time in any field. Soil water content was not strongly correlated with inoculum levels of *P. capsici* in any field. Results demonstrate the importance of soil water in epidemic development.

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EFFECT OF TEMPERATURE ON CONIDIAL PRODUCTION ON SCLEROTIA OF *COLLETOTRICHUM COCCODES* . S. Sanogo, S.P. Pennypacker, and R. Stevenson. Department of Plant Pathology, The Pennsylvania State University, PA 16802.

Conidial production on sclerotia of *Colletotrichum coccodes* was assessed in temperature-controlled chambers set at 14, 22, and 30 C with a regime of 14 hrs light followed by 10 hrs darkness. The average irradiance, provided by cool-white fluorescent lamps, was about 30 wm^{-2} at the surface of sclerotia. Two 9-cm glass petri plates, sealed with parafilm and containing 30 sclerotia placed onto moistened filter papers, were maintained in each temperature treatment chamber for 5 days. Then, sclerotia from each plate were suspended and mixed for 30 sec in 10 ml distilled water contained in 30-ml glass test tubes. To facilitate release of conidia from sclerotia during mixing, about 100 mg of white sand were added to each tube. The resulting suspension was passed through three layers of cheesecloth and a 1 ml-aliquot of the filtered suspension was spread over the surface of acidified potato-dextrose-agar medium in petri plates and incubated at 22 C for 72 hrs. The average numbers of colonies/30 sclerotia were 55, 325, and 195 at 14, 22, and 30 C, respectively. These results suggest that 22 C may be near the optimum temperature for conidial production on sclerotia of *C. coccodes*.

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SPORE DISPERSAL AND INFECTION BY DOWNY MILDEW OF LETTUCE DURING MORNINGS WITH PROLONGED LEAF WETNESS. H. Scherm and A.H.C. van Bruggen, Plant Pathology, University of California, Davis 95616

The ability of downy mildew of lettuce (*Bremia lactucae*) to disperse and infect during mornings with prolonged leaf wetness was tested on potted plants outdoors. The experiments were done at Davis, CA, during radiation fog in the winter. At sunset, healthy test plants (10-days-old) were exposed next to diseased plants with fresh downy mildew lesions and a volumetric spore sampler. Lesions on diseased plants sporulated at night, and spore dispersal began after sunrise. After leaf wetness had dried late in the morning, the test plants were moved to a growth chamber and incubated in conditions conducive to colonization by *B. lactucae* but not to infection. Plants were inspected for disease 10 to 14 days after exposure. Infected plants were observed in 5 out of 13 tests. On these plants, infection can only have occurred during the morning of exposure, concurrently with spore dispersal. Although it had been proposed previously that some downy mildews can disperse and infect during the same morning, this is one of the first reports in which this hypothesis is supported by experimental data. Since a metalaxyl-resistant isolate of *B. lactucae* was used and plants were grown in a metalaxyl-amended nutrient solution, background contamination could be ruled out.

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INTRACLUSTER CORRELATION AND THE PROBLEM OF SAMPLE SIZE DETERMINATION G. Hughes and L.V. Madden. School of Agriculture, University of Edinburgh, Edinburgh EH9 3JG, Scotland, U.K., and Department of Plant Pathology, OARDC, Ohio State University, Wooster, Ohio, USA.

The intracluster correlation coefficient (ρ) provides a measure of the tendency of neighbouring plants, or plant parts, to have similar disease status. If the frequency distribution of diseased plants, or plant parts, per sample is adequately described by the beta-binomial distribution, with parameter estimates $\hat{p}$ (mean disease incidence) and $\hat{\theta}$ (an index of aggregation), an estimate of ρ is provided by $\hat{\theta}/(1+\hat{\theta})$. Then the standard error of $\hat{p}$ is $\sqrt{[\hat{p}(1-\hat{p})(1+\hat{\rho}\{n-1\})/nN]}$ in which N is the number of samples and n is the number of observations per sample. This can be used to determine the sample size required to estimate disease incidence with a prespecified degree of reliability. Generally, the required sample size increases as ρ increases.

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SPATIAL PATTERN OF THE INCIDENCE OF GRAPE DOWNY MILDEW. [1]L. V. Madden, [2]G. Hughes, and [1]M. A. Ellis. [1]Dept. of Plant Pathology, Ohio State University, OARDC, Wooster, 44691, [2]Inst. of Ecology and Resource Management, University of Edinburgh, Scotland.

Aggregation of the incidence of downy mildew of grape, caused by *Plasmopara viticola*, was quantified in an experimental Ohio vineyard. The proportion of leaves on each of 15 shoots (sampling units) per plot was determined for 18 plots at two times (Aug. and Sept.) over 3 yr. Based on the binary data analogue of Taylor's power law, in which the log of the observed variance is regressed on the log of the theoretical variance for a binomial (random) distribution, diseased leaves were aggregated. The extent of aggregation varied with mean incidence consistently over assessment times and years. The overall regression slope (b) was 1.30 (s.e.=0.04). Aggregation in individual plots was measured by the aggregation parameter (θ) of the beta-binomial distribution fitted to the data. Estimates of θ were variable, but were highest between 20 and 60% mean disease incidence.

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OBSERVERS'S BIAS IN THE ASSESSMENT OF ALTERNARIA BLOTCH ON APPLE LEAVES. N. Filajdić and T. B. Sutton, Department of Plant Pathology, North Carolina State University, Raleigh 27695-7616.

The ability of eight observers to accurately assess severity of Alternaria blotch (*Alternaria mali*) on apple leaves and to repeat results obtained in the first assessment was examined. Usefulness of the lower portion of the Horsfall-Barratt scale (0 = no disease, 1 = 1 - 3% of the leaf area covered with lesions, 2 = 4 - 6%, 3 = 7 - 12%, 4 = 13 - 25%, and 5 = 26 - 50%) and a continuous scale (0 - 100% leaf area covered with lesions) was compared as well. Four observers with extensive training in plant pathology did not assess disease more accurately (relative to video image analysis) than did four observers with no previous training, although trained observers needed less time to complete the task. All observers except one underestimated the amount of disease when the Horsfall-Barratt scale was used. Use of the Horsfall-Barratt scale did not prove advantageous in either time efficiency or accuracy, although observers had better repeatability when this scale was used compared to continuous scale of 0 - 100%. A new scale based on preferred numbers (0 - 10, 15, 20, 25, 30, 40, 50, 60, 70, and 75) to assess disease severity of Alternaria blotch is proposed.

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DISPERSAL OF *PHYTOPHTHORA CAPSICI* IN BELL PEPPER SUPPRESSED BY A NO-TILL WHEAT COVER CROP. J. B. Ristaino, G. Parra, and C. L. Campbell. Dept. of Plant Pathology, North Carolina State University, Raleigh.

Spatial and temporal dynamics of disease development were monitored in bell pepper in artificially infested plots with one of three soil cover types: none, black plastic, or stubble from a no-till winter wheat cover crop. Final disease incidence was 72% and spread occurred within and across rows when all dispersal mechanisms were operative in plots with bare soil. Dispersal of soil inoculum was suppressed and disease was only 2.5% in plots with the wheat stubble. Final disease was 42% in plastic mulched plots where a sporulating pepper fruit was placed on the surface, and within and across row spread occurred. Ridomil applied in the drip system did not suppress within row spread of surface inoculum from a sporulating fruit but did limit across row spread and final disease was 11.5%. With plastic mulch, disease was 17% in plots infested with soil inoculum and spread occurred primarily within rows, whereas disease was 9.6% and limited to rows adjacent to initial inoculum when inoculum was placed in furrows. Stubble from a no-till wheat cover crop suppressed Phytophthora epidemics.

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EPIDEMIOLOGY OF BLACK SIGATOKA DISEASE ON PLANTAIN IN NIGERIA. F. Gauhl and C. Pasberg-Gauhl, International Institute of Tropical Agriculture, PMB 5320, Ibadan, Nigeria

Africa contributes more than 50% of the world plantain production. Black sigatoka disease (Mycosphaerella fijiensis) is one of the major constraints to plantain production worldwide, reducing yield by 30-50%. During 1992 and 1993, development of black sigatoka was evaluated in a plot of False Horn plantain cv. Agbagba at IITA Onne Station, southeast Nigeria. Airborne spores of M. fijiensis were collected using a volumetric spore trap. Disease development was highly correlated with rainfall. Incubation time of black sigatoka was 14.1 days in the rainy season and 23.8 days in the dry season, symptom evolution time was 21.9 and 27.8 days, respectively. Seasonality of disease development was also reflected in ascospore counts. Counts peaked during the rainy season and were lowest during the dry season. However, the pattern of spore counts differed over the two years. In 1992, counts of ascospores were highest from May when rain started until November when the rainy season ended. In 1993, the rainy season lasted from March to September, but ascospore counts were only high from June to October. This indicates that although rain is an important factor, other climatic factors may also influence ascospore production. Conidiospore counts remained very low throughout the year, indicating that conidiospores play a secondary role in the spread of M. fijiensis.

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REGIONAL CROP FORECASTING SYSTEMS BASED ON COMPUTED REAL-TIME METEOROLOGICAL VARIABLES. I.A Woodard, Glades Crop Care, 949 Turner Quay, Jupiter, FL, 33458 and C.M. Liddell, New Mexico State University, Las Cruces, NM 88003.

Site specific crop models based on meteorological conditions are currently limited in their spatial implementation due to the need for relatively expensive on-site weather monitoring stations. We have developed a Regional Prediction System (RPS) to implement these models using existing weather stations. The RPS uses MERCURY, a program developed by the Computing Research Laboratory of New Mexico State University to provide real-time computed weather data at all desired locations lacking weather stations. MERCURY uses interpolation and extrapolation techniques and simple heuristic functions of distance, elevation, and land use. The prototype RPS implements a site-specific peanut web blotch model, BLOTCHCAST, and the PNUTGRO peanut yield prediction model. The BLOTCHCAST-RPS was able to predict the initial onset of web blotch to within five days in two of five test plots monitored in 1990 and all four test sites monitored in 1991. Prediction of the disease progress curve was satisfactory. The site specific tests of PNUTGRO and the PNUTGRO-RPS failed to predict peanut yields in the test region of eastern New Mexico.

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CLONING AND MUTAGENESIS OF A PATHOGENICITY FACTOR FROM *STREPTOMYCES SCABIES* IN *STREPTOMYCES LIVIDANS* TK24. R. A. Bukhalid and R. Loria, Department of Plant Pathology, Cornell University, Ithaca NY 14853.

Streptomyces scabies causes the disease potato scab. To identify pathogenicity factors from *S. scabies*, a cosmid (pKC505) library of total genomic DNA from *S. scabies*, strain 84-34, was expressed in *S. lividans* TK24, a non-pathogen. One clone, pKC87.5, which was pathogenic in a tuber slice assay, contained an insert of 12 kb. A

9.4 kb *Bam*H1 fragment was subcloned on the high-copy-number shuttle plasmid vector pWHM3. The resulting clone, pRB101, was more virulent on potato slices than the original cosmid clone or strain 84-34. Cultures of pRB101, and cell-free preparations of those cultures, produced necrosis on tuber slices. pRB101 was mutagenized using Mu dII1734. Approximately half of the clones with insertions in the cloned DNA fragment did not produce necrosis on tuber slices; these insertions are being mapped.

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CLONING A LOCUS FROM *PANTOEA CITREA* RESPONSIBLE FOR THE PINK DISEASE OF PINEAPPLE. J.-S. Cha and C. I. Kado, Dept. of Plant Pathology, University of California, Davis, CA 95616

Historically, the cause of the pink disease of pineapple remained a mystery. The pink disease is characterized by the generation of dark brown pigmentation on pineapple fruit during the canning process making the commodity unmarketable. Of the complex of bacteria claimed to be the causal agents of this disease, *Pantoea citrea* is now considered the major organism responsible for the elaboration of components that catalyze the formation of the discoloration. Thus, mutants deficient in their ability to form the pineapple discoloration were generated by nitrosoguanidine mutagenesis. These mutants were screened by complementation with a genomic bank of the wild type strain and the DNA of a complementing strain was subcloned to a 3.5 kb DNA fragment containing the active locus. Sequencing and characterization of this locus has revealed an interesting function responsible for the pink disease problem.

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ORGANIZATION OF THE *HRP* GENE CLUSTER AND NUCLEOTIDE SEQUENCE OF THE *HRPL* GENE FROM *PSEUDOMONAS SYRINGAE* PV. *MORSPRUNORUM*. L. Z. Liang and A. L. Jones, Department of Botany and Plant Pathology, Michigan State University, East Lansing, 48824

Pseudomonas syringae pv. *morsprunorum* PM7 causes bacterial canker of cherry and induces the hypersensitive response in tobacco. Tn*3*-spice mutagenesis and complementation studies revealed that the *hrp* region of *P. s. morsprunorum* PM7 is organized into seven putative transcriptional units. Units II, VI, and VII exhibited DNA homology with the *hrpI*, *hrpH*, and *hrpZ* genes, respectively, of *P. s. syringae* 61. The nucleotide sequence of *hrpL*, the first transcriptional unit in the *hrp* cluster of *P. s. morsprunorum* PM7, encoded a polypeptide of 185 amino acids. This polypeptide exhibited 94% identity with HrpL of *P. s. syringae* 61. The HrpL protein regulated its own expression and the expression of transcriptional units IV and VI. Two transcriptional start sites, P1 and P2, located 63 and 11 bp upstream of the *hrpL* start codon, were identified by primer extension analysis. The -12 and -24 region of the putative P2 promoter resembled a σ^{54} consensus sequence.

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avrPphC: AN *avr*C HOMOLOGUE THAT IS CLOSELY LINKED TO AN *avr*D ALLELE IN *PSEUDOMONAS SYRINGAE* PV. *PHASEOLICOLA* 3121. I. Yucel, D. Slaymaker, C. Boyd, J. Murillo, R. I Buzzell* and N. T. Keen. University of California, Riverside, CA 92521 and *Research Station, Harrow, Ontario, Canada.

Cosmid clone Psp01 from *Pseudomonas syringae* pv. *phaseolicola* 3121 conferred a unique avirulence phenotype pattern on soybean cultivars when expressed in *P. s.* pv. *glycinea* R4. Psp01 was shown to contain an *avr*D allele as well as a second avirulence gene located ca. 5-kb upstream. The new gene, called *avrPphC*, is highly homologous and phenotypically identical to *avrC*, previously cloned from *P.s.* pv. *glycinea* race 0. Both *avrD* and *avrPphC* occur on the same ca. 120-kb indigenous plasmid in pathovar *phaseolicola* 3121. While *avr*D appears to be widely distributed among different pathovars of *Pseudomonas syringae*, only DNA from *P. syringae* pv. *glycinea* races 0 and 6 hybridized to *avrPphC*. Although previously observed in *Xanthomonas campestris*, this is the first noted occurrence of multiple avirulence genes on a single plasmid in *P. syringae*.

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LOSS OF *avrXa10* ACTIVITY ON RICE BY AMINO ACID SUBSTITUTION IN AVRXa10 PROTEIN W. Zhu and F. F. White, Department of Plant Pathology, Kansas State University, Manhattan, KS, 66506-5502

Xanthomonas oryzae **pv. oryzae (Xoo) carrying *avrXa10* can cause hypersensitive response (HR) on rice BB10 variety containing corresponding resistance gene Xa10. *avrXa10* has 15.5 near-identical, 102bp repeats in the coding region. Amino acids No. 13 and 14 in each repeat are variable. In order to understand the importance of arrangements of repeats on avirulence gene activity, *avrXa10* mutants were obtained with site-directed mutagenesis using a synthesized 34mer oligonucleotide which is complementary to repeat No. 13. Mutants that had mutations at single positions in repeats No. 1, 2, 5, 9, 10, 12, respectively, abolished the ability to give HR on BB10 rice. Single mutations in repeats No. 3, 4, 6, 7, 11, 14, 15, 16, respectively, did not affect avirulence activity. No mutants could cause HR on rice IR24, a susceptible host of Xoo with *avrXa10*.**

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CHARACTERIZATION OF A CHITINASE GENE CLONED FROM XANTHOMONAS MALTOPHILIA ISOLATE 34S1. D. Kobayashi, Dept. of Plant Pathology, Rutgers University, New Brunswick, NJ 08903

Xanthomonas maltophilia isolate 34S1 was previously identified as a biocontrol agent of summer patch disease, caused by *Magnaporthe poae*. *X. maltophilia* 34S1, as well as all other *X. maltophilia* isolates screened, tested positive for chitinolytic activity as detected by clearing zones in agar media supplemented with colloidal chitin. A genomic library of *X. maltophilia* 34S1 was constructed in the cosmid vector pLAFR3 and screened for chitinase activity. No cosmid clones that expressed chitinase activity were identified when the library was screened in *E. coli*. Two overlapping library clones, however, were found to express chitinase activity when the library was mobilized into *Pseudomonas cepacia*. The chitinase gene was subcloned to a 3 kb *Xho*I-*Sac*I fragment, in which expression of activity was observed only in *P. cepacia* and certain other selected *Pseudomonas* strains. Weak expression of chitinase activity could be observed in *E. coli* that contained the gene positioned behind the lac promoter, but only after prolonged incubations of several days, suggesting a problem with secretion of the protein.

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CLONING OF PUTATIVE CHITINASE GENES FROM *BACILLUS* TO CONTROL TOXIN PRODUCING FUNGI. R. Boyapati[1], A.L. Moyne[1], T.E. Cleveland [2] and S. Tuzun[1], Department of [1]Plant Pathology, Auburn University, AL 36849 and [2]Southern Regional Research Center, USDA/ARS, New Orleans, LA 70124.

Success of transgenic expression for the control of fungal pathogens relies upon the availability of genes encoding antifungal proteins that are active against pathogens as well as transportation of the active protein to the site of infection. Chitinases are antifungal enzymes that degrade fungal cell walls by hydrolyzing the β-1-4 linkages of chitin. Chitinolytic bacteria, as sources of antifungal genes, were tested *in vitro* for their antagonistic effects against the aflatoxin-producing fungi *Aspergillus flavus* and *A. parasiticus* and against *Fusarium moniliforme*, a fungus which produces a wide range of toxins. Work done in our laboratories identified an isolate of *Bacillus chitinosporus* (AU192) with very high lytic activity against *Aspergillus* spp. and *F. moniliforme* (Karyala et al., Phytopath. 83:1360). Microscopic observations showed hyphal tip bulging and lysis as evidence of chitinolytic activity. The aim of this project is to clone and characterize chitinase genes from AU192. The strategy adopted for cloning the *Bacillus* chitinase genes, is based on the screening of a gene library for the presence of chitin clearing zone. DNA from AU192 was isolated, partially cleaved with *Sau*3AI and fragments ranging from 2-10Kb in size were purified. The DNA fragments were ligated into pUC18 and the ligation mixture was used to transform *E. coli*. Presence of a clearing zone around colonies on a chitin-containing media has been used to identify putative chitinase clones. The ultimate goal of our project is to express a bacterium-derived antifungal chitinase gene constitutively in transgenic peanut and cotton and to reduce aflatoxin contamination.

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CHARACTERIZATION OF *PSEUDOMONAS SYRINGAE* PV. APII BY RAPD ANALYSIS AND DEVELOPMENT OF PATHOVAR-SPECIFIC PCR PRIMERS. E. L. Little[1], S. T. Koike[2], and R. L. Gilbertson[1]. [1]Plant Pathology Dept., Univ. of California, Davis 95616 [2]Cooperative Extension, Univ. of California, Salinas 93901.

Pseudomonas syringae pv. apii (Psa) causes celery bacterial blight, a potentially severe disease of greenhouse-produced transplants. RAPD primers and PCR were used to examine the genetic diversity of Psa isolates from California, and to determine their relatedness to isolates of *P. s.* tomato (Pst), *P. s.* maculicola (Psm), and isolates of Psa from eastern celery growing regions. No differences were detected among California Psa isolates, but some primers revealed differences between isolates from California and eastern celery growing regions. Pst was differentiated from Psa, although for some primers similar sized fragments were amplified. Pst isolates were genetically more diverse than Psa isolates. An 800 bp band amplified from all Psa isolates, but not from other pathovars, was cloned and partially sequenced. Psa-specific PCR primers were designed that amplified this 800 bp DNA fragment from all Psa isolates tested and a 1.2 kb DNA fragment from Pst isolates, but no fragments from other bacterial species and pathovars. The primers amplified the Psa-specific fragment from boiled bacterial colonies, extracts of diseased tissue, and contaminated seeds.

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A Diffusible Signal Molecule Regulates Phenazine Expression in *Pseudomonas aureofaciens* 30-84. D.W. Wood and L.S. Pierson III. Department of Plant Pathology, University of Arizona, Tucson, AZ 85721.

The expression of phenazine antibiotic genes in the biological control bacterium *Pseudomonas aureofaciens* strain 30-84 is regulated in response to cell density. A positive regulatory gene, *phzR*, has been identified previously whose deduced amino acid sequence shares homology with the LuxR family of regulatory proteins. Proteins in this class regulate the expression of their target genes in a density dependent manner by sensing the accumulation of a diffusible homoserine lactone (HSL) autoinducer. We have identified cloned and sequenced a second gene, *phzI*, responsible for the production of a diffusible signal molecule. The predicted amino acid sequence of this gene shares homology with proteins of the *luxI* family involved in production of the diffusible homoserine lactone signal molecules. Cell free supernatants from *Escherichia coli* strains carrying the *phzI* gene induce phenazine gene expression at lower cell densities than controls lacking *phzI*. These results suggest that *phzI* may be involved in the production of a specific intercellular signal which regulates phenazine gene expression. We are currently examining other rhizosphere organisms to determine if they produce similar signal molecules which are able to affect expression of the phenazine biosynthetic genes in 30-84.

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CHARACTERIZATION OF THE IRON-REGULATED AEROBACTIN PROMOTER OF *ERWINIA CAROTOVORA*. M. J. Boehm and J. E. Loper, USDA-ARS Horticultural Crops Research Lab., 3420 N.W. Orchard Ave., Corvallis, Oregon 97330.

The promoterless ice nucleation activity gene (*inaZ*) was used as a reporter system to evaluate the expression of genes encoding the production of aerobactin, a hydroxamate siderophore, produced by *Erwinia carotovora*. Many but not all strains of *E. carotovora* expressed ice nucleation activity (INA) from an introduced *inaZ* gene transcribed from the *lac* promoter of *Escherichia coli*. It appeared, therefore, that the *inaZ* reporter would be useful in most strains of this plant pathogen. Insertions of Tn*3*-Spice in the same orientation into six sites within the cloned aerobactin biosynthesis genes (*iuc*) of *E. carotovora* disrupted aerobactin biosynthesis and conferred iron-regulated INA. Two additional insertions, which were in the opposite orientation from those described above, disrupted aerobactin biosynthesis but did not confer INA. A 0.9-kb fragment upstream of the six ice-active insertions had promoter activity when cloned into a chloramphenicol acetyltransferase promoter probe vector. A region within this 0.9-kb fragment was significantly similar to the promoter region of the aerobactin operon of *E. coli*.

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COMPARISON OF FOUR PROMISING BIOLOGICAL CONTROL AGENTS FOR MANAGING POSTHARVEST DISEASES OF APPLES AND PEARS. S. N. Jeffers and T. S. Wright. EcoScience Corporation, 377 Plantation St., Worcester, MA 01605.

Four biological control agents, each with demonstrated efficacy against postharvest diseases of apples or pears, were compared in trials conducted in 1992 in Massachusetts, Michigan, and Washington. The four agents, two bacteria (isolate ESC-10 discovered by EcoScience Corp. and isolate ESC-11 discovered by W. J. Janisiewicz, USDA, Kearneysville, WV) and two yeasts (isolates ESC-15 and ESC-230 discovered by R. G. Roberts, USDA, Wenatchee, WA), were compared for ability to protect Red and Golden Delicious apples and d'Anjou pears from postharvest decay by *Penicillium expansum*, *Botrytis cinerea*, and *Mucor piriformis* (all at 10^3-10^4 conidia/ml). Fruits were wounded; dipped in suspensions of only pathogens or pathogens mixed with bacteria (10^7-10^9 cfu/ml), yeasts (10^7-10^8 cfu/ml), or thiabendazole (label rate); and incubated in cold storage for up to 18 wk or in controlled atmosphere storage for up to 35 wk. The four agents significantly reduced disease incidence from all three pathogens and usually provided better control than thiabendazole. ESC-11 and ESC-15 provided the best protection of d'Anjou pears. Differences in efficacy among agents on apple cultivars occurred in 7 of 10 trials. Treatments that gave superior protection were ESC-15 in 8 of 10 trials, ESC-10 in 7 of 10 trials, ESC-11 in 6 of 10 trials, and ESC-230 in 4 of 5 trials. Additional trials are ongoing and include wettable powder formulations of both ESC-10 and ESC-11.

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AVIRULENT GEOTRICHUM CANDIDUM FOR BIOLOGICAL CONTROL OF GREEN MOLD ON GRAPEFRUIT. C. G. Eayre, and Mani Skaria. USDA ARS, Subtropical Agricultural Research Laboratory, Weslaco, Texas 78596, and Texas A&M University-Kingsville Citrus Center, Weslaco, TX 78599.

An avirulent culture of Geotrichum candidum reduced the incidence of green mold, caused by Penicillium digitatum, on grapefruit. Green mold is the most prevalent postharvest disease of citrus in Texas. Harvested grapefruit were not washed, leaving any naturally occurring P. digitatum inocula. Fruit were wounded with a nail, and the wounds were treated with 10 µl of G. candidum spore suspension (10^4 spores/µl), made from the avirulent culture. The fruit were stored in plastic bags at 22C. Control fruit were wounded and treated with sterile distilled water. Disease incidence was recorded after 7 days. Each test included 5 replications of 10 fruit each, and tests were run three times. Disease incidence varied, but the reduction in green mold incidence in fruit treated with the avirulent G. candidum was always significant. The avirulent G. candidum culture was obtained by frequent subculturing of a virulent culture on potato dextrose agar in the lab.

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INDUCED SYSTEMIC RESISTANCE AGAINST BACTERIAL WILT OF CUCUMBER BY SELECT PLANT GROWTH-PROMOTING RHIZOBACTERIA. C. Yao, G. Wei, G. W. Zehnder, R. A. Shelby, and J. W. Kloepper. Dept. of Plant Pathology, Biological Control Inst., Ala. Agric. Exp. Sta., Auburn Univ., AL 36849.

In 1992 field trials, select plant growth-promoting rhizobacteria (PGPR), which previously exhibited induced systemic resistance (ISR) against cucumber anthracnose (Phytopathology 81:1508-1512), demonstrated significant (P=0.05) protection from naturally occurring bacterial wilt of cucumber, caused by *Erwinia tracheiphila*. In follow-up studies, two PGPR strains repeated significant protection from the wilt disease in the greenhouse. Mean disease incidence 17 days after inoculation of *E. tracheiphila* was reduced 30-50 % by seed treatments plus soil drenches with these two PGPR strains. In contrast, the "classical ISR control" (previously inoculated with *C. orbiculare*) did not induce resistance to bacterial wilt in the field test. In 1993 field trials, there were significantly fewer cucumber beetles (vectors of *E. tracheiphila*) in the PGPR-treated plots compared to those of the noninduced controls. In investigations of the mechanisms for resistance, the concentration of cucurbitacin, which induces cucumber beetle feeding, was found to be significantly reduced by one of the PGPR strains. Work is continuing to determine the potential of incorporating PGPR with ISR activity into an IPM system for controlling bacterial wilt and cucumber beetles.

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Antibiotic-producing strain of *Erwinia herbicola* as a potential biological control agent for *Alternaria solani*. L. S. Sujkowski, Y. Ophir, W. E. Fry and S. V. Beer. Department of Plant Pathology, Cornell University, Ithaca, NY 14853

Erwinia herbicola Eh425, an epiphytic strain isolated from pear flowers in Israel produces substances *in vitro* that inhibit the growth of several phytopathogenic bacteria and fungi. In a dual culture assay system, spore germination and mycelial growth of *Alternaria solani* were both completely inhibited by the bacterium and by supernatant from the bacterial culture. Application of bacterial suspensions suppressed early blight caused by *Alternaria solani* on tomato foliage in a controlled environment chamber. Lesion diameter decreased by 60% and lesion numbers by 35%, relative to untreated plants. Transposon-induced mutants of Eh425, deficient in antibiotic production and culture supernatant from these mutants, were not inhibitory to bacteria and fungi inhibited by the wild-type strain. Strain Eh425 and its antibiotic have potential as biological control agents for early blight and possibly other organisms.

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ISOLATION OF A GENE FROM *ENTEROBACTER CLOACAE* THAT AFFECTS BIOLOGICAL CONTROL OF *PYTHIUM ULTIMUM* SEED ROT. Alan P. Maloney and Eric B. Nelson, Department of Plant Pathology, Cornell University, Ithaca, NY 14853.

Bacterial gene products corresponding to *phoA*$^+$ mutants should reside in the bacterial periplasm, outer cell membrane or cell wall and are thus potentially involved in biological control interactions between a cell and its surrounding environment. A mini-Tn*5*/*phoA* mutagenesis strategy and complementation with a wild-type cosmid library were used to identify a gene in *Enterobacter cloacae* strain EcCT-501 that plays a major role in the biological control *Pythium ultimum* seed rot of cucumber. Of about

2000 mutants recovered, 5% were *phoA*$^+$, of which four had reduced ability to suppress *P. ultimum* seed infection in a bioassay. One mutant, V58, exhibited a complete loss of biocontrol as well as loss of the ability to utilize several structurally unrelated carbon sources, including several amino acids, carbohydrates, and acetate. Populations of V58 persisted in sand-based bioassays, and, on undefined media, grew nearly as well as the parental strain. A wild type cosmid corresponding to V58's mutation was hybrid-selected and conjugationally transferred to the mutant, which restored both biological control and catabolic functions to wild-type levels. The isolated gene, *psp1*, appears to be different from most other genes known to affect biological control activity in individual bacterial isolates.

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INDUCTION OF SYSTEMIC RESISTANCE IN CUCUMBER AGAINST *COLLETOTRICHUM ORBICULARE* BY BIOLUMINESCENT DERIVATIVES OF PLANT GROWTH-PROMOTING RHIZOBACTERIA (PGPR) STRAIN 89B-27. L. Liu[1], J.W. Kloepper[1], J.J. Shaw[2], and S. Tuzun[1]. [1]Dept. of Plant Pathology and [2]Dept. of Botany and Microbiology, Auburn University, AL 36849.

Six bioluminescent derivatives of PGPR strain 89B-27 (*Pseudomonas putida*) were tested for their abilities to induce systemic resistance (ISR) in cucumber against anthracnose caused by *C. orbiculare*, and were compared to wild-type 89B-27 and a classical ISR control (induced by *C. orbiculare*). The bioluminescent derivatives were obtained by biparental matings between 89B-27 and *E. coli* strain DH5α (pUCD623); pUCD623 is a plasmid which carries Tn*4431* with the *luxCDABE* genes of *Vibrio fischeri*. Light emitted by the bioluminescent derivatives was detected with a charge-coupled device (CCD) camera. Bioassays for ISR activity of the derivatives were conducted in soilless Pro-Mix mix in a greenhouse, and total lesion diameter (TLD) was analyzed using the general linear model. Four of the six derivatives protected cucumber significantly compared to the nonbacterized control in each of 3 experiments. One derivative even showed significant reduction of TLD compared to wild-type 89B-27. Mean TLD was 260.3 mm for the disease control, 185.9 mm for wild-type 89B-27, and 160.2 mm and 144.7 mm for the 2 best bioluminescent derivative strains. The results indicates that most bioluminescent derivatives retained their activity to induce systemic resistance in cucumber, hence, *lux* is a good genetic marker system for investigation of colonization and population dynamics of PGPR which induce systemic resistance.

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A STANDARD METHOD FOR STUDYING THE MICROBIAL ECOLOGY OF FRUITS. Tara Chand-Goyal and Robert A. Spotts, Mid-Columbia Research and Extension Center, Oregon State University, 3005 Experiment Station Drive, Hood River, OR 97031.

A standard method was developed to study the surface microflora of unsprayed fruits. Apple fruits (Golden Delicious) were individually placed in each beaker containing 150 ml of sterile phosphate buffer (0.05M, pH 7.0) and Tween 80 (0.005%). Beakers were placed on a rotary shaker (150 rpm) for 5 min before taking samples for plating. After shaking, the beakers containing fruits and buffer tween were placed in an ultrasonic bath (43 KHz, 270 Watts) and samples were removed after 1, 2, 5, 10 and 15 minutes of ultrasonication. Serial dilutions of samples were plated on diluted nutrient broth agar for bacteria and diluted yeast malt agar for yeasts. The bacterial and yeast colonies were enumerated and characterized after 14 and 7 days of incubation at 20 C, respectively. Washing followed by 5 minutes of ultrasonication was optimum for isolating most diverse bacterial and yeast microflora from fruit surface. More than 5 minutes of ultrasonication resulted in an increase of filamentous fungal propagules that created difficulty in the accurate enumeration and characterization of bacteria and yeast.

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INCREASED PEROXIDASE ACTIVITY IN BEAN SEEDLINGS PROTECTED FROM *RHIZOCTONIA SOLANI* INFECTION WITH NON-PATHOGENIC BINUCLEATE *RHIZOCTONIA* SPECIES. S. J.-Hare[1], P. Masilamany[1], & P.M. Charest[2]. 1. Dept. of Plant Science, McGill University, Ste.-Anne-de-Bellevue, 2. Dept. de Phytologie, Université Laval, Ste.-Foy, Quebec, Canada

Non-pathogenic binucleate *Rhizoctonia* species (BNR) can protect bean seedlings from Rhizoctonia root rot. Although the mechanism of protection in this system is not well understood, activation of host-defence mechanisms or induced resistance appears to be involved. Enhanced peroxidase activity has been associated with induced resistance in many host-parasite interactions, but little is known about the role of peroxidases in the *R. solani*-bean-BNR interaction. We have estimated peroxidase activity in protected and non-protected bean tissues. Total peroxidases were estimated in etiolated bean hypocotyls that were: (i) inoculated with BNR, (ii) inoculated with BNR and then infected with *R. solani*, (iii) infected with *R. solani*, and (iv) untreated (control). Significantly higher soluble peroxidase activity ($P < 0.05$) was detected in protected bean tissues as compared to tissues infected with *R. solani* and the control. Ionically- and covalently-bound peroxidases followed the same trend. Bean tissues with high peroxidase activity were resistant to *R. solani* infection. Disease severity of *R. solani* was negatively correlated with increased peroxidase activity. These results suggest that protection of bean seedling by BNR is related to enhanced peroxidase activity.

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ENZYMATIC ACTIVITY OF *TRICHODERMA HAMATUM* GROWN ON SCLEROTIA OF *SCLEROTINIA SCLEROTIORUM* DAMAGED BY FUNGUS GNATS. J.A. Gracia[1], B. Bailey[2], T.C. Paulitz[1], R.D. Lumsden[2], D. Roberts[2] [1]McGill University, Ste. Anne de Bellevue, Québec, Canada H9X 3V9. [2]USDA-ARS, Biocontrol of Plant Diseases Laboratory, Beltsville, MD, 20705.

In previous work, sclerotia of *Sclerotinia sclerotiorum* simultaneously exposed to the biocontrol agent *Trichoderma hamatum* and fungus gnats *(Bradysia coprophila)* were degraded faster than when each was acting individually. A series of tests were carried out to evaluate the susceptibility of sclerotia, previously damaged by the larval activity of *B. coprophila*, to degradation by *T. hamatum*. Sclerotia were subjected to different levels of mechanical or larval feeding damage. Mycelial growth of *T. hamatum* and its enzymatic activity of glucanase and chitinase were measured. As damage increased, the activity of both ß-1,3-glucanase and chitinase decreased. In contrast, mycelial growth increased as sclerotial damage increased. Sclerotia with a high degree of mechanical or larval damage (50 to 100% of the rind removed) exuded greater amounts of amino acids, carbohydrates, proteins and electrolytes. Damaged sclerotia may release higher amounts of exudates that increase the mycelial growth of *T. hamatum* without the induction of high levels of fungal cell wall-degrading enzymes.

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EFFECT OF VOLATILES PRODUCED BY SOME SOIL FUNGI ON WHEAT BRAN ON GROWTH OF *Pythium dimorphum*, CAUSE OF LONGLEAF PINE DAMPING-OFF DURING COLD STORAGE. Xiaoan Sun and J.P. Jones, Dept. of Plant Pathology and Crop Physiology, LA. Expt Stn., LA State University Agr. Center, Baton Rouge, LA 70803

The observation that some *Trichoderma* isolates inhibited *Pythium dimorphum* without coming in physical contact in dual inoculation tests on a wheat bran substrate indicated that some mechanisms other than parasitism were involved. In an enclosed petri dish system, *Trichoderma harzianum, T. koningii, T. piluliferum, T. hamatum, Gliocladium* sp. and *Pestalotia* sp. growing on wheat bran killed or inhibited *Pythium dimorphum* growing on corn meal agar (CMA) without physical contact, while isolates of *P. dimorphum* did not. Sterile CMA, which had been suspended over wheat-bran cultures of the above fungi also inhibited the growth of *P. dimorphum*. Low oxygen concentration apparently was not correlated with inhibition of *P. dimorphum* even when it changed significantly in the inhibitory tests. Water extract of the wheat bran inoculated with individual fungi was not toxic to *P. dimorphum* in preliminary tests. These results suggest that volatiles may play a role in the biocontrol processes.

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FIELD EVALUATION OF BIOLOGICAL CONTROL AGENTS AGAINST RHIZOCTONIA SHEATH BLIGHT OF RICE. T.W Mew, A.M.Rosales, G.V. Maningas, I.F.Telan[1], and T.H.Xuan[1].International Rice Research Institute, Manila, and [1]Philippine Rice Research Institute, Munoz, Nueva Ecija, Philippines.

The effect of biocontrol agents (BCA) in suppressing sheath blight (ShB) was evaluated during 1992 and 1993 wet seasons and 1993 dry season at IRRI and during 1992 wet and 1994 dry seasons at PhilRice, Nueva Ecija. ShB incidence and severity were not significantly affected by BCA in wet season 1992. However, treatment with BCA either singly or in combination reduced significantly secondary infection and sclerotial infectivity in the same year. An increase in yield was observed associated with BCA application for all three seasons. In 1992 wet season trial at PhilRice, a mixture of two *Bacillus* strains reduced ShB incidence. In the 1994 dry season, plants treated singly with *Pseudomonas cepacia* and a mixture of two *Bacillus* strains had lower ShB incidence and severity than untreated plants at 55 days after transplanting at 90 kg N/ha. The percent increase in tiller count in plants treated with mixtures of isolates ranges from 4.13% to 9.34% at zero N application. At 180 kg N/ha, the percent increase in tiller count ranges from 8.1% to 13.96 % in BCA-treated plants either singly or in mixtures.

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FUNGAL COLONIZATION OF GRAPEVINE PRUNING WOUNDS. Coleman, P. M. and Marois, J. J., Department of Plant Pathology, University of California - Davis, 95616.

Pruning wounds provide an infection court for the fungus *Eutypa lata*, cause of Eutypa dieback of grapevines. Non-pathogenic fungi were applied to pruning wounds to determine their ability to colonize a wound surface. Wounds treated with 10^5 spores/ml *Fusarium lateritium*, *Penicillium* sp. or *Cladosporium herbarum* had higher numbers of these fungi than did untreated wounds over the 23 day sampling period, both at 21C and at 10C. In other experiments, application of 10^6 spores/ml of *F. lateritium* on 0 or 7 days after pruning resulted in higher populations on treated than on untreated wounds. Application of 10^6 spores/ml of *C. herbarum* on 0 days resulted in higher populations on the treated than untreated wounds, but treatment on 7 days had no effect. Fungi that colonize the wound are potential biological control agents for *Eutypa*.

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STUDY OF MICROBIAL ECOLOGY IN A CROP ROTATION PROGRAM WITH SOYBEAN, VELVETBEAN, AND WINTER CROPS. Roberto Vargas, R. Rodríguez-Kábana, and J.W. Kloepper. Dept. of Plant Pathology, Biological Control Institute, Ala. Agric. Exp. Sta., Auburn University, AL 36849.

A rotation program was established to assess shifts in populations of microorganisms in the soil and rhizosphere. Field microplots were planted with velvetbean (*Mucuna deeringiana*) or cowpea (*Vigna unguiculata*), followed by wheat (*Triticum aestivum*), clover (*Trifolium* sp.), or winter fallow. Soybean (*Glycine max*) was planted in the second year. Bacteria and fungi were isolated from the soil and rhizosphere of velvetbean, cowpea, wheat, clover, and soybean and plated on Tryptic Soy Agar and Ohio Agar at the end of each season. Predominant bacteria were identified using fatty acid analysis. Lower populations of bacteria and fungi were found in microplots planted with velvetbean compared to those with cowpea. Rotations with the highest yield and lowest nematode population were correlated with higher populations of bacteria and fungi. Microplots planted with velvetbean and clover showed a high incidence of fungi associated with nematode-parasitism in the second year. No significant differences were found in bacterial or fungal populations using richness and evenness indices. Results suggest that velvetbean decreased microbial populations in soil and stimulated the development of beneficial microorganisms.

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EFFECTS OF COVER CROP DECOMPOSITION STAGES ON GROWTH OF TWO FUNGI IN VITRO. F. Workneh, N.J. Grünwald, and A.H.C. van Bruggen, Plant Pathology Department, University of California, Davis, CA 95616

Soils were sampled from organic and conventional field plots in a replicated experiment with 4 blocks. All plots had been cover cropped with an oat-vetch mix. Samples were collected 1 wk before incorporation of cover crops, and 1, 3, and 5 wk after incorporation. Fifteen ml of each soil sample were either sterilized or not sterilized and then spread on sterile cellophane strapped around a metal ring in a petri dish. Cooled, molten agar (0.8% water agar) was poured over the soil and incubated overnight. The agar-ring was turned over, and a mycelial plug of *Phytium aphanidermatum* or *Rhizoctonia solani* was then placed on the cellophane. Radial growth rates of both fungi on nonsterilized soil were lowest before incorporation of cover crops, highest 1 wk after incorporation and declined 3 and 5 wk after incorporation. Suppression of growth in nonsterilized relative to sterilized soil increased 3 and 5 wk after incorporation compared to 1 wk after incorporation and was greater in organically than conventionally managed soil. Placement of germinated seeds under the cellophane decreased soil suppressiveness but differences between organically and conventionally managed soils remained.

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TRAITS OF *Bacillus megaterium* B153-2-2 AFFECTING ITS SOYBEAN ROOT COLONIZATION. X. Y. Zheng and J. B. Sinclair, Department of Plant Pathology, University of Illinois at Urbana-Champaign, 1101 W. Peabody Drive, Urbana, IL 61801.

Traits of B153-2-2 for motility (Mot), chemotactic response (Che), sporulation (Spo) and antagonism (Ant) to *Rhizoctonia solani* were studied for their effect on soybean root colonization in clay and sandy soils in the greenhouse. Altered B153-2-2 using 0.1 M ethylmethane sulfonate resulted in mutants 1013LR ($Ant^-Mot^{+/-}Che^{+/-}Spo^+$), 1013R ($Ant^+Mot^+Che^+Spo^+$) and SDD12 ($Ant^+Mot^+Che^-Spo^+$). These plus M2144 ($Ant^+Mot^+Che^+Spo^+$, a nitrosoquanidine-induced mutant), and B153-2-2 ($Ant^+Mot^+Che^{+/-}Spo^{+/-}$) were applied to seed or soil without or with *R. solani*. Results showed that Ant^+ mutants were better root colonizers than Ant^- mutants in both soils; the greater the Che and Mot, the greater was root colonization in clay soil; seed was more efficient than soil inoculation at 0 to 5 cm soil; B153-2-2 populations were higher in 0 to 5 cm soil; and Spo^- did not survive.

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RHIZOSPHERE COLONIZATION PATTERNS OBSERVED WITH TWO *BACILLUS SUBTILIS* SEED INOCULANTS OF COTTON. P. M. Brannen and P. A. Backman, Dept. of Plant Pathology, Auburn University, AL 36849-5409.

In a 2-year field study, GB03 and GB07 (Gustafson, Inc., Plano, TX) strains of *Bacillus subtilis* were applied at different inoculum levels to cotton seeds, and temporal/spacial rhizosphere colonization patterns were recorded. All seed were pretreated with metalaxyl-PCNB-carboxin. Initial bacterial counts obtained on seed for 1992 and 1993 ranged from log 4.5-7.0 and log 6.2-7.0 CFU g^{-1} seed, respectively. For both years and both strains, the initial inoculum levels applied on the seed were correlated with rhizosphere colonization of young (<35 DAP) and end-of-season root systems. The relationship between inoculum level and early rhizosphere colonization when using GB03 was better described by a logistic dose-response curve [$Y = 0.92 + 3.20/(1+(X/5.50)^{-113.92})$]($R^2 = 0.93$) than a linear trend. With GB07, insufficient intermediate inoculum points were achieved, so the relationship could only be defined as linear ($R^2 = 0.80$). Rhizosphere colonization was reduced for both GB03 and GB07 strains on lateral roots and lower taproots (> 5 cm soil depth). However, GB07 was a more effective colonist of feeder roots distal to the point of inoculation.

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DIRECT EXTRACTION AND ANALYSIS OF FATTY ACIDS TO STUDY MICROBIAL COMMUNITIES IN RHIZOSPHERES OF DIFFERENT CROPS. P.A.H.M. Bakker, J.E. Lawrence, D.D. Jurkonie and D.A. Kluepfel, Dept. of Plant Pathology and Physiology, Clemson University, 120 Long Hall, Clemson, SC 29634-0377.

Crop specificity with regard to microbial communities that develop in the rhizosphere has been suggested in literature, but so far conclusive data have not been reported. Direct extraction of fatty acids and analysis of their GC retention profiles offers a tool to study diversity in microbial communities without the necessity to culture the micro-organisms prior to analysis (Presting *et al.* 1993, Phytopathology 83: 1367). In the present study we evaluate the sensitivity of fatty acid extraction in the detection of differences in microbial communities in soils and rhizospheres and its use to study communities in rhizospheres of tomato and wheat grown in the same soil.

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ROOT BORDER CELL GENE EXPRESSION: IMPLICATIONS FOR RHIZOSPHERE INTERACTIONS. Lindy A. Brigham, Ho-Hyung Woo, and Martha C. Hawes. Departments of Plant Pathology and Molecular and Cellular Biology, University of Arizona, Tucson, AZ 85721, USA

Roots of many plant species release thousands of individual, metabolically active cells into the rhizosphere. We propose that root border cells regulate the microbial populations in the environment of the growing root by selective attraction, repulsion, or nutrient accessibility. Our approach to test these hypotheses is to manipulate gene expression in root border cells. We have demonstrated that root border cells undergo a rapid and dramatic change in protein synthesis upon separation from the root cap: numerous proteins synthesized in abundance by root border cells are undetectable in progenitor root cap cells, and vice versa. Results using mRNA differential display assays indicate that this change in protein profiles is, at least in part, regulated at the level of transcription. Expression of several mRNAs in root caps and root border cells have been analyzed with respect to temporal and spatial regulation.

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FIELD INOCULATION WITH VAM FUNGI TO REVEGETATE COARSE TACONITE TAILING. R. K. Noyd[1], F. L. Pfleger[1], and M. R. Norland[2] [1]University of Minnesota, St. Paul, MN 55108. [2]U. S. Bureau of Mines Research Center, Minneapolis, MN 55417.

Revegetation of coarse taconite tailing, or crushed bedrock, is constrained by extremely low amounts of available P (<1 mg kg^{-1}) organic matter (~0%), and low water retention (1% w/w). Inoculation with vesicular-arbuscular mycorrhizal fungi (VAMF) significantly improved the growth of native grasses in tailing in pot experiments. In the field, a factorial experiment was conducted to study the effect of 3 revegetation treatments (VAMF inoculation, 3 rates of fertilizer, and 3 rates of composted yard waste) on plant cover of 5 seeded native prairie species, big bluestem (*Andropogon gerardi*), little bluestem (*Schizachyrium scoparium*), Kalm's brome, (*Bromus kalmii*), Canada wild rye, (*Elymus canadensis*), bush clover (*Lespedeza capitata*) and nonseeded volunteer species. Each inoculated plot received spores and 2.5 g of root segments of sudan grass (*Sorghum sudanense*) infected with *Glomus intraradix*, a VAMF species frequently associated with existing vegetation at the site. After 2 growing seasons, over all treatment combinations, total plant cover was significantly greater in inoculated plots (35%) than non-inoculated plots (29%). Using *E. canadensis* as a host, infectivity bioassays from inoculated plots had a mean percent root length colonization of 27%. Field inoculation of *G. intraradix* promotes the successful establishment of native prairie plants on coarse taconite tailing.

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ISOLATION OF SOIL BACTERIA THAT DEGRADE THE FUNGICIDE CARBENDAZIM. M.A. Holtman, and D. Y. Kobayashi. Dept. of Plant Pathology, Rutgers University, New Brunswick, NJ 08903

Bacteria capable of degrading carbendazim were isolated from continuous enrichment cultures consisting of minimal medium supplemented with the fungicide as a sole carbon source. Soil inocula for the cultures were obtained from field plots or golf courses previously subjected to heavy applications of benzimidazole class fungicides. At least 15 different bacterial isolates were identified by their ability to grow on a minimal solid medium containing 0.1% carbendazim. Clearing zones of particulate carbendazim were observed for 9 of

the 15 isolates. HPLC analysis of culture filtrates from these bacteria grown in minimal medium broth supplemented with 84 μM carbendazim indicated loss of the compound over a 30 day period. Isolates varied according to rates of degradation, of which the slowest was 30% in 30 days. The most rapid degradation was achieved by two *Rhodococcus* spp., in which 100% loss was detected within 15 days. Growth studies indicated population increases that corresponded with loss of carbendazim in cultures.

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SURVIVAL OF *VERTICILLIUM DAHLIAE* MICROSCLEROTIA IN DIFFERENT SOILS. T.A. Wheeler, R.C. Rowe, and L.V. Madden, Dept. of Plant Pathology, OARDC, Ohio State University, Wooster, OH 44691.

Soil from four potato fields (2 silt loam [12% sand, 68% silt, and 21% clay; 19% sand, 58% silt, and 23% clay], 1 peat [76% organic matter], 1 loamy sand [81% sand, 13% silt, 6% clay] were placed in mesh pouches, infested with equal numbers of microsclerotia of *V. dahliae* obtained from dried potato stems, and buried in the same soil in the pouches at a depth of 16 cm. After 7 and 12 mo pouches were removed and assayed for *V. dahliae*. Microsclerotial survival (as compared to assayed in microsclerotia density at the time of pouch burial) was significantly higher in the two silt loam soils (44% and 29% after 7 mo; 26% and 24% after 12 mo) than in the loamy sand (15% and 10% after 7 and 12 mo) or peat soil (6% and 7% after 7 and 12 mo). Rate of decrease in viable microsclerotial density for the first 6 mo (Nov. to May) was 8-10%/mo in silt loam soils, 12%/mo in a loamy sand and 13%/mo in a peat. However, during May to Oct., rate of microsclerotial decline was only 1-3.5%/mo in the silt loam soils, 0% in the peat and 1% in the loamy sand.

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WATER RELATIONS OF *PHYTOPHTHORA CAPSICI* ZOOSPORES AND MYCELIA DURING VEGETATIVE GROWTH AND ZOOSPOROGENESIS. C.M. Liddell, T.L. Jones, and H. Palmer. New Mexico State University, Department of Entomology, Plant Pathology, and Weed Science, Box 3BE, Las Cruces, NM 88003, U. S. A.

Irrigation practices are known to influence the progress of *Phytophthora* diseases such as root rot of peppers caused by *Phytophthora capsici*. Laboratory experiments were conducted to understand the water relations mechanisms acting in the pepper-*P. capsici* pathosystem. Fifty percent of *P. capsici* zoospores were motile after 4 hours in dilute (2%) soil extract (SE) and all encysted by 10 hours. Zoospores remained motile longer in SE of moderate ionic strength (similar to irrigation water) but motility decreased rapidly in high ionic strength SE. Vegetative growth of *P. capsici* occurred down to -10kPa in natural soil and sporangium development on living roots was found to be very sensitive to cyclic changes in matric potential. Experiments on the quantitation of *P. capsici* from soil showed that absolute counts are difficult to obtain but relative counts by the dilution plate method are reproducible for use in modelling studies. Zoosporogenesis is the only stage of the *P. capsici* life cycle that is sufficiently influenced by soil water to explain the effect of irrigation management on disease development.

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SUPPRESSION OF SUMMER PATCH IN TURF WITH ACIDIFYING NITROGEN FERTILITY PROGRAMS. D. C. Thompson[1], B. B. Clarke[1], J. R. Heckman[2] and J. A. Murphy[2], Departments of Plant Pathology[1] and Plant Science[2], Rutgers University, New Brunswick, NJ, USA 08903

The impact of nitrogen (N) source, nitrification inhibition, and the timing and rate of N application on the severity of summer patch was evaluated in the field and in controlled environments. Ammonium N provided the greatest level of disease suppression, followed by sulfur coated urea and ureaform. The highest level of disease occurred using nitrate N. Urea, methylene urea, and no nitrogen resulted in an intermediate level of disease. The severity of summer patch decreased as the rate of ammonium N increased. In contrast, as the rates of urea, methylene urea, and nitrate N were increased, disease severity increased. The timing of N application did not significantly affect disease severity. Bulk soil pH and rhizosphere pH were positively correlated with summer patch severity, and little disease was observed below pH 6.0 and 5.5, respectively. *M. poae* was difficult to detect on turf roots in the field after 3 yr of ammonium N application. Controlled environment studies confirmed that ammonium N suppressed summer patch development. Addition of a nitrification inhibitor enhanced disease suppression by ammonium N in the controlled environment, but not in the field.

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MICROENVIRONMENTAL FACTORS AFFECTING BROWN PATCH DISEASE SEVERITY IN TALL FESCUE. L.J. Giesler, G.Y. Yuen, and G.L. Horst, Departments of Plant Pathology and Horticulture, University of Nebraska-Lincoln, Lincoln, NE 68583-0722.

In field plots of tall fescue (*Festuca arudinacea*) 'Fawn', planted at three densities, severity of brown patch disease, caused by *Rhizoctonia solani* AG 1-IA, increased with increasing plant density. Canopy temperature, leaf wetness duration, relative humidity, and canopy air temperature were measured during the experiment using microenvironmental instrumentation. No differences were found for any of the environmental parameters among the three plant densities that could explain observed differences in disease. In a growth chamber experiment, 'Fawn' planted at three densities was provided with uniform environmental conditions. *R. solani* mycelial growth and spread of necrosis through these canopies was observed. Hyphal growth between grass blades occurred more readily in high seedling densities, and differences in bridging ability was related to rate of necrosis development. We conclude that the affect of plant density on disease in the field was not due to canopy microclimatic influences, but was related to proximity of grass blades within the canopies.

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INFLUENCE OF TEMPERATURE AND RELATIVE HUMIDITY ON GERMINATION OF CONIDIA OF *OIDIUM* SPP. ON POINSETTIA. M.K. Hausbeck and J. Kalishek. Dept. of Botany and Plant Pathology, Michigan State University, East Lansing 48824.

Powdery mildew (*Oidium* spp.) on poinsettias (*Euphorbia pulcherrima*) growing in commercial greenhouses was first observed in Michigan in 1991. Effects of temperature and relative humidity (RH) on germination, appressorium formation, and shriveling of conidia were studied in 15, 20, and 25 C growth chambers. Conidia were incubated in sealed containers for 24 hr on 9-mm-diameter leaf disks cut from mature leaves of poinsettia 'Freedom'. The disks were placed on 2.5 x 1.25 cm pieces of water agar on screening over saturated salt solutions that provided 95, 85, 75, 65, 55, 45, and 35% RH. A minimum of 100 conidia on each of three leaf disks were observed and each experiment was conducted three times. Maximum germination of conidia was 72, 61, and 42% at 25, 20, and 15 C, respectively, when RH was 85%. Appressorium formation was not significantly affected by temperature or RH with >89% of the germinated conidia forming appressoria. Shriveling of conidia was least (6%) at 25 C and 95% RH; greatest (39%) at 20 C and 35% RH.

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IMPACT OF TEMPERATURE AND WATER POTENTIAL ON THE GROWTH RATE OF THREE ECTOTROPHIC ROOT INFECTING PATHOGENS OF TURFGRASS. K. A. Plumley and B. B. Clarke. Department of Plant Pathology, Cook College, Rutgers University, New Brunswick, NJ 08903.

Three fungi with an ectotrophic growth habit, *Magnaporthe poae, Leptosphaeria korrae*, and *Gaeumannomyces incrustans*, are known to infect the roots of turfgrass species under a variety of cultural and enviromental conditions. These fungi were utilized in a laboratory study to (1) evaluate the impact of temperature and water potential, both independently and interactively, on fungal growth rates, and (2) determine the limits of growth imposed by these parameters. Fungal growth rates were assessed in a complete factorial design (15 to 35 C; -0.12 to -5.0 Mpa) using a basal salt medium. The growth of *M. poae* and *G. incrustans* was optimal at 30 C, whereas *L. korrae* grew best at 25 C. At these temperatures, the growth of all fungal species decreased with decreasing water potential and was detectable at the lowest water potential measured (-5.0 Mpa). When the temperature was not optimal, growth of all fungal species decreased dramatically at -2.0 Mpa and was not observed for most isolates at -5.0 Mpa.

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GERMINATION OF *PYTHIUM* OOSPORES IN RESPONSE TO TURFGRASS SEED AND ROOT EXUDATES. David Y. Han and Eric B. Nelson, Department of Plant Pathology, Cornell University, Ithaca, N.Y. 14853.

Oospore responses to seed and root exudates of *Pythium torulosum* and *P. graminicola* were studied in order to understand how seed and root infections are initiated. Exudates from seeds and roots of perennial ryegrass (*Lolium perenne* L.) and creeping bentgrass (*Agrostis palustrus* Huds.) were found to stimulate oospore germination of *P. torulosum*, but not of *P. graminicola*. The germination rate of *P. torulosum* was dependent on oospore age. Oospores less than 7 days old did not germinate whereas about 80% germination was observed in 15 day old oospores. Older oospores showed increasing germination in response to buffer controls but not to exudate. Only low levels of *P. graminicola* oospore germination (<5%) were observed even when aged for three months. Chromatographic analysis of seed and root exudates indicated several active fractions. Several sugars, amino acids and fatty acids were stimulatory to *P torulosum* oospore germination when tested alone. No synergistic or inhibitory effects were observed with various combinations of sugars, amino acids or fatty acids. No germination of *P. graminicola* oospores was observed in response to sugars, amino or fatty acids. The high germinability of *P.torulosum* oospores may account for its high recovery from turfgrass roots and abundance in turfgrass ecosystems.

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EVALUATION OF NON-PATHOGENIC STRAINS OF *GAEUMANNOMYCES GRAMINIS* VAR. *GRAMINIS* DERIVED BY PROTOPLASTING AND CHEMICAL

MUTAGENESIS. M. L. Elliott, and S. A. Chasse, University of Florida, Fort Lauderdale Research and Education Center, Fort Lauderdale, FL 33314

An isolate of *Gaeumannomyces graminis* var. *graminis*, pathogenic to warm-season turfgrasses, was subjected to protoplasting and chemical (MNNG) mutagenesis. The resulting 170 strains were evaluated for: 1) growth on PDA, Czapeks agar and Czapeks agar supplemented with yeast extract and 2) pathogenicity to wheat using an *in vitro* assay - wheat grown on water agar. Based on these results, twelve strains were compared with the original parental isolate. Growth rate and formation of lobed hyphopodia were not correlated with pathogenicity of these strains. Some strains that were initially identified as non-pathogenic were pathogenic when yeast extract was added to the water agar. However, these strains remained non-pathogenic to wheat and bermudagrass when used as inoculum in a standard topsoil mix.

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GENOTYPIC DIVERSITY OF OPHIOSPHAERELLA HERPOTRICHA. K.M. McCann, N.A. Tisserat, and S.H. Hulbert, Department of Plant Pathology, Kansas State University, Manhattan, KS 66506-5502.

Eighty isolates of Ophiosphaerella herpotricha, a cause of spring dead spot of bermudagrass, were collected from multiple, but distinct, circular dead patches of turf at two locations in Kansas and from samples collected from a wide geographic area in the United States. DNA extracted from isolates was amplified with oligonucleotide primers derived from the multicopy probe pOH29. The primers reproducibly amplified 6-9 DNA fragments of various sizes from all isolates. PCR products were identical for isolates collected from the same patch, or from clusters of individual patches in each of two plots (each plot ca. 930 m^2). Banding patterns from isolates collected from widely scattered locations, however, were polymorphic. Results suggest each diseased bermudagrass patch results from the vegetative spread of an individual clone of the fungus, that localized areas have a mosaic of clones, and the fungus has broad genotypic diversity across its range.

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ASSESSMENT OF RESISTANCE IN CREEPING BENTGRASS (*AGROSTIS PALUSTRIS* HUDS.) TO *PYTHIUM APHANIDERMATUM* (EDSON) FITZP. A. H. Icard and L. L. Burpee, University of Georgia, Athens, Georgia 30602.

Fifteen cultivars of creeping bentgrass (*Agrostis palustris* Huds.) were assessed for resistance to foliar blight incited by *Pythium aphanidermatum* (Edson) Fitzp. in field plots during the summer of 1992. Eighteen cultivars were evaluated in 1993. Disease assessment was conducted by remote sensing with a multispectral radiometer in 1993 and visually, using the Horsfall-Barratt scale, in both years. In 1992, Putter, Cobra Late, Cobra, Penneagle, Emerald, Providence, and National were the most resistant cultivars, and Penncross was the most susceptible. In 1993, no significant differences ($\alpha \leq 0.05$) among cultivars were detected from visual assessment; however, remote sensing with the multispectral radiometer revealed significant differences. The cultivars JH Bent, Providence, Penneagle, Putter, Emerald, Syn-1, Syn-3, Forbes-89-12, SR 1020, AP89140, and Cobra were more resistant to *P. aphanidermatum* in 1993 than Penncross, Pennlinks, AP89150, Cobra Early, Cobra Late, Syn-4, and National. Remote sensing was more precise than visual assessment in detecting differences in the intensity of foliar necrosis as a measure of resistance.

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DEVELOPMENT OF A DIAGNOSTIC ASSAY FOR WHITEFLY-TRANSMITTED GEMINIVIRUSES USING PCR. A. M. Idris, G. K. Banks, and J. K. Brown. Department of Plant Sciences, University of Arizona, Tucson, AZ 85721.

Diseases caused by whitefly-transmitted (WFT) geminiviruses often result from infections caused by two or more viruses. A sensitive PCR-based assay was developed which allows for detection and identification of several WFT geminiviruses occurring in tomato and/or pepper. Degenerate primers were designed over conserved regions of the A and B components of WFT geminiviruses. For virus differentiation, four criteria were used: presence, absence, size and number of PCR products. Diagnostic PCR patterns were obtained for five WFT geminiviruses: chino del tomate virus (CdTV), pepper mild tigre virus (PMTV), serrano golden mosaic virus (SGMV), pepper hausteco virus (PHV), and tomato yellow leaf curl virus-Thailand (TYLCV-Th). These results indicate that geminiviruses can be identified at early stages of plant growth, information which may be useful in designing disease management strategies.

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DETECTION OF LATENT VIRUSES OF ROSACEOUS FRUIT TREES USING RT-PCR. G. R. Kinard and S. W. Scott. Department of Plant Pathology and Physiology, Clemson University, Clemson, SC 29634-0377.

Apple chlorotic leafspot closterovirus (ACLSV) and apple stem grooving capillovirus (ASGV) infect rosaceous fruit trees. Because virus concentration is low, ELISA and graft detection methods have limitations. Therefore, reverse transcriptase polymerase chain reaction (RT-PCR) procedures have been developed for rapid and sensitive detection. Primers specific for the ACLSV genome amplify a 548 nt fragment which encompasses 94% of the coat protein gene, whereas ASGV-specific primers amplify a 419 nt fragment in the putative coat protein region. Picogram quantities of untreated, purified virions can be amplified. Detection in fruit trees depends on the methods used for RNA extraction. ACLSV has been detected in young leaves of ornamental and commercial cultivars of *Malus*; the ASGV fragment has been amplified from leaves of *Malus* and *Pyrus* species. To date, amplification has been achieved using 200 ng of total RNA from infected leaves. A more extensive range of isolates of ASGV is being tested and amplified fragments from several isolates are being sequenced. A U.S. isolate of ASGV was found to have 90% nucleotide sequence similarity with that of an isolate from Japan.

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PCR-BASED DETECTION OF CITRUS EXOCORTIS AND GROUP II CITRUS VIROIDS DIRECTLY FROM FIELD-GROWN SWEET ORANGE TREES. S. M. Garnsey, and D. L. Zies, USDA, ARS, Orlando, FL 32803, M. Irey, U. S. Sugar Corporation, Clewiston, FL, 33440, L. Levy, USDA, ARS, Beltsville, MD 20705 and M. E. Hilf, USDA, ARS, Orlando, FL 32803.

A practical reverse transcription-polymerase chain reaction (RT-PCR) method was developed to test field-grown orange trees directly for viroid infection. Samples of bark tissue (0.2g) were collected from budsticks from healthy and viroid-infected trees. The tissue was homogenized in Tris buffer and extracted by a SDS potassium acetate procedure. Exocortis viroid (CEV) and hop stunt viroid (HSV) primers were used respectively for RT-PCR amplification of CEV and group II citrus viroids (CVII) from total nucleic acids. The amplification products were analyzed by agarose gel electrophoresis or by Southern blotting with digoxigenin-labeled DNA probes specific for CEV and HSV. CEV and cachexia (CVIIb) were detected in valencia, hamlin and navel sweet oranges infected with pure isolates. One hundred field trees (viroid-free or with single or mixed infections of CEV and group II citrus viroids) were sampled, tested twice by RT-PCR in blind assays, and indexed to Etrog citron. All 49 viroid-free trees tested negatively. CEV was detected by RT-PCR in 33 of 35 trees which indexed positively for CEV in Etrog citron. Forty-seven of 50 trees with known or presumed CVII viroid infections tested positively by RT-PCR for CVII viroids.

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Nested-PCR for the specific and sensitive detection of *Xanthomonas campestris* pv. citri. John S. Hartung [1] and Olivier Pruvost [2]. USDA ARS Beltsville, MD USA [1] and CIRAD-FLHOR St. Pierre, La Reunion, FRANCE [2]

Sensitivity of our previously published PCR detection protocol for *X. campestris* pv. citri (Appl. Environ. Microbiol. 59 (4): 1143-1148), has been improved by incorporating a second cycle of PCR using primers internal to the first set of primers. This approach eliminates the need for Southern blotting to achieve detection of fewer than 10 cfu/sample and is therefore faster and easier to carry out. When one of the internal primer pairs is 5' labled with biotin and the other 5' labeled with the *lac* operator sequence from *E. coli*, colorimetric detection in an ELISA format is possible based on a commercially available lac operator/ß-galactosidase fusion protein.

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IMMUNOCAPTURE AND DNA AMPLIFICATION OF XANTHOMONAS ALBILINEANS FROM VASCULAR SAP OF SUGARCANE LEAVES. S. A. Lopes and K. E. Damann, Dept. of Plant Pathology & Crop Physiology, LSU Agricultural Ctr., Baton Rouge, LA 70803-1720.

Xanthomonas albilineans causes leaf scald disease of sugarcane. Diagnostic techniques involve the use of indicator plants, symptomatology, serology and pathogen isolation. PCR amplification of the bacterial DNA directly from vascular sap also has been used (Lopes and Damann, Phytopathology 83:1398). However, vascular sap contains unknown compounds which inhibit the polymerase reaction. To overcome this problem we have adapted the protocol of Nolasco et al (J. Virol. Methods 45:201-218) for immunocapture of X. albilineans in microtiter plates.

DNA amplification using outward facing concensus t-RNA primers (Welsh and McClelland, Nucleic Acids Res. 19:861-866) is performed in the same plate. The bacteria was readly detected in all symptomatic (16/16), in 6 asymptomatic (6/16), and in 1 apparently healthy (1/16) sugarcane leaf. Results will be presented and the potential use of the technique for early disease diagnosis will be discussed.

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PCR-MEDIATED DETECTION OF BEAN COMMON BLIGHT CAUSED BY *XANTHOMONAS CAMPESTRIS* PV. *PHASEOLI*. P. Audy, A. Laroche, G. Saindon, H.C. Huang and R.L. Gilbertson. Agriculture and Agri-Food Canada, Research Centre, Lethbridge, Alberta, T1J 4B1 and Department of Plant Pathology, University of California, Davis, CA 95616.

Bean common blight caused by *Xanthomonas campestris* pv. *phaseoli* (Xcp), is a major seed-borne disease worldwide. A few infected seeds can lead to outbreaks of common blight in the field and cause important yield reductions. Preventing disease spread through contaminated seedlots requires development of a highly sensitive detection assay. A 3.4 kb plasmid fragment of Xcp previously identified as being specific to common blight bacteria, was partially sequences, and primers containing high G-C content were devised and used in PCR-assays under stringent conditions of annealing. Two pairs of primers led to the amplification of specific DNA fragments from Xcp strains whereas no discrete fragments were amplified from other strains of *X. campestris*, or species of *Pseudomonas*, *Clavibacter*, *Erwinia* and *Agrobacterium*. DNA corresponding to as few as 10 genomes of Xcp could be detected on ethidium bromide-stained agarose gel following 35 cycles of PCR-amplification.

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SPECIFIC DETECTION OF *CLAVIBACTER MICHIGANENSIS* SUBSP. *SEPEDONICUS* WITH THE POLYMERASE CHAIN REACTION. J. L. Drennan, S. A. Slack, A. A. G. Westra, A. Collmer, N. C. Gudmestad, and A. E. Oleson, Cornell University, Ithaca, NY 14853 and North Dakota State University, Fargo ND 58105.

Clavibacter michiganensis subsp. *sepedonicus*, the causal agent of bacterial ring rot of potato, was specifically detected in field-grown potatoes with a 20 bp synthetic oligomer derived from an inverted repeat region of the high-copy-number repeated sequence of plasmid pCS1 that was used as a PCR primer. Stems from potato cultivars 'BelRus' and 'Russet Burbank', inoculated with 0, 10^2, or 10^9 colony forming units (cfu) and grown in New York (NY) and North Dakota (ND), were collected at 35 and 90 days after planting (DAP), frozen (-80 C), ground in Tris-EDTA buffer, and tested by PCR and ELISA. Detection by PCR was similar to that observed with ELISA (32 vs. 34% positive, 95% agreement between assays) and ranged from 0% (BelRus/10^2 cfu/NY/35 DAP) for both assays to 83 and 90% (Russet Burbank/10^9 cfu/ND/90 DAP) for PCR and ELISA, respectively. Detection by both assays was affected by inoculum dose, cultivar, location, and sampling date. Detection was favored by high inoculum dose and late sampling date, and was higher in the susceptible cultivar 'Russet Burbank' than the tolerant cultivar 'BelRus'. Detection was higher in samples from ND than in those from NY, with differences between locations being most pronounced early in the growing season.

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DETECTION OF ASTER YELLOWS MYCOPLASMA LIKE ORGANISM (MLO) IN ITS LEAFHOPPER VECTOR AND IN VEGETABLE CROPS IN OHIO BY THE POLYMERASE CHAIN REACTION (PCR). S.A. Miller[1], D.J. Murral[2], L.R. Nault[2], C.W. Hoy[2], and R.E. Davis[3], Depts. of Plant Path.[1] and Entom.[2], Ohio State University, OARDC, Wooster, OH 44691, and USDA-ARS[3], MPPL, Beltsville, MD 20705.

Aster yellows MLO was detected reliably in single laboratory-reared AY MLO-exposed aster leafhopppers (*Macrosteles quadrilineatus*) by PCR, using the AY MLO 16S rRNA-specific primer pair r16F4R1. AY MLO was detected in infected, non-inoculative leafhoppers as early as 8 days after the initiation of the acquisition-access period (AAP). For leafhoppers that had become inoculative (20-22 days post AAP), detection of AY MLO by PCR compared favorably to detection by bioassay. AY MLO was also detected in field collected, symptomatic carrots, celery and leaf lettuce by PCR. Restriction fragment length polymorphism (RFLP) analysis of DNA amplified by the MLO 16S rRNA-specific primer pair r16F2R2, using the restriction enzymes HaeIII and HhaI, indicated that strains representing at least two AY MLO genetic subclusters were present in Ohio vegetable crops in 1993.

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EVALUATION OF A NON-ISOTOPIC PCR-COUPLED LIGASE CHAIN REACTION ASSAY FOR SPECIFICITY TO *ERWINIA STEWARTII*. W. J. Wilson[1], C. A. Batt[2], and H. R. Dillard[1]. Department of Plant Pathology, Cornell University, Geneva, NY 14456[1], and Department of Food Science, Cornell University, Ithaca, NY 14853[2].

A non-isotopic polymerase chain reaction (PCR)- coupled ligase chain reaction (LCR) assay was developed to directly detect the plant pathogenic bacterium *Erwinia stewartii* in plant and vector material. The technique is based on a single-base-pair difference in the 16S rRNA gene which is unique to *E. stewartii*, allowing identification to species level. The PCR-coupled LCR assay was evaluated for specificity with a collection of 120 bacterial corn isolates. Seventy corn isolates were verified as *E. stewartii* by colonial morphology on tryptone-yeast extract-phosphate medium, pathogenicity to sweet corn cultivar Jubilee, oxidative-fermentative reaction, lack of motility, and response to a set of 95 carbon sources (Biolog system). The PCR-coupled LCR assay revealed 100% specificity for the *E. stewartii* isolates and no reaction to the corn epiphytes. From the Biolog data, a dendrogram was constructed of the *E. stewartii* isolates to reveal 3 closely related groups with no apparent geographical correlations within groups.

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A PCR APPROACH FOR EARLY AND SPECIFIC DETECTION OF *ERWINIA AMYLOVORA* IN MONITORING STUDIES IN TURKEY. E. A. Momol[1], M. T. Momol[1], and E. Hacioglu, Dept. of Plant Pathology, Akdeniz Univ., Antalya, Turkey and [1]Dept. of Plant Pathology, Cornell Univ., Geneva, NY 14456.

Monitoring *Erwinia amylovora* populations in orchards, nurseries and quarantine stations was necessary for several purposes. Sensitive and species-specific detection of *E. amylovora* by PCR had been developed earlier. Primer A and B were obtained from S. Bereswill. Some modifications of the earlier PCR procedure have been made and amplification of *E. amylovora* obtained from pear flowers will be discussed. Monitoring of bacterial populations using PCR was compared with use of modified Miller-Schroth medium. Sensitive detection of *E. amylovora* by PCR analysis could improve forecasting system for fire blight and data obtained from this type of study could lead to a better understanding of the epidemiology of fire blight.

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DETECTION OF *ALTERNARIA RADICINA* AND *A. DAUCI* USING A PCR-BASED ASSAY. B. M. Pryor, R. M. Davis, and R. L. Gilbertson, Dept. of Plant Pathology, University of California, Davis, CA 95616

Two fungal pathogens of carrots, *Alternaria radicina* (Ar) which causes carrot black rot, and *A. dauci* (Ad), which causes Alternaria leaf blight, were subjected to random amplified polymorphic DNA (RAPD) analysis. Most primers allowed for the differentiation of Ar and Ad isolates. With some primers, two groups of Ar isolates were identified and these correlated with two morphologically distinct culture types of this fungus, referred to as type I and II. Both types are pathogenic on carrots. No polymorphisms were detected among *A. dauci* isolates. Unique RAPD fragments from Ar and Ad were cloned and partially sequenced. Selected sequences from each clone were used to develop Ar- and Ad-specific primers that were used in PCR-based detection assays. Ar-specific primers amplified expected size DNA fragment from both type I and II Ar isolates but not from *A. dauci*, *A. alternata*, *Ulocladium atrum*, or *Stemphylium vesicarium* isolates, or from isolates of the closely related fungus *A. radicina* var. *petroselini*, which is pathogenic on parsley but not carrot. Ad-specific primers amplified the expected size DNA fragment from Ad and not from the other fungi mentioned. These primers are being used in the development of PCR-based assays for the detection of these pathogens on carrot seed.

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DEVELOPMENT OF SPECIFIC PRIMERS FOR IDENTIFICATION AND DETECTION OF PYTHIUM ARRHENOMANES. W. Chen, Illinois Natural History Survey, 607 East Peabody Drive, Champaign, IL 61820.

Previous research showed that P. arrhenomanes is genetically distinct from morphologically similar species such as P. graminicola, P. myriotylum, and P. aphanidermatum based on RFLPs of PCR-amplified rDNAs. The internal transcribed spacer region of these species was specifically amplified and the nucleotide sequences were determined. A PCR primer was designed specifically for P. arrhenomanes. This specific primer in combination with a universal primer amplifies the DNA region from isolates of P. arrhenomanes and does not amplify DNA from other Pythium species. When corn seedlings were artificially inoculated with P. arrhenomanes, use of the primers amplified the targeted DNA region from infected seedlings, and did not amplify any DNA from non-inoculated healthy plants.

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INCORPORATION OF NUCLEOSIDE TRIPHOSPHATES (NTPs) INTO TRICHLOROACETIC ACID (TCA)-PRECIPITABLE PRODUCTS BY TOMATO SPOTTED WILT VIRUS (TSWV). Scott T. Adkins[1], René Quadt[1,2], Paul G.

Ahlquist[1,2] and Thomas L. German[1]. [1]Dept. of Plant Pathology and [2]Institute for Molecular Virology, Univ. of Wisconsin-Madison, Madison, WI 53706.

Detergent-disrupted purified TSWV virions were found to support incorporation of radiolabeled NTPs into TCA-precipitable products. Similar fractions from mock inoculated plants did not support NTP incorporation. Manganese appeared to be the preferred divalent metal cation although magnesium also facilitated NTP incorporation, albeit at a greatly reduced level. When included as a component of the reaction mixture, RNAse but not DNAse, rifampicin, actinomycin D or α-amanitin significantly reduced incorporation of NTPs. Radiolabeled products hybridized to TSWV genomic RNA but not tobacco mosaic virus genomic RNA. TSWV-supported incorporation of NTPs increased with incubation time and virus concentration.

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GENETIC DIVERSITY OF "AFRICAN STREAK GEMINIVIRUSES" R.W.Briddon, P.Lunness, L.C.L.Chamberlin, R.Hull and P.G.Markham. Dept. of Virus Research, John Innes Institute, Colney Lane, Norwich, NR4 7UH, UK.

Streak diseases of cereals and grasses across central and southern Africa have been shown to be caused by members of the Geminiviridae. Three different viruses have been identified to date (maize streak [MSV], *Panicum* streak [PSV] and sugarcane streak viruses [SSV]), of which MSV is the most serious pathogen of food crops. The genetic diversity of streak viruses has been investigated by determining the nucleotide sequence of the coat protein gene of 15 streak virus isolates. The results are discussed with respect to evolution, resistance breeding and the detection and differentiation of streak viruses.

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STRAIN-SPECIFIC RESCUE AND AMPLIFICATION OF A TRANSGENIC DEFECTIVE-INTERFERING DNA OF THE GEMINIVIRUS BEET CURLY TOP. D. C. Stenger, Dept. of Biological Sciences, Northern Illinois University, DeKalb, IL 60115.

Transgenic *Nicotiana benthamiana* plants have been constructed which bear integrated, tandemly repeated copies of a beet curly top virus (BCTV) defective-interfering (DI) DNA derived from the Logan strain. Transgenic DI plant lines challenge inoculated with BCTV-Logan exhibited delayed and attenuated symptoms compared to non-transformed plants. Infection of transgenic DI plants with the Logan strain resulted in the rescue of the integrated DI sequence which was subsequently amplified as a native DI DNA. The accumulation of Logan standard viral DNA forms was reduced in transgenic DI plants, relative to non-transformed plants. In contrast, no delay or attenuation of symptoms was observed for transgenic DI plants challenge inoculated with the BCTV strains CFH and Worland. Infection by the CFH and Worland strains did not result in the rescue or amplification of the integrated Logan DI sequence, and no difference in the accumulation of CFH or Worland standard viral DNA forms was observed among transformed and non-transformed plants. These results and sequence analyses suggest that the three BCTV strains encode distinct replication proteins which exhibit specificity with respect to recognition of DNA replication origin *cis*-elements.

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DETECTION AND LOCALIZATION OF SYNV-ENCODED PROTEINS IN SYSTEMICALLY INFECTED *NICOTIANA EDWARDSONII* PLANTS.

K.-B. G. Scholthof and A. O. Jackson, Dept. of Plant Biology, University of California, Berkeley, CA 94720.

Sonchus yellow net virus (SYNV), a rhabdovirus, encodes six viral proteins from a minus-sense single stranded RNA genome. This virus replicates in the nucleus and acquires an envelope by budding through the inner nuclear membrane to the perinuclear space. SYNV-infected leaves of N. edwardsonii were fractionated by differential centrifugation to separate the cellular constituents into four components: P1 (nuclei), P30 (membranes and SYNV virions), S30 (soluble proteins), and CW (cell wall proteins). Western blots were used in conjunction with polyclonal antibodies to either disrupted virions or a fusion protein to detect SYNV encoded proteins. A recently identified sixth protein, sc4, whose function is unknown, was localized to the P30 fraction. Further analyses show that sc4 is a component of SYNV and it is also associated with the virus envelope. The other proteins of SYNV fractionated to P1, P30, and S30 and the nucleocapsid protein also fractionated to the CW. The roles of each of the viral proteins will be discussed.

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CHARACTERIZATION OF THE SONCHUS YELLOW NET RHABDOVIRUS M2 PHOSPHOPROTEIN, A COMPONENT OF THE VIRAL TRANSCRIPTASE.
John D. Wagner and Andrew O. Jackson, Department of Plant Biology, 111 Koshland Hall, University of California, Berkeley CA 94720.

Sonchus yellow net virus (SYNV) is the best characterized member of the large group of nuclear associated plant rhabdoviruses. We have isolated an *in vitro* viral transcriptase from nuclear extracts of SYNV infected tobacco leaf tissue and SYNV-infected propoplasts. Three viral proteins, the nucleoprotein, N, the putative core polymerase, L, and an accessory protein, M2, are associated with the transcriptase activity. The transcriptase complex sediments as a core preparation with a size similar to the inactive cores derived from purified virus. Immunoprecipitation of the transcriptase activity with an antibody raised against the SYNV M2 protein provides strong evidence for physical association of the M2, N and L proteins with the active complex. The M2 protein is phosphorylated both *in vivo* and *in vitro*, and phosphoamino acid analysis suggests that threonine residues are phosphorylated. The phosphorylation status of M2 protein appears to appears to be essential for enzymatic activity of the complex.

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ASSOCIATION OF CMV COAT PROTEIN AND RNAS WITH CHLOROPLASTS IN TOBACCO. Y. H. Hsu, W. S. Tsai, and C. L. Chu. Agricultural Biotechnology Laboratories, National Chung Hsing University, Taichung, Taiwan, 402, R. O. C.

The accumulations of viral coat protein and RNAs in chloroplasts of tobacco (*Nicotiana tobacum* cv. Van-Hicks) leaves infected by a severe (NT9), a mild (M48) and two pseudorecombinant strains of cucumber mosaic virus (CMV) were examined. Pseudorecombinants, NNM and MMN, were constructed by exchange of RNA3 between NT9 and M48. Like NT9, MMN produced severe mosaic on tobacco, while NNM and M48 both caused symptomless infection. Intact chloroplasts from either inoculated or systemically infected leaves were isolated on Percoll gradients and treated with pancreatic RNase and thermolysin. By Western and Northern blot analyses, CMV coat protein and RNAs were found within chloroplasts prepared from leaves infected by NT9 and MMN, but not by M48 and NNM. This study indicates that symptom expression in CMV infected tobacco may be due to altered chloroplast function.

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IMMUNOLOGICAL DETECTION AND LOCALIZATION OF BAMBOO MOSAIC POTEXVIRUS SATELLITE RNA-ENCODED PROTEIN IN INFECTED PROTOPLASTS. Lin, N. S.[1], Chai, Y. J.[1], Chen, J. M.[1] and Hsu, Y. H.[2] ([1]Inst. of Botany, Academia Sinica, Taipei; [2]Agricultural Biotechnology Lab., National Chung Hsing University, Taiwan, R. O. C.)

Satellite RNA associated with bamboo mosaic potexvirus encodes a 20 kD non-structural protein. The open reading frame of the satellite-encoded protein was cloned into a T7 expression vector and an antiserum against this protein was raised. The antiserum specifically recognized satellite-encoded protein synthesized in in vitro rabbit reticulocyte lysate and also in infected barley protoplasts. In infected protoplasts, the protein was detected transiently, reaching its maximum between 8 to 16 hr post-infection and decreasing at the very low level at 48 hr post-infection. By immunoelectron microscopy, the satellite-encoded protein was mainly localized in the nucleus and cytoplasm of the infected cells.

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IDENTIFICATION OF A TOMATO BUSHY STUNT VIRUS SYMPTOM DETERMINANT USING A POTATO VIRUS X EXPRESSION VECTOR.
H.B. Scholthof, K.-B. G. Scholthof, and A.O. Jackson. Dept. of Plant Biology, Univ. of California, Berkeley, CA 94720 and NSF-CEPRAP, Davis, CA 95616

Tomato bushy stunt virus (TBSV) is a tombusvirus with a single stranded positive sense RNA genome of ca. 4.8 kb encoding five genes. Viral replication requires two 5' proximal genes, the capsid gene located in middle of the genome is dispensable for infection, and virus spread is mediated by a membrane associated protein (p22) encoded at the 3' end of the genome. A small gene of unknown function, residing within the p22 gene, codes a product of approximately 19 kDa (p19) which by western analyses is shown to be predominantly present in the soluble fraction of infected tissue. The p19 gene is not required for infectivity but inactivation of this gene prevents the induction of lethal necrotic symptoms elicited by TBSV in some systemic hosts. Expression of p19 by potato virus X (PVX), which by itself does not induce lethal symptoms, also results in the induction of a lethal necrosis in the same hosts. These results strongly suggest that p19 is the sole viral determinant responsible for eliciting a total collapse of certain plants upon infection with TBSV.

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SEQUENCE OF THE CAPSID PROTEIN CISTRON FROM A PVY FIELD ISOLATE REVEALS A HETEROLOGOUS POPULATION. J.A. Abad, O.W. Barnett, A.K. Weissinger, G. M. Hellmann, and S. A. Lommel, Dept. of Plant Pathology, and Dept. of Crop Science, NCSU Raleigh, NC 27695-7616.

Potato virus Y (PVY) is the type member of the *Potyvirus* genus and an important pathogen of cultivated solanaceous plants. Sequence of the capsid protein (CP) cistron of many isolates of this virus has been published with identities ranging from 82 to 100 % (>80 % are strains of the same virus). In an attempt to characterize the CP cistron of a PVY field isolate (PVY-L) obtained from tobacco plants in North Carolina, we found 9 different sequences at the nucleotide (n) level from 22 cDNA clones varying from 83 to 100% identity. Sequences were obtained from the 5' terminal 300 n of the variable region of the CP cistron. The cDNA clones were amplified by PCR after reverse transcription of the purified PVY-L RNA. From the predicted amino acids (aa) a dominant population of 11 identical sequences (50%) was identified. Two other groups with 4 and 2 identical sequences were also found. The last was the less conserved when compared to the others. The remaining clones were different in at least one aa. This is the first report of such a variation in a CP cistron sequence from a single potyvirus field isolate. A study is in progress to determine the phenotypes of the distinct genotypes obtained from the field isolate.

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NUCLEOTIDE SEQUENCE OF THE COAT PROTEIN GENE AND 3' UNTRANSLATED REGION OF AZUKI BEAN MOSAIC VIRUS SHOWS CLOSE RELATIONSHIP WITH BEAN COMMON MOSAIC VIRUS. C. W. Collmer[1,2], E. J. Vesely[1], M. C. Israel[1], S. E. Ruuska[1], H. A. Maville[1], S. M. Albert[1], S. Bajaj[1], and M. M. Kyle[2]. [1]Department of Biology, Wells College, Aurora, NY 13026 and [2]Cornell University, Ithaca, NY 14853.

Nucleotide sequencing has allowed the reliable establishment of taxonomic relationships among some potyviruses in the cluster including bean common mosaic virus (BCMV), azuki bean mosaic virus (AzMV), blackeye cowpea mosaic virus (BlCMV), and cowpea aphid borne mosaic virus. Degenerate oligonucleotide primers and the polymerase chain reaction were used to amplify and clone the coat protein (CP) gene and 3' untranslated region (UTR) of AzMV. Nucleotide sequencing of the 3' UTR of AzMV showed a 92% sequence similarity with BCMV strain NY15, and the sequence of the 3' half of the coat protein gene showed an amino acid similarity of greater than 90%. Based on the sequence of the 3' UTR and preliminary data from the remaining coat protein sequence, it can be concluded that AzMV, like BlCMV, should be considered a strain of BCMV.

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EFFECTS OF SELECTED POSTEMERGENCE SOYBEAN HERBICIDES ON *HETERODERA GLYCINES*. L. L. Wainwright and G. L. Tylka. Dept. Plant Pathology, Iowa State University, Ames IA 50011

Blazer, a postemergence herbicide for soybeans, has been previously shown to inhibit hatching of free soybean cyst nematode, *Heterodera glycines*, eggs in vitro. Experiments were conducted to further investigate the effects of Blazer on *H. glycines* egg hatch and to determine whether Blazer affected the infectivity of second-stage juveniles (J2) or the fecundity of females developing from those J2. Although virtually no hatch occurred when eggs were incubated for 14 days in 500 µg/ml Blazer, subsequent egg hatch proceeded normally once eggs were removed from the herbicide. Percent infection of susceptible soybean roots by J2 hatched from eggs incubated in Blazer was not significantly different from infection by J2 hatched from eggs incubated in deionized water or 3 mM zinc sulfate control solutions. Likewise, there was no difference in fecundity of females which had developed from juveniles hatched from eggs incubated in Blazer and those which had developed from eggs incubated in control solutions. Similar trends have been observed in preliminary evaluations of other herbicides with active ingredients similar to acifluorfen, the active ingredient in Blazer.

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RESISTANCE TO *HETERODERA GLYCINES* RACES 3 and 5 IN SOYBEAN. S. C. Anand, S. B. Sharma, and J. A. Wrather. University of Missouri, Delta Center, Portageville, MO 63873

Heterodera glycines races 3 and 5 are widespread in the United States. Soybean plant introduction (PI) 90763 is resistant to both races and PI 424595 is resistant to only race 5. Crosses involving the two PI lines and the susceptible cultivar Essex were studied in the F_1, F_2, and F_3 generations to determine their genetic relationships for resistance to races 3 and 5. The plants were evaluated for resistance using conventional screening techniques based on the index of parasitism and data were analyzed using the chi-square test to determine goodness of fit between observed and expected genetic ratios. The cross PI 90763 x Essex segregated 3 resistant:13 susceptible plants in the F_2 generation for race 3 resistance indicating the presence of a dominant and a recessive gene, whereas, the cross PI 90763 x PI 424595 gave monogenic inheritance. All plants of the cross PI 424595 x Essex were susceptible. Resistance to race 5 in PI 90763 and PI 424595 was conditioned by different set of genes. The data suggested that some of the alleles in PI 90763 which controlled resistance to race 5 also provided resistance to race 3, whereas, in PI 424595 the race 5 resistance alleles did not provide resistance to race 3.

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USE OF "CATCH" CROPS TO CONTROL THE SUGAR BEET NEMATODE, *HETERODERA SCHACHTII*. F.A. Gray, D.W. Koch and J.M. Krall, Dept. of PSIS, Univ. of Wyo., Laramie, WY 82071-3354.

A radish, cv. Pegletta, "catch" crop was planted in late summer following malting barley, silage corn and dry bean harvest and sugar beets were planted the following spring. Reduction of soil populations of *H. schachtii* and increase in sugar beet yields were associated with the amount of radish growth prior to fall plowdown. The greatest response occurred when radishes were planted on July 28, 1992 following malting barley. The following spring there were 57% fewer nematodes recovered from soil in the radish treatment compared to the traditional fallow (no radish) treatment, 3.2 second stage juveniles (J2)/cc soil and 1.4 J2/cc, respectively. The damage threshold for *H. schachtii* in sugar beet for Wyoming is estimated at 3 J2/cc soil. At beet harvest on Sept 17, 1993, soil population of *H. schachtii* for the no radish and radish treatments were 12.7 J2/cc and 8.6 J2/cc, respectively. Sugar beet yields were 14.1 T/A in the no radish treatment and 18.0 T/A in the radish treatment, nearly a 4 T/A increase. Similar, but less dramatic responses were obtained following silage corn and dry beans when radish was planted on Sept 9 and 15, 1992, respectively.

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COKER 371 GOLD - AN ADDITIONAL FLUE-CURED TOBACCO CULTIVAR RESISTANT TO TOBACCO CYST NEMATODES IN VIRGINIA. C.S. Johnson, Virginia Polytechnic Institute and State University, Southern Piedmont Agricultural Research & Extension Center, P.O. Box 448, Blackstone, VA 23824.

Population densities of tobacco cyst nematodes (Globodera tabacum solanacearum, or TCN) and development of 10 popular flue-cured tobacco cultivars were monitored in 1992 and 1993 in plots either untreated or sprayed with 6.7-7.5 L/ha of fosthiazate before transplanting. Fosthiazate reduced TCN

population densities 5 or more weeks after transplanting. TCN population densities developed similarly on all cultivars early in the growing season, but were dramatically lower for Coker 371-Gold late in the growing season. Fosthiazate significantly increased yield of all cultivars, including Coker 371-Gold. Suppression of TCN reproduction by Coker 371-Gold was not associated with improved growth, yield, or quality. However, Coker 371-Gold possesses better agronomic traits than other TCN-resistant cultivars, and should be useful in reducing nematode population levels in infested fields.

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INTERACTIVE EFFECTS OF *PRATYLENCHUS PENETRANS* AND *VERTICILLIUM DAHLIAE* ON POTATO GAS EXCHANGE. Ibrahim A. M. Saeed, A. E. MacGuidwin, and D. I. Rouse, Department of Plant Pathology, University of Wisconsin, Madison, WI 53706.

Growth-chamber experiments were conducted to study the effects of solitary or concurrent infection of *Pratylenchus penetrans* (Pp) and *Verticillium dahliae* (Vd) on gas exchange of Russet Burbank potato. Treatments were three levels of nematodes (1,2, and 4 nematodes/g soil), one level of Vd alone (500 ppg soil), and Vd in combinations with the three levels of nematodes, and a non-treated control. Gas exchange measurements were done 2-4 times per week on leaves of similar ages. Plants infected with Vd alone showed a reduction of 25% in carbon assimilation rate (A) and leaf light use efficiency (LUE) compared to non-infected plants at 55 days after planting (DAP). Individual leaves from nematode-infected plants occasionally exhibited reduction in the above parameters. Co-infection with both pathogens resulted in a consistent synergistic effect in which A and LUE were reduced by 50 to 100% at 55 DAP irrespective of the nematode level. These results confirm our earlier studies showing synergistic interactions of Vd and Pp for yield and symptom expression.

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INTERRELATIONSHIPS BETWEEN *MACROPHOMINA PHASEOLINA* AND NEMATODES ASSOCIATED WITH SORGHUM IN LOUISIANA. L. Wenefrida, J.S. Russin, and E.C. McGawley. Dept. Plant Pathology and Crop Physiology, LSU Agricultural Center, Baton Rouge, LA 70803.

A series of microplot experiments was established to investigate the relationships between *M. phaseolina* and nematodes found commonly on sorghum in Louisiana. A factorial treatment arrangement was used and consisted of two sorghum hybrids (Dekalb-Pfizer 50 and Pioneer 8333), three levels of *M. phaseolina* (0, 10, and 100 colony forming unit (cfu)/g of vapam fumigated soil) and two nematode infestation levels (1,266 and 2,332 nematodes/pot comprised of 5.2% *Helicotylenchus*, 37.9% *Tylenchorhynchus*, 52.1% *Criconemella*, and 4.7% *Pratylenchus*). Plants were harvested 105 days after transplanting. Dry weights of roots and heads were reduced by *M. phaseolina* at 10 cfu/g; no further reduction was observed at 100 cfu/g. Stem dry weight was decreased by nematodes at both levels but only in the absence of *M. phaseolina*. Populations of *Tylenchorhynchus* and *Criconemella* in soil decreased as levels of *M. phaseolina* increased. Neither *Helicotylenchus* nor *Pratylenchus* were recovered from soil at harvest. Results indicate a consistent antagonistic relationship between *M. phaseolina* and nematode genera with regard to plant growth and nematode colonization of sorghum roots.

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POPULATION DYNAMICS OF BURSAPHELENCHUS XYLOPHILUS IN AIR-DRIED LOBLOLLY PINE WOOD. L. D. Dwinell, USDA Forest Service, Southeast. For. Expt. Sta., 320 Green St., Athens, GA 30602

The response of pinewood nematodes (PWN) to wood moisture content (WMC) in loblolly pine was tracted in 2 studies. In the laboratory, sixty 5-cm square blocks of (PWN)-infested loblolly pine were dried over anhydrous calcium chloride; every 2 wk for 12 wk, WMC and PWN density were determined for 10 of the blocks. In a sawn wood study, 28 10 x 10 x 91 cm PWN-infested boards were stored and sampled monthly over a year. WMC was determined by drying samples at 105°C for 24 h. The Baermann funnel procedure was used to assay for the PWN. The initial WMC (dry weight basis) and PWN density of the blocks averaged 53.5% and 16 PWN/g(dw), respectively. After two wk, the mean WMC was 21.4%, but PWN density did not change significantly. After 6 wk, the mean WMC was 4.1% and no nematodes were extracted. In the sawn wood, the PWN population remained constant at about 24/g(dw) for five months and then declined the next month to a mean of 8/g(dw); the average WMC decreased from 53% to 37%. The PWN population continued to decline thereafter. The population dynamics of the PWN in sawn wood being air-dried is governed by factors other than slowly changing WMC.

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EFFECT OF HOST-PLANT RESISTANCE AND A NEMATODE-PATHOGENIC FUNGUS ON *PRATYLENCHUS PENETRANS*. P. Timper and B. B. Brodie, USDA, ARS, Cornell University, Ithaca, NY 14853.

The nematode *Pratylenchus penetrans* is a serious pest of potatoes in the northeastern United States. A factorial experiment was conducted in a growth chamber to investigate the combined effectiveness of a resistant potato clone and the fungus *Hirsutella rhossiliensis* in reducing numbers of this nematode. Susceptible (NY85) and resistant (L118) potatoes were grown in pots with (+Hr) and without (-Hr) the fungus, and inoculated with *P. penetrans*. After 60 days, nematodes in the roots and soil were counted. Compared to the NY85-Hr treatment, nematode numbers were reduced by 7% (NY85+Hr), 33% (L118-Hr), and 63% (L118+Hr). The greater effectiveness of *H. rhossiliensis* with the resistant than with the susceptible clone indicates a synergistic interaction.

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AVAILABILITY OF FENAMIPHOS AND ITS TOXIC METABOLITES TO SOIL WATER. R. F. Davis, R. D. Wauchope, and A. W. Johnson. USDA ARS, University of Georgia Coastal Plain Experiment Station, Tifton, GA 31793.

Field and greenhouse experiments were conducted to determine the extent to which fenamiphos and its nematicidal degradation products, fenamiphos sulfoxide and fenamiphos sulfone, are available to contact nematodes in the soil water. Although much larger amounts of these chemicals were present in the soil, only small amounts of fenamiphos and f. sulfone could be extracted by water; virtually all of the f. sulfoxide present in the soil was extractable by water. Three days after fenamiphos (3EC) was applied at 6.7 kg a.i./ha to field plots, 6% of the fenamiphos, 14% of the f. sulfone, and 100% of the f. sulfoxide present in the soil was extracted by water. In greenhouse tests with soil from the same field, f. sulfoxide became completely available for water extraction 3-4 days after application, whereas fenamiphos remained relatively unextractable by water. Its availability to soil water indicates that f. sulfoxide plays a major role in fenamiphos' control of nematodes.

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BIOCONTROL ACTIVITY OF BACILLUS SP. STRAIN L324-92 TO ROOT PATHOGENS OF WHEAT. **Dal-Soo Kim[1], David M. Weller[2], R. James Cook[2]. Washington State University[1] and USDA-ARS[2], Pullman, WA 99164-6430**

Bacillus sp. strain L324-92 suppressed rhizoctonia root rot, pythium root rot and take-all of wheat in growth chamber tests and increased the yield of wheat by 23.6% in field tests in 1993. All strains of Rhizoctonia solani (41 isolates from 7 anastomosis groups), Pythium spp. (14 species) and Gaeumannomyces graminis (35 isolates) tested were inhibited by L324-92 on dilute potato dextrose agar. However, the amount of inhibition differed among isolates. Populations of L324-92 in both the rhizosphere and spermosphere were 10- or 100-fold lower, respectively, as compared to populations of Pseudomonas fluorescens 2-79, but the population difference decreased over time. The optimum temperature for growth of L324-92 was 30 °C, but growth was observed down to 10 °C. Strain L324-92 reached a cell density of 5.7 x 10^9 cfu/ml in tryptic soy broth. A minimum dose of 10^6 cfu/seed was necessary to obtain maximum suppression of the root diseases in growth chamber tests.

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CONTROL OF SILVER SCURF DISEASE OF POTATOES CAUSED BY *HELMINTHOSPORIUM SOLANI* DUR. & MONT. WITH *PSEUDOMONAS CORRUGATA*. W. W. C. Chun[1] and K. K. Shetty[2], Department of Plant, Soil and Entomological Sciences. Plant Pathology[1] and Plant Science[2] Divisions, University of Idaho, Moscow, ID 83844-2339.

The tomato pith necrosis pathogen, *Pseudomonas corrugata*, exhibits a broad spectrum of antimicrobial activity that inhibits mycelial growth of fungi *in vitro*. Treatment of whole potato tubers naturally infected with *Helminthosporium solani* Dur. & Mont. with *P. corrugata*, reduced disease severity (the percent of the tuber surface infected) from 45.3% to 28.3%. Approximately 17% of the *P. corrugata*-treated tubers were free of silver scurf symptoms while no disease-free tubers were observed in the non-treated controls. A reduction in secondary transfer of the pathogen to daughter tubers from 18.6% to 2.7% was observed when *P. corrugata*-treated tubers were grown in field soil under greenhouse conditions. The

number of sprouts per tuber, mean shoot height, and yield were not significantly affected by treatment with *P. corrugata*.

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DIFFERENTIAL SENSITIVITY OF *GAEUMANNOMYCES GRAMINIS* POPULATIONS TO ANTIBIOTICS PRODUCED BY BIOCONTROL FLUORESCENT PSEUDOMONADS. M. Mazzola, D. K. Fujimoto, and R. J. Cook, USDA-ARS, Root Disease & Biological Control Unit, Pullman, WA 99164-6430.

Production of phenazine-1-carboxylic acid (PCA) and 2,4-diacetylphluoroglucinol (Phl) by *Pseudomonas fluorescens* 2-79 and *P. fluorescens* Q2-87, respectively, is the primary mechanism by which these bacteria suppress take-all of wheat caused by *G. graminis* var. *tritici* (*Ggt*). Sensitivity of strains of *G. graminis* to PCA and Phl was assessed. Growth of some strains of *Ggt* was completely inhibited by PCA at 0.2 ug/ml, while growth of several strains was unaffected at 1.0 ug/ml. Similarly, strains of *Ggt* responded differentially to Phl; growth of strain MV118 was suppressed at a concentration of 0.5 µg/ml and growth of strain MV113 was not restricted in the presence of 3.0 µg/ml Phl. All strains of *G. g.* var. *avenae* and *G. g.* var. *graminis* were insensitive to PCA. Insensitivity to PCA and Phl among certain populations of the target pathogen may provide an additional explanation for the inconsistent field performance of biocontrol rhizobacteria, and gives further justification for the use of biological control systems that employ multiple strains or multiple mechanisms.

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MECHANISMS OF BIOCONTROL OF *PHYTOPHTHORA CITROPHTHORA* ROOT ROT OF CITRUS BY *PSEUDOMONAS PUTIDA*. J. K. Turney, C.-H. Yang, D. A. Cooksey, and J. A. Menge. Dept. of Plant Pathology, University of California, Riverside 92521.

The mechanisms of biocontrol were investigated using three Tn*5* mutants of *Pseudomonas putida* (Pp), one defective for adhesion (Pp-mot$^-$) to *Phytophthora citrophthora* (Pc) mycelium, one defective for siderophore production (Pp-sid$^-$) and one unchanged marker strain (Pp-mar). Pp-sid$^-$ was unable to reduce root infection of Troyer citrange, propagule production by Pc or improve visible root rot ratings compared to the infected control; whereas Pp-mar did decrease disease parameters and increase seedling growth. Pp-mot$^-$ was less effective at reducing root rot compared to Pp-mar, but was still effective in reducing disease parameters and increasing seedling growth. Treatment with ethylenediamine di(o-hydroxy-phenylacetic acid), an iron chelator, mimicked the activity of Pp-mar in reducing disease parameters and increasing seedling growth. Treatment with (ethylenedinitrilo)-tetraacetic acid ferric sodium salt did not reverse the effects of treatment with Pp-mar on propagule production, root infection, or visible healthy roots but did prevent increases in seedling growth. Iron competition appears to play a significant role in the biocontrol of Pc root rot of citrus by Pp while the adhesion of Pp to Pc hyphae may be a less important component of biocontrol.

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LOSS OF BIOLOGICAL CONTROL ABILITY IN AN *ENTEROBACTER CLOACAE* MUTANT UNABLE TO CATABOLIZE LINOLEIC ACID. Karin V. van Dijk and Eric B. Nelson. Department of Plant Pathology, Cornell University, Ithaca, NY 14853.

Enterobacter cloacae strain EcCT-501 is an effective biological control agent against *Pythium ultimum*-incited seed and seedling rots. This strain inactivates the stimulatory activity of cotton seed exudate, thus preventing sporangial germination of *P. ultimum*. Recent data from our lab suggest that long chain fatty acids act as principal stimulants of sporangial germination. This study was initiated to examine the role of fatty acid catabolism in the expression of biological control properties in *E. cloacae*. In intial studies, EcCt-501 reduced the stimulatory activity of a long chain fatty acid, linoleic acid. A series of Tn*phoA* mutants (Kan^r) were screened for growth on linoleic acid as a sole carbon source. One out of 5000 Kan^r colonies was difficient in the ability to inactivate the stimulatory activity of both cotton seed exudate and linoleic acid. This mutant, 21-1, no longer protected cotton from Pythium seed rot and damping-off. A cosmid, pKV1, complemented the linoleic acid catabolic deficiencies of 21-1 and restored the ability to inactivate the stimulatory activity of cotton exudate. Furthermore, this clone fully restored wild-type biological control properties. These data suggest that the metabolism by EcCT-501 of fatty acids released from seeds may be important in the biological control of Pythium seed and seedling rots.

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TRICHODERMA ATROVIRIDE, A POTENTIAL BIOLOGICAL CONTROL AGENT OF *VERTICILLIUM DAHLIAE*. J. H. McBeath, Department of Plant, Animal and Soil Sciences, University of Alaska Fairbanks, Fairbanks AK 99775-7200.

The potential use of *Trichoderma atroviride* (a wide spectrum, cold tolerant mycoparasite found in Alaska) as a biological control agent of *V. dahliae* was evaluated under laboratory conditions. Five isolates (two wild types and three biotypes) of *T. atroviride* were found capable of inhibiting the growth and development of five *V. dahliae* strains, obtained from Illinois and California. Penetration of mycelia of *T. atroviride* into the mycelia and microsclerotia of *V. dahliae* was observed. This mycoparasitism appeared to cause the lyses of conidia, mycelia, and microsclerotia of *V. dahliae*. At the later stage of colonization, the entire colony of *V. dahliae* was covered by profusely sporulating conidiophores of *T. atroviride*. Although all five isolates of *T. atroviride* were capable of controlling the *V. dahliae* strains tested, certain *T. atroviride* isolates are more effective than others.

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SUPPRESSION OF PRE- AND POSTEMERGENCE DAMPING-OFF IN MAIZE BY *BURKHOLDERIA CEPACIA*. K. P. Hebbar[1], M. H. Martel[2], R. D. Lumsden[1], T. Heulin[2], [1]USDA, ARS, Biocontrol of Plant Diseases Laboratory, Beltsville, MD 20705, [2]Centre de Pédologie Biologique, CNRS, BP5, 54501 Vandoeuvre les Nancy Cedex, France.

Burkholderia cepacia (syn. *Pseudomonas cepacia*) isolates from various maize monoculture soils were used to suppress pre- and postemergence damping-off in maize caused by *Pythium* sp. and *Fusarium* sp. in France. In soils naturally infested with *Pythium* sp., *B. cepacia* reduced preemergence damping-off and increased seedling emergence by 30-70% compared to the diseased control. When used in conjunction with the fungicide Thiram, a synergistic effect was noted. In soils naturally infested with *Fusarium* sp., when used as seed inoculants five different *B. cepacia* strains decreased root and mesocotyl necrosis by 15-67% and increased plant dry weights by 16-30% compared to the disease control. The maize cultivar used had a significant effect on the results obtained although root colonization levels of *B. cepacia* were similar in three different cultivars.

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BIOLOGICAL CONTROL OF RHIZOCTONIA INCITED DISEASES OF TABLE BEETS WITH BINUCLEATE RHIZOCTONIA ISOLATES. G. Olaya and G. S. Abawi. Department of Plant Pathology, Cornell University, Geneva, NY 14456.

Nine binucleate Rhizoctonia isolates were previously obtained from roots of table beets collected from commercial fields in central New York State. The efficacy of these isolates in controlling seed decay and damping-off diseases caused by *Rhizoctonia solani* were evaluated in the greenhouse. Inoculum of a highly virulent isolate of *R. solani* (No. 521) was prepared according to the soil-potato medium of Ko and Hora and used to infest a potting soil mixture at a rate of 2% (v/v). Seedballs of table beet cv. Ruby Queen were placed on the surface of pasteurized soil mixture in 10-cm clay pots (400 ml/pot) and then were covered with a 3-cm layer of the *R. solani* infested soil (100 ml/pot). The number of emerged seedlings and the incidence of post-emergence damping-off were recorded for 4 weeks. The binucleate Rhizoctonia isolates were effective in increasing emergence and reducing post-emergence damping-off when they were applied as hyphal fragments to seeds with methylcellulose (seed treatment) or mixed into the *R. solani* infested soil as colonized beet seedballs (whole or ground) or infested soil potato inoculum preparations. These binucleate isolates were also effective when used with untreated beet seedballs or seedballs treated with Apron 25W (1.4 g/kg) and Thiram 42S (3.8 g/kg). In addition, all the binucleate Rhizoctonia isolates significantly increased the dry weight of beet seedlings as compared to those growing in the control treatment (*R. solani* infested soil).

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MECHANISMS IN THE BIOCONTROL OF *RHIZOCTONIA SOLANI* INDUCED SEEDLING DISEASE BY *GLIOCLADIUM VIRENS*: GLIOTOXIN. C. R. Howell and R. D. Stipanovic, USDA, ARS, Southern Crops Research Laboratory, Route 5, Box 805, College Station, TX 77845.

Mutant strains of *Gliocladium virens* deficient for antibiotic activity were obtained by UV irradiation of conidia and isolation of resulting colonies without surrounding clear zones in a lawn of *Bacillus subtilis* on an agar medium. HPLC analyses of extracts from cultures of the mutant strains revealed that they no longer produced the antibiotic gliotoxin. Assay of extracts for antibiotic activity against *Rhizoctonia solani* showed that those from parent strains were inhibitory to the fungus, while those from mutant strains were not. However, a comparison of the biocontrol efficacies of parent and mutant strains against *R. solani* induced seedling disease showed no significant differences in performance. This brings into question the role of gliotoxin in the biocontrol process.

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GROWTH INHIBITION AND ANTAGONISM IN VITRO BY *GLIOCLADIUM VIRENS* (GLX$^+$) AND GLIOTOXIN NON-PRODUCING (GLX$^-$) MUTANTS. R. D. Lumsden[1], S. Wilhite[2], C. Ding[2], and D. Straney[2], [1]USDA, ARS, Biocontrol of Plant Diseases Laboratory, Beltsville, MD 20705; [2]Dept. of Botany, Univ. of Maryland, College Park, MD 20742.

Gliotoxin, a diketopiperazine antibiotic produced by *G. virens*, inhibited radial growth of several pathogens (10 µg/ml in PDA medium). *Pythium ultimum, P. aphanidermatum, Aphanomyces euteiches* (4 strains), *Phytophthora megasperma* f. sp. *medicaginis* (4 strains), *P. capsici, Sclerotinia sclerotiorum, S. minor* and *Rhizoctonia solani* (3 strains) were inhibited 100%. An AG-4 strain of *R. solani* was inhibited 75%, *Sclerotium rolfsii* 41%, *Fusarium solani* f. sp. *pisi* 16% and *F. oxysporum* f. sp. *lycopersici* 12%. In challenge cultures on PDA medium of wild type *G. virens* (GLX$^+$) and two *G. virens*

gliotoxin-minus mutants (GLX$^-$) against the above pathogens, growth was distinctly inhibited but only with the wild type GLX$^+$ strain and only with pathogens inhibited 100% by gliotoxin. This confirms that gliotoxin can be a significant antifungal factor produced by *G. virens*. Those with less than 100% inhibition showed no challenge inhibition and were not sensitive to GLX$^-$ strains. This information may be useful in screening effective strains of *G. virens*.

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DETECTION OF PRESUMPTIVE MYCOPARASITES IN SOIL ON HOST-COLONIZED AGAR PLATES. D. F. C. Mulligan, C. R. Howell and J. W. Deacon*, USDA, ARS, Cotton Pathology Research Unit, Route 5, Box 805, College Station, Texas 77845 and *Institute of Cell and Molecular Biology, University of Edinburgh, Edinburgh, Scotland.

Soil samples were assayed for the presence of presumptive mycoparasites by incubation on agar pre-colonized by different phytopathogenic fungal hosts. In a survey of British soils, only *Pythium oligandrum*, *Gliocladium roseum*, *Trichoderma* spp. and *Papulaspora* sp. were detected routinely from 56%, 100%, 88% and 79% of soils, respectively. The efficiency of detection of the mycoparasites was influenced by the host fungus used and by competition between the mycoparasites. Baiting of soils with fungal-colonized substrata only partly increased the detection efficiency. The effectiveness of the pre-colonized plate method in detecting specific mycoparasites that could be used in the biocontrol of cotton plant pathogens (*Pythium ultimum* and *Rhizoctonia solani*) was also investigated, using American soils sampled from fields previously cropped to cotton. The results will be discussed in relation to the advantages and limitations of the pre-colonized plate method.

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A SCREENING PROCEDURE FOR BIOLOGICAL CONTROL OF SEEDLING DISEASE OF WATER-SEEDED RICE. R. W. Schneider, B.-J. Moon, and C. G. Giles. Dept. Plant Pathology and Crop Physiology, Louisiana State University Agric. Center, Baton Rouge, LA 70803.

The following variations to a greenhouse screening procedure were evaluated: nitrogen fertilization, different forms and concentrations of inoculum, shading, use of naturally infested field soil, variations of seed inoculation, different methods of seed surface sterilization, and others. In addition, several in vitro assays were evaluated. All of these procedures were evaluated with reference to 36 bacterial isolates that had been tested in the field. Mycelial mats of Pythium arrhenomanes were triturated, and the turbidity was standardized in buffered water. Meanwhile, sterilized soil was added to disposable salad trays, and the trays were filled with deionized water and infested with the inoculum. Lots of 50 seed were surface sterilized by immersion in unbuffered 50% household bleach for 2 hours, rinsed three times with unbuffered sterilized water, and allowed to soak overnight in the fourth rinse. The seed were then treated with a standardized suspension of candidate bacteria by soaking the seed in bacterial suspensions for 2 hours, draining, and incubating overnight at 28C. The seed were then planted in the flooded salad trays which were covered with newspaper for at least 5 days. Percent emergence was determined after about 2 weeks.

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EFFECTS OF DENSITY AND AGGREGATION OF SEEDLINGS ON YIELD OF WATERMELON. Jim Duthie and Warren Roberts, Wes Watkins Agricultural Research and Extension Center, Oklahoma State University, P.O. Box 128, Lane, OK 74555.

Pest attacks affect density and aggregation of watermelon plants. Accounting for aggregation in density-yield relationships may be important if compensatory yield declines with increased aggregation. To evaluate effects of seedling density and aggregation on yield, 64 seeds of watermelon (cv. Sugar Baby) were planted 0.15 m apart in a row oriented along the long axis of each of 96 plots (9.6m x 3.7m). Seedlings were thinned in a uniform, random, or aggregated pattern to average densities of 3.2, 2.8, 2.4, 2.0, 1.6, 1.2, 0.8, 0.4 plants per m of row. Aggregation with varying average density was simulated by establishing, in individual plots, 1 to 8 clumps of 4 adjacent seedlings. Each density-pattern combination was assigned randomly to one plot in each of 4 replicate blocks. Density and aggregation were measured one month before harvest. With decreasing density, total weight of fruit per m of row decreased. The number of fruit per m of row declined sigmoidally from an upper asymptote of 3.7. Average weight of fruit increased linearly. There was no evidence that compensation was affected by aggregation at the scale that was investigated. Further work is needed to evaluate aggregation at other scales and to evaluate effects of damage to plants nearing maturity.

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ETIOLOGY OF PINK ROOT DISEASE OF CANTALOUPE AND WATERMELON. *B. D. Bruton[1], J. A. Duthie[2] and E. V. Wann[1]. [1]USDA-ARS, Lane, OK 74555; and [2]Oklahoma State University, Lane, OK.*

Numerous fungal genera have been associated with the pink to reddish lesions (2-10mm) on roots of cantaloupe and watermelon. Lesions are most common on the secondary and tertiary roots. Often, the root beyond the lesion is killed. Numerous isolations onto water agar and later transferred to V-8 agar consistently yielded colonies of Phoma terrestris Hansen. In greenhouse pathogenicity tests, cantaloupe and watermelon isolates from Oklahoma and Texas produced high levels of pink lesions on roots of cantaloupe, watermelon, and onion. In additional studies, the cucurbit isolates appeared identical to ATCC cultures of P. terrestris (#64033 & #16993) in virulence, symptom expression, and microsclerotial production in the lesions. None of the isolates reduced root weight of the respective hosts grown for 60 days in infested soil.

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SCREENING WATERMELON FOR RESISTANCE TO THREE RACES OF COLLETOTRICHUM ORBICULARE. **L.A. Wasilwa, J.C. Correll, and [1]T.E.Morelock, Depts. of Plant Pathology and [1]Horticulture, Univ. of Arkansas, Fayetteville, AR 72701.**

The anthracnose pathogen, Colletotrichum orbiculare, causes severe damage to watermelon, particularly in the southeastern U.S. C. orbiculare is composed of two genetically distinct races which have been differentiated by both virulence and vegetative compatibility tests (Phytopathology 83:1192-1193). Race 1 consists of cucumber and cantaloupe isolates which belong to VCG 1 and VCG 3, race 2 consists of watermelon isolates in VCG 2. Recently, a third race designated 2B has been differentiated among certain watermelon and cucuzzi gourd isolates in VCG 2. The three races can be differentiated in a seedling cotyledon test using the cucumber cultivars Arkansas Little Leaf (H19) and Marketer and the watermelon cultivars Charleston Grey and Black Diamond. Marketer and Black Diamond are highly susceptible to all three races. H19 is highly resistant to race 2 and 2B but is susceptible to race 1; Charleston Grey is resistant to race 1 and 2B and is susceptible to race 2. A total of 70 watermelon cultivars and plant introductions were screened for resistance to each of the three races in a cotyledon test using a composite of six geographically diverse isolates for each race. All watermelon cultivars examined were highly susceptible to race 2. Most watermelon cultivars were susceptible to race 1 and 2B. The watermelon cultivars highly resistant to race 1 also were highly resistant to race 2B.

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EFFECTS OF THRIPS FEEDING ON PURPLE BLOTCH SEVERITY AND ONION BULB YIELD. M. E. Miller, Texas A&M University, Weslaco, TX 78596, B. Cartwright, C. L. McKenzie, and J. V. Edelson, Oklahoma State University, Lane, OK 74555.

Effects of thrips (*Thrips tabaci*) populations on purple blotch (*Alternaria porri*) severity and bulb yield were determined on onion cv. Texas Grano 1015Y from 1990-92. Purple blotch severity levels were maintained by weekly treatments of either iprodione at 1.12 kg (ai)/ha, anilazine at 1.12 kg (ai)/ha, mancozeb at 2.69 kg (ai)/ha or no fungicide. Thrips populations were maintained as follows: 1) cypermethrin at 89.7 g (ai)/ha + endosulfan at 1.12 kg (ai)/ha when populations reached 0-5/plant, 2) cypermethrin at 89.7 g (ai)/ha + endosulfan at 1.12 kg (ai)/ha when populations reached 5-10/plant, 3) cypermethrin at 89.7 g (ai)/ha when populations reached 10-25/plant and 4) no insecticide. There was a positive linear relationship between thrips populations and purple blotch severity and a negative linear relationship between thrips populations ($p<0.001$) and bulb yield.

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EFFECTS OF BROCCOLI RESIDUE ON *VERTICILLIUM DAHLIAE* MICROSCLEROTIA AND WILT INCIDENCE IN CAULIFLOWER. K. V. Subbarao, J. C. Hubbard, and S. T. Koike. Dept. of Plant Path., Univ. of California, Davis, c/o U. S. Agric. Res. Stn., Salinas, CA 93905.

Wilt incited by *Verticillium dahliae* has become an important disease on cauliflower in the Salinas Valley in recent years. Although broccoli is closely related to cauliflower, wilt has not occurred on this host. The *V. dahliae* isolates from cauliflower were weakly pathogenic on broccoli. We therefore determined the effectiveness of broccoli residues on propagule attrition and wilt incidence on cauliflower in a field with known infestation by *V. dahliae* microsclerotia. The treatments in the experiment were broccoli residue with tarp, broccoli residue without tarp, methyl bromide + chloropicrin, vapam, control with tarp, and control without tarp, and were arranged in a randomized block design with four replications. Approximately 200 Kg chopped broccoli (in 36 m^2) was uniformly spread and incorporated into the corresponding plots by disking. For treatments with tarping, clear plastic was spread over the plots and sealed at the edges. Tarps were removed after two weeks. Pre- and post-treatment densities of *V. dahliae* microsclerotia were determined at 0, 35, 95, and 150 days using the modified Anderson sampler technique. Plant height, number of marketable heads, head weight, and wilt severity were determined at maturity. The number of *V. dahliae* propagules in broccoli-treated plots were lower than the control plots and were comparable to that of the fumigated plots. Similarly, plant height, marketable heads, and head weight were significantly higher in broccoli treatments than in check plots. Tarping alone did not reduce the number of propagules. These results suggest that broccoli residue has the potential to replace standard fumigants for Verticillium wilt control. The ideal means of exploiting this may be by rotating cauliflower with broccoli.

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REDUCED DISEASE SEVERITY OF COMMON ROOT ROT IN PEA FOLLOWING SWEET CORN AND OAT ROTATION. J.L. Williams and F.L. Pfleger, Dept. of Plant Pathology, University of Minnesota, St. Paul 55108.

Rotation crops and tillage treatments were evaluated in the field for effects on common root rot of pea caused by *Aphanomyces euteiches* in Minnesota in 1993. Residue of sweet corn and oat crops that were plowed significantly reduced the percent infected pea plants 3-weeks after planting as compared to all other treatments. However, the entire field became infected with *A. euteiches* regardless of crop/tillage treatment due to flooding. Residual treatment effects were measured in the greenhouse by planting seed of a susceptible and resistant pea cultivar in samples of soil from each plot taken in early fall. Sweet corn and oat crops, using susceptible and resistant pea cultivars, significantly reduced *A. euteiches* soil inoculum potential by 67% and 11%, and 70% and 46%, respectively, and increased fresh pea vine weights by 192% and 216%, and 154% and 178%, respectively, as compared to consecutive pea cropping, indicating that corn and oat residues can reduce disease severity in the next pea crop. Pea yield and fresh and dry vine weights over all crop treatments were significantly higher and disease index and inoculum potential values were significantly lower for the resistant than the susceptible pea cultivar.

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DEVELOPMENT OF CELERY LINES WITH INCREASED RESISTANCE TO *FUSARIUM OXYSPORUM* F.SP. *APII* INFECTION USING SOMACLONAL VARIATION. K.F. Toth, S.L. Krebs, M.L. Lacy, R. Grumet, E.J. Hudgins, Departments of Botany & Plant Pathology and Horticulture, Michigan State University, East Lansing, MI 48824.

Fusarium yellows (*Fusarium oxysporum* f.sp. *apii* race 2) limits celery (*Apium graveolens* var. *dulce*) production in Michigan. Host resistance is an important method for controlling this disease. Somaclonal variation was used to increase the level of resistance in celery. Callus was initiated from sterile axillary buds on modified Murashige-Skoog agar. Shaken somatic cell suspensions initiated with callus yielded embryoids which were regenerated into whole plants. These plants were screened for disease reaction in the field and some individuals proved more resistant than the parent cultivar. Successive cycles of vernalization and selfing followed by field screening and selection for 5-6 years stabilized resistance. Five somaclone lines derived from Tall Utah 52-70 HK are in their final year of field testing for release as germplasm in 1995. Somaclone lines derived from Florida 683, which are expected to be more horticulturally desirable than the Tall Utah 52-70 HK-derived lines, are also in advanced development.

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DEVELOPMENT OF TOMATO IRREGULAR RIPENING SYMPTOMS AFTER FIELD HARVEST. C. A. Powell and P. J. Stoffella, Univ. of Florida, AREC, POB 248, Fort Pierce, FL 34954.

Tomato irregular ripening (TIR) is a disorder of tomatoes associated with the feeding of the sweet potato whitefly (SPWF). Symptoms, which are limited to the mature fruit, are external (green or white streaks) and internal (white or green discoloration). Tomato fruit, harvested from SPWF infested plants at the first sign of pink color and allowed to ripen at 20-22°C for 3 wks, had mean external TIR of 37.1% and mean internal TIR of 66.0%. Tomato fruit that were stored at 10-13°C for 21 days prior to ripening at 20-22°C had mean external TIR of 50.2% and a mean internal TIR of 69.9%. Of the fruit exhibiting external TIR during the ripening process, 89% recovered from the external symptoms. However, about half of these "recovered" tomatoes had internal TIR. The data indicate that tomatoes can develop TIR after harvest when the SPWF is no longer present, and that tomatoes can recover from external TIR, appearing satisfactory for the fresh market, but may still have a high incidence of internal TIR.

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SUPPRESSION OF APHID-VECTORED VIRUS DISEASES AND INCREASED MARKETABLE YIELD OF ZUCCHINI SQUASH USING REFLECTORIZED SPRAY MULCHES. J. J. Stapleton[1], C. G. Summers[2], R. A. Duncan[1], and A. S. Newton[2]. Statewide Integrated Pest Management Project[1] and Department of Entomology[2], University of California, Kearney Agricultural Center, Parlier, CA 93648.

Reflectorized plastic mulches are known to protect susceptible vegetable crops against early-season, aphid-vectored virus diseases. Biodegradable, water-soluble spray mulch (Styrofan®, BASF Corp.) painted silver was applied to beds of zucchini (*Cucurbita pepo* var. *melopepo* cv. Sunre 7918) squash and compared to other silver and white mulches for virus disease suppression and effect on crop yield. The experiment was conducted in Aug.-Oct. 1993 near Fresno, and 12 pickings of squash were made. A flight of cotton/melon aphid (*Aphis gossypii*) vectoring zucchini yellows mosaic, watermelon mosaic-2, and cucumber mosaic viruses occurred during the experiment. At the first picking, general treatment differences in suppressing foliar virus symptoms were silver mulches > white mulches > plants on bare soil or insecticide sprayed controls. All mulch treatments gave significant ($P<0.05$), 3 to 5-fold increases in yield of marketable squash over controls. The silver spray mulch provided the highest numerical yield.

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DETECTION OF TWO SEROTYPES OF POTATO VIRUS Y (PVY) IN POTATO FARMERS' FIELDS IN BOLIVIA. V. Alvarez, and E. N. Fernandez-Northcote, PROINPA (IBTA-CIP-COTESU), Casilla 4285, Cochabamba, Bolivia.

Potato leaves were sampled from different cultivars in four Departments of Bolivia. In direct DAS-ELISA, PVY-A and PVY-B serotypes were specifically detected by using immunogamma globulins and their conjugates with alkaline phosphatase, prepared at the International Potato Center (CIP) from isolate PVY^N 201, Cuzco, Peru, for PVY-A, and one commercially available prepared from the Swiss isolate PVY^N 605, for PVY-B. In Bolivia the most commonly detected serotype is PVY-A. In the Departments of Cochabamba, Tarija, and La Paz, in native potato cultivars and above 3,400 masl the incidence is usually of 100% for PVY-A and 0% for PVY-B. In other areas where PVY-A incidence is about 30-60%, PVY-B incidence is about 1-4%. However, in the Department of Potosi, the incidence is of 72% for PVY-B, and 17% for PVY-A. The evidence indicates that PVY-B was introduced to Potosi in a foreign cultivar. Both serotypes are PVY^N according to their reaction in ***Nicotiana tabacum*** 'White Burley.'

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Purification and Analysis of Amorphous Xylem Occlusions from Citrus Trees with Blight. R. H. Brlansky[1], M. M. Sanwo[1], C. Elliott[2] and C. A. Powell[3]. [1]Univ. of FL, CREC, Lake Alfred, FL 33850, [2]DIAGTEC, Loxahatchee, FL 33470, [3]Univ. of FL, AREC, Ft. Pierce, FL 34954.

Amorphous xylem occlusions were extracted from transverse sections of trunk cores from citrus trees affected with blight by maceration of the tissue sections followed by multiple cycles of sonication at output readings ranging from 22-50 watts. Separation of the amorphous occlusions from filamentous occlusions and the wood was accomplished by centrifugation on cesium chloride gradients. Amorphous and filamentous occlusions banded at the 67% and 65% cesium layers, respectively. The occlusions were removed, washed and concentrated on 0.4 μm polycarbonate filters. Identity and purity were confirmed using light and scanning electron microscopy. Occlusions were solubilized in thioglycolic acid/HCl at 100 C followed by dissolution in NaOH, reprecipitation in concentrated HCl, and redissolution in NaOH. The characteristic solubility profile on the UV spectrophotometer data suggests that the occlusions are composed of a polyphenol (lignin).

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RELATIONSHIP OF CUTICLE DEVELOPMENT TO RESISTANCE OF LEAVES TO STOMATAL FLOODING, BACTERIAL INGRESS AND DEVELOPMENT OF CITRUS CANKER. J. Graham, T. Gottwald,[1] T. Riley and D. Achor. Univ. Florida, Lake Alfred 33850 and [1]USDA-ARS, Orlando, FL 32803

Cuticle thickness on the abaxial surface of Duncan grapefruit leaves increased from 0.06 to 1.06 μm as leaves grew from half to fully expanded. The rapid development of cuticle coincided with an increase in resistance of leaves to stomatal flooding and ingress by *Xanthomonas campestris* pv. *citri*. Leaf surfaces were brushed with Vapor Gard® (VG, polyterpene) to create an epicuticular film to increase cuticle resistance on half-expanded leaves. To lower cuticle resistance, leaves were sprayed with Herbex® (HX) containing a siloxane surfactant (SS) and a penetrant (P), or with each component alone. Treated leaves were inoculated with 2×10^5 cfu/ml in a stomatal inoculation chamber at an inoculation pressure of 9.81 KPa against the abaxial leaf surface. Discrete infections of stomata and lesions developed after 168 h. For half-expanded leaves, VG treatment decreased, SS had no effect, and HX and P increased bacterial ingress, internal populations, and lesion number compared to the water control. HX and P decreased resistance of leaves at half, three-fourths, and full expansion to bacterial ingress and increased bacterial populations and lesion number due to stomatal flooding.

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EXACERBATION OF CITRUS BACTERIA SPOT DISEASE BY STOMATAL FLOODING CAUSED BY THE USE OF SURFACTANTS. T. R. Gottwald[1], J. H. Graham[2], and T. D. Riley[1]. [1] USDA-ARS, Orlando, Florida, and [2]Citrus Research and Education Center, University of Florida, Lake Alfred, Florida, USA.

Swingle citrumelo plants inoculated with *Xanthomonas campestris* pv. *citrumelo* were arranged as line source of inoculum at one end of nursery plots. A speed sprayer was used to simulate a wind-blown rain by spraying water over the source plants and down the nursery rows. Individual rows were treated with a water control, Herbex® (a common commercial surfactant-penetrant), or the penetrant or surfactant components of Herbex alone. To estimate the dispersal gradient immediately after the simulated rainstorm, surface bacteria were quantified by washing leaf samples. Bacterial dispersal gradients in all rows were similar and extended the full 7m of the nursery rows. Disease incidence and mean lesion diameter were estimated for selected assay points in each row 28 days after the event. Herbex and its surfactant component significantly increased the total number of lesions per plant and mean lesion diameter compared to the water control. The disease gradient slopes associated with Herbex, and its surfactant component, were significantly flatter and more extensive than the water control, whereas its penetrant component was not significantly different from the water control. These results suggest that surfactants which cause stomatal flooding can enhance infection and exacerbate citrus bacterial epidemics.

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DIFFERENTIAL INTERACTION BETWEEN STRAINS OF RHIZOMONAS SUBERIFACIENS OR RELATED SPECIES AND ACCESSIONS OF LACTUCA SPP. WITH RESPECT TO SEVERITY OF CORKY ROOT DISEASE. A. H. C. van Bruggen, Department of Plant Pathology, R. W. Michelmore and O. E. Ochoa, Department of Vegetable Crops, University of California at Davis, CA 95616.

Fifty three strains of Rhizomonas suberifaciens and 57 strains of unnamed species related to R. suberifaciens were tested for induction of corky root on lettuce (Lactuca sativa L.) cv. Salinas and breeding line 440-8, susceptible and resistant, respectively, to strain CA1^T of R. suberifaciens. One strain of R. suberifaciens (CA3) and three strains of unnamed species (CA15, CA32, and NL2) were equally virulent to Salinas and 440-8. Twenty three accessions of L. sativa, L. serriola, L. saligna, and L. virosa were then tested for resistance to strains CA1^T, CA3, CA15 and NL2. All lines with resistance to strain CA1^T were susceptible to strains CA3, CA15, and/or NL2. The only two lines with moderate resistance to all four strains were lettuce cultivars Raleigh and South Bay. In a randomized complete block experiment, there was a significant differential interaction between eight Lactuca lines and ten strains of R. suberifaciens and related species with respect to corky root severity. Thus, it would be prudent to use several different bacterial strains to breed lettuce for resistance to corky root.

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A LEAF SPOT OF VINCA CAUSED BY *PSEUDOMONAS CICHORII*. S. M. McCarter, Department of Plant Pathology, University of Georgia, Athens, GA 30602-7274.

In March of 1993 a leaf spot was observed on *Vinca rosea* cv. Peppermint plants being produced in commercial greenhouses for sale as bedding plants. Streak-plate isolations made on King Medium B from diseased tissue macerated in water consistently yielded a fluorescent pseudomonad that tested +, —, and + for oxidase, arginine dihydrolase, and tobacco hypersensitivity, respectively. Steps in Koch's Postulates were successfully fulfilled. The bacterium was identified as *Pseudomonas cichorii* based on both fatty acid analysis and carbon source utilization (Biolog). In growth chamber tests cultivars in the Cooler series of *V. rosea* were generally more susceptible than other types. The disease developed at 20, 25, and 30 C with an optimum of 25. Wounding was not necessary for infection but greatly increased disease incidence and severity. Both streptomycin and cupric hydroxide provided moderate control on nonwounded plants, but only streptomycin was effective on wounded plants.

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POPULATION DYNAMICS OF EPIPHYTIC STRESS TOLERANCE MUTANTS OF *PSEUDOMONAS SYRINGAE* UNDER FIELD CONDITIONS. G. A. Beattie and S. E. Lindow. University of California, Department of Environmental Science, Policy, and Management, Berkeley, CA 94720.

The epiphytic fitness of four Tn*5* mutants of *Pseudomonas syringae* that exhibited reduced epiphytic fitness in the laboratory was evaluated under field conditions. The mutants differed more from the parental strain under field conditions than under laboratory conditions in their survival immediately following inoculation onto bean leaves and in the sizes of the epiphytic populations that they established and maintained in the 8 days following inoculation. These results demonstrate that their fitness was reduced more in the field than in the laboratory. The presence of the parental strain, B728a, did not influence the survival or growth of three of the mutants; however, one mutant, an auxotroph, established larger populations in the presence of B728a than in its absence, possibly due to cross-feeding by B728a *in planta*. Three of the mutants behaved similarly to two nonpathogenic strains of *P. syringae*, suggesting that the mutants may be altered in traits that are missing or poorly expressed in naturally occurring nonpathogenic epiphytes.

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A NEW DISEASE OF SEEDLING COTTON CAUSED BY *PSEUDOMONAS SYRINGAE*. J. Mertely, J. Gannaway, and H. Kaufman. TAMU Agricultural Research and Extension Center, Route 3 Box 219, Lubbock, TX 79413.

On the High Plains of Texas, the passage of cool fronts and associated wet weather often precedes leaf spot development on cotton seedlings. The circular, 2-4 mm-dia spots consist of tan necrotic centers surrounded by dark brown to purple borders. Large, irregular, necrotic areas appear on severely diseased leaves. *Ascochyta gossypii*, the causal agent of Ascochyta or wet weather blight, produces similar symptoms on cotton seedlings. However, isolations from typical leaf spots in 1993 yielded a fast growing, gram negative, oxidase negative bacterium which fluoresced on King's medium B. Two isolates from different locations were identified as *Pseudomonas syringae* by fatty acid profile analysis done at the Plant Disease Diagnostic Laboratory at Texas A&M University. When Paymaster HS-26 seedlings were pressure spray inoculated with a 10^8 cfu/ml suspension of either isolate, typical leaf spots developed after 10-12 d. Koch's postulates were completed by the recovery of *P. sryingae* from these spots. This is the first report of a Pseudomonas leaf spot disease of cotton.

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AGGRESSIVENESS OF *XANTHOMONAS CAMPESTRIS* PV. *VESICATORIA* TOMATO RACE 3 (T3) STRAINS OVER TOMATO RACE 1 (T1) STRAINS: EVIDENCE FOR ANTAGONISM. G. A. El-Morsy, G. C. Somodi, J. W. Scott, R. E. Stall and J. B. Jones. Plant Path. Res. Inst., Agric. Res. Center, Giza, Egypt and GCREC, Univ. of Florida, Bradenton 34203.

Until recently, T1 was the only tomato race found in Florida. In 1991 a new race, T3, was identified from strains collected in several locations and has since become more prevalent. The two races can be identified by differential amylolytic activity. A field study in 1993 was initiated to determine if T3 strains were more aggressive than T1 strains. The genotypes Walter (susceptible (S) to both races), Hawaii 7998 (resistant (R) to T1, S to T3) and two other genotypes, PI 126932 and Hawaii 7981 (S to T1 and R to T3) were inoculated with both strains and transplanted to the field. Overall, 92%, 99% and approximately 50% of *X. c. vesicatoria* strains isolated from lesions on Walter, Hawaii 7998, and the T3 resistant genotypes, respectively, were T3. T3 strains from 11 different fields were determined to be inhibitory to a T1 strain on nutrient agar. Five additional T3 strains were inhibitory to ten T1 strains. In a greenhouse study PI 126932 and Hawaii 7981 plants were treated with a T3 strain or with buffer, placed in polyethylene bags at 28 C, and 24 hr later, some of the plants were inoculated with a T1 strain. Plants sprayed with the T3 suspension 24 hr prior to inoculating with T1 had significantly less disease (<1%) than those inoculated with T1 alone (>9%). Plants sprayed with T3 alone also had less than 1% defoliation. The prevalence of T3 strains on plants in fields where both T1 and T3 are present may in part be due to the antagonistic nature of T3 to T1 strains.

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RACE SHIFT IN *XANTHOMONAS CAMPESTRIS* PV. *VESICATORIA* WITHIN A SEASON IN THE FIELD. C. S. Kousik, D. C. Sanders and D. F. Ritchie, North Carolina State University, Raleigh, NC 27695.

Race shifts of *Xanthomonas campestris* pv. *vesicatoria*, the bacterial spot pathogen of bell peppers, was studied in the field during 1993. Early Calwonder (ECW) plants inoculated with rifampicin-resistant race 1 strain (Xcv 33^{rif}) carrying the avirulence gene *avrBs3* were planted as inoculum sources in a plot of ECW-30R, which carries the *Bs3* gene for resistance. Two wk after inoculation, disease was observed on plants adjacent to the inoculum plants. Within eight wk all plants of ECW-30R were diseased. Bacteria were isolated from diseased plants, and 425 rifampicin-resistant single colonies were screened for race reaction on pepper differentials. All colonies exhibited a race 3 phenotype. Plasmid DNA from 25% of the single colonies hybridized with the *avrBs3* gene probe. The plasmid carrying *avrBs3* was not detected in colonies that did not hybridize to *avrBs3*. Analysis of *in planta* growth of the strains which had shifted from race 1 to race 3 confirmed the ability of these strains to grow in ECW-30R. The original strain of Xcv 33^{rif} and representatives of the 425 single colonies did not cause hypersensitive reaction on tomato cv. 'Bonny Best'. Strain Xcv 33^{rif} did not undergo a race shift when exposed to a susceptible cv. Thus, a race 1 population rapidly shifted to a race 3 within a growing season, when exposed only to a resistant host carrying the corresponding single gene (*Bs3*) for resistance. This shift was due to loss of a plasmid carrying the avirulence gene (*avrBs3*) or to inactivation of this gene.

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INDUCTION OF BLACK LEAF-VEIN ON GERANIUM BY XANTHOMONAS CAMPESTRIS PV. PELARGONII. D. J. Kim[1], S. H. Kim, and T. N. Olson. [1]Central Dauphin High School, Harrisburg, and Pennsylvania Department of Agriculture, Harrisburg, 17110-9408.

A black leaf-vein symptom was induced when seedlings of Pelargonium x hortorum L. H. Bailey cv. 'Orbit White' were inoculated with Xanthomonas campestris pv. pelargonii (Xcp). The symptom was pronounced when Xcp, 1E5 colony-forming units (cfu) in 10 ul .01 M PO4, pH 7.2, was placed on a needle-punctured vein of a young leaf, and incubated at 20C-8hr dark and 30C-16hr light, RH 100 % for 4 days. The symptom was induced with 145 isolates from 71 geranium cultivars including P. x hortorum, P. domesticum, P. peltatum, and Geranium sanguineum. The lowest Xcp population that caused the black vein was 1.5±0.8 cfu in 10 ul; the symptom was visible when the Xcp reached 1.6E6 cfu.

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RICE CATIONIC PEROXIDASE ACCUMULATES IN XYLEM FLUIDS DURING INCOMPATIBLE INTERACTIONS WITH *XANTHOMONAS ORYZAE* PV. ORYZAE. Scott A. Young, Ailan Guo, James A. Guikema, and Jan E. Leach, Department of Plant Pathology, Kansas State University, Manhattan, KS 66506-5502

A cationic peroxidase, PO-C1 (MW 43 kD, pI 8.6), which is induced in incompatible interactions between the vascular pathogen *Xanthomonas oryzae* pv. oryzae and rice (*Oryza sativa* L.), was purified. Amino acid sequences from chemically cleaved fragments of PO-C1 exhibited a high percentage of identity with sequences of peroxidases from rice, barley, and wheat. Polyclonal

antibodies were raised to a synthetic oligopeptide (a 12-mer, called POC1a) that was derived from a region where the cationic peroxidase diverged from other known peroxidases. The anti-POC1a antibodies reacted with POC1a peptide and guttation fluids from plants undergoing an incompatible response. Guttation fluids from compatible interactions did not react to the anti-POC1a antibodies. The antiserum specifically reacted with PO-C1 from extracts of induced plants when separated on native cathodic gels. Thus, PO-C1 activity increases in xylem fluids preferentially during incompatible interactions.

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INTRARACIAL VARIATION IN VIRULENCE AND HOST ADAPTATION OF *XANTHOMONAS ORYZAE* PV. *ORYZAE* ON RICE. C. C. Mundt, M. R. Finckh, and R. F. Alfonso. International Rice Research Institute, Manila, Philippines.

Isolates of *Xanthomonas oryzae* pv. *oryzae*, causal agent of bacterial blight of rice (*Oryza sativa*), were collected from two near-isogenic rice lines and their 1:1 mixture during naturally-occurring epidemics at two sites in the Philippines. Almost all isolates were virulent on both hosts when tested in the greenhouse, a reaction typical of of *X. oryzae* pv. *oryzae* race 3. A continuous range of aggressiveness (as measured by lesion length) was found, and variance components analysis indicated that the majority of this variation was due to genetic differences among isolates. Evidence for adaptation to the host of origin was found at both sites. At one site, virulence of populations originating from the host mixture was intermediate between that of populations obtained from the two pure stands. At the other site, populations from the mixture showed strong adaptation towards one component of the mixture. Spatial diversity for adaptation to the two rice lines was found at each site. Contingency analyses indicated that virulence on each of the two hosts are independent. We conclude that, despite the clonal nature of *X. oryzae* pv. *oryzae*, there is much selectable variation for virulence present even within a single race of the pathogen.

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WATER STRESS AND ISOLATE EFFECTS ON DISEASE DEVELOPMENT BY *SEPTORIA MUSIVA* ON *POPULUS* HYBRIDS. D.L. Maxwell and G.R. Stanosz. Dept. Plant Pathology, University of Wisconsin-Madison, Madison, WI 53706.

Septoria musiva Peck (teleomorph *Mycosphaerella populorum* G.E. Thompson) causes a leaf spot and canker disease of poplars. The disease has been reported to be more severe on harsher sites. To determine if water stress predisposes hybrid poplars to colonization by *S. musiva*, we conducted a greenhouse study on 2-month-old greenwood cuttings of clones NM-6 (nigra X maximowiczii) and NC-11396 (maximowiczii X berolinensis) rooted in Fafard Mix #2. Half of the trees were watered 3 times/wk; the rest were watered only when the lowest measured predawn water potential fell below -1.5 MPa. We wounded trees by removing the fourth fully expanded leaf from the apex and inoculated them by placing a colonized agar plug on the wound. Inoculum consisted of two single-conidial isolates that originated from the same leafspot. We also included wounded and nonwounded controls. After 80 days, we measured the lengths of the cankers that resulted. Analysis of variance indicated that the effects of inoculation treatment, water stress, and treatment by water stress interaction were significant ($p<.01$). Mean canker length differed between isolates and was greater for water-stressed trees. These results suggest that host moisture status influences canker development in hybrid poplars colonized by *Septoria musiva*.

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ASCOSPORE MATURATION AND DISCHARGE IN TWO POPULATIONS OF *VENTURIA POPULINA* ON HYBIRD POPLAR IN THE PACIFIC NORTHWEST. G. Newcombe and G. A. Chastagner, Wash. State Univ. Res. & Ext. Center, Puyallup, 98371.

Venturia populina is a pathogen of *Populus trichocarpa*, the native cottonwood of the Pacific Northwest. It now causes leaf and shoot blight of hybrid poplar (*P. trichocarpa* X *P. deltoides*) in spring in short-rotation plantations managed for pulp. Ascospore maturation and discharge of pseudothecia found on hybrid poplar clone, Hyb 5, near Pack Forest, WA, and near Clatskanie, OR, 115 km apart, were studied in three springs, 1991-1993. In all three years, at Clatskanie, ascospores matured by early March and were discharged between mid-March and mid-April. In contrast, at Pack Forest, ascospore maturation and discharge began in March but increased only gradually through April and May; immature ascospores were still found in June. The difference in the two populations was examined in light of host phenology, local climate, a common-garden experiment, and local blight resistance in *P. trichocarpa*.

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TEMPERATURE EFFECTS ON PITCH CANKER CAUSED BY *FUSARIUM SUBGLUTINANS* ON JUVENILE MONTEREY PINES (*PINUS RADIATA*). M. J. McDonald[1], T. R. Gordon[2], and W. E. Bros[1]. [1]Dept. of Biology, San Jose State University, San Jose, CA 95112. [2]Dept. of Plant Pathology, University of California, Berkeley, CA 94720.

Pine pitch canker disease, caused by Fusarium *subglutinans* f. sp. *pini*, is prevalent on Monterey pine (*Pinus radiata*) in California where it also infects twelve other native conifers. This study examined the effects of temperature on *F. s. pini* in artificially inoculated juvenile *P. radiata* branches. A 5 ul suspension of microconidia (2.5×10^6 microconidia/ml) was injected into three branches/tree on 79 trees and incubated for 21 days concurrently at 14, 18, or 26°C. The tree branches were evaluated, then dissected and the stain lengths were measured. A nested one-way ANOVA showed that incubation temperature did have an effect on *F. s. pini* induced stain length. The least mean squares stain lengths were 0.28, 0.86 and 2.5 cm for 14, 18 and 26°C respectively. A Pearson correlation test indicated no correlation between stain length and branch age, branch length, branch height, or branch circumference.

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FUNGAL ROOT PATHOGEN INTERACTIONS IN A MIXED CONIFER FOREST IN NORTHEASTERN OREGON. Brennan A. Ferguson and Everett M. Hansen. Dept. of Forest Science, Oregon State University, Corvallis, OR. 97331.

Forest disease surveys have noted the presence of two or more root pathogens infecting the same stump or root, and it has been suggested that these fungi may be interacting synergistically. To test this hypothesis, we studied three separate root disease centers where the dominant pathogens appeared to be either Armillaria ostoyae, Heterobasidion annosum, or Phellinus weirii. H. annosum was found infecting trees and stumps within the P. weirii and A. ostoyae centers, and was isolated from some of the same roots as A. ostoyae. Three experiments studied interactions between A. ostoyae, P. weirii, the S and P intersterility groups of H. annosum, and Perenniporia subacida. Hyphal interactions were studied microscopically on agar filled culture slides, macroscopically on 2% and enhanced malt agar, and within wood blocks of true fir and ponderosa pine. Individual hyphal contact occurred between fungal species on the culture slides, resulting in occasional hyphal vacuolization, but no other discernible responses. Interactions on media resulted in formation of zones of inhibition, walling off, abutting growth, or overgrowth of one isolate over the other. Colony interactions were inconsistent between the two media types. Interactions within wood blocks showed formation of dark lines between opposing fungi, and isolations showed the colonizing fungi remained separate. We conclude that these fungi do not act synergistically, but appear together independently in nature as conditions permit.

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THE SUSCEPTIBILITY OF PACIFIC YEW TO *PHYTOPHTHORA LATERALIS*. Marion Murray and Everett Hansen. Department of Botany and Plant Pathology, Oregon State University, Corvallis, OR 97331.

In 1990 Pacific yew was reported as a new host for *Phytophthora lateralis*, (DeNitto and Kliejunas, Plant Disease, 75: 986) an aggressive root rot pathogen of Port Orford cedar. This study compares the pathogenicity of *P. lateralis* on the two hosts. Root colonization and mortality were compared on inoculated seedlings, and lesion expansion was compared on inoculated, harvested branches. Seedling mortality averaged 90% for cedar and 10% for yew. Root colonization averaged 90% for cedar and 30% for yew. Lesion expansion on the cedar branches exceeded lesion expansion on yew branches by nearly twofold. Zoospore attraction to freshly cut cedar and yew rootlets was examined microscopically. Abundant zoospore aggregation occurred on cedar rootlets behind the root caps and along the cut ends. Zoospores encysted randomly along the yew rootlets in far fewer numbers. A field study of yew mortality in *P. lateralis*-infected drainages of southwest Oregon and northern California revealed that mortality is correlated with distance to high water. We conclude that Pacific yew is less susceptible to *P. lateralis* than Port Orford cedar, but can be killed in areas where seasonally flooded soils correspond with high inoculum concentration.

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SHORT-TERM VECTOR CONTROL FAILS TO PREVENT PROCERUM ROOT DISEASE IN WHITE PINE. J.A. Carlson and S.A. Alexander, Department of Plant Pathology, Physiology and Weed Science, VPI&SU, Blacksburg, VA 24061-0330.

Leptographium procerum causes procerum root disease (PRD) and is carried by pales weevils (*Hylobius pales*) in the field. Studies have shown that the weevil can transmit *L. procerum* to eastern white pine. The pathogen is thought to be introduced into healthy mature Christmas trees via weevil oviposition or feeding, with trees becoming symptomatic and dying a short time later. The insecticide lindane is effective against weevils and recommended for PRD control in Christmas tree plantations. Paired plots were established at five field sites to determine if annual lindane applications affected PRD epidemiology. Neither weevil populations nor PRD mortality were reduced during or following three years of treatment. A recent survey of Christmas tree plantations found pales weevil feeding on the stem and roots of newly planted seedlings. Isolations from the feeding areas yielded *L. procerum*. These findings suggest that trees may be infected by *L. procerum* some years prior to symptom expression and death.

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A BOTRYOSPHAERIA CANKER EPIDEMIC IN NORTHERN RED OAK. R. W. Roncadori[1], T. L. Krugner[2], P. P. Kormanik [3], E. C. Whiting[1], and K. L. Reynolds[1]. [1]Dept. of Plant Pathology, University of Georgia, Athens, GA 30602-7274. [2]Departamento de Fitopatologia, Universidade de São Paulo, Piracicaba, S.P. Brasil, and [3]U. S. Forest Service, Southeastern Forest Experiment Station, Athens, GA 30602-2044.

A 4-year-old experimental plantation of eight half-sib families of Northern red oak (*Quercus rubra*) growing in Oconee County, SC suffered extensive damage from Botryosphaeria canker during the spring and summer of 1992. Cankers were evident on 2- or 3-year-old wood both on the main stem or branches of 80% of the 794 trees. The outer and inner bark of young cankers were dark brown to black and the infected area sunken, often girdling the stem or branch and causing a dieback. Many of the older cankers were partially or completely healed. *Botryosphaeria dothidea* (=*Botryosphaeria ribis*) was the only suspected pathogen isolated (growing from 50% of the lesion and canker margin samples) and Koch's Postulates were satisfied by inoculating saplings in the plantation. There was no correlation between tree cylindrical volume and disease severity and no apparent family effect on disease susceptibility. Mortality averaged 2% primary as a result of sprouting by trees suffering extensive dieback.

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Pathogenicity and histopathology of *Botryosphaeria ribis* in *Melaleuca quinquenervia* ramets. M.B. Rayachhetry and R.S. Webb. School of Forest Res. and Conservation, Univ. of Florida, Gainesville, FL 32611.

Single-spored isolates of *Botryosphaeria ribis* obtained from cankered *Melaleuca quinquenervia* trees in South Florida were cultured on PDB and inoculated into wounded ramets, and the ramets were incubated in the greenhouse. Cankered stem tissues were fixed in FPA, sectioned, stained with Pianeze's IIIB, and observed using light microscopy. Hyphal colonization in longitudinal, radial, and tangential directions through inter- and intra-cellular spaces of tracheids, vessels, and parenchymatous cells was evident. Stem cankers similar to those observed under field conditions were produced in greenhouse trials. Canker extension was greatest distally and tangentially from inoculation point. Virulence of isolates was tested on seven melaleuca clones.

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INFECTION OF SLASH AND LOBLOLLY PINE SEEDLINGS BY BASIDIOSPORES OF CRONARTIUM QUERCUUM F. SP. FUSIFORME THROUGH STEM WOUNDS. T. Miller and R. A. Schmidt. Department of Forestry. University of Florida, Gainesville, FL 32611.

A method for inoculating 6-wk-old seedlings of slash and loblolly pines by applying basidiospores of Cronartium quercuum f. sp. fusiforme to wounds created by severing the upper 5-10 mm of stems was tested and compared to the concentrated basidiospore spray (CBS) inoculation method. Seedlings of resistant (R) and susceptible (S) families were wound-inoculated with 100 basidiospores/5 microliter droplet/seedling (80 seedlings/family). A second test used 50, 100, 1,000 and 5,000 spores/seedling of the same R and S families. The wound technique was successful in separating the R and S families and produced the same phenotypic symptom types as the CBS method. Pine family responses to increasing basidiospore concentrations on wounds were similar to those using the CBS method with different inoculum concentrations. The potential value of the wound technique in research on mechanisms of resistance and host/parasite interactions are discussed.

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INFECTION OF SLASH PINE SEEDLINGS BY VEGETATIVE HYPHAE DERIVED FROM SINGLE BASIDIOSPORES OF CRONARTIUM QUERCUUM F. SP. FUSIFORME. T. Miller, G. M. Blakeslee, R. A. Schmidt and W. E. Lante. School of Forest Resources and Conservation, University of Florida, Gainesville, FL 32611.

Infection and typical symptom development after inoculation of slash and loblolly pine seedlings through stem wounds (severed epicotyls) with basidiospores of Cronartium quercuum f. sp. fusiforme prompted similar wound inoculations using mycelial cultures grown from mass and single (haploid) basidiospores. Hyphae from mass spore cultures produced 50% galled seedlings on susceptible slash pines. Wound inoculations of 58 slash pine seedlings using cultures from single basidiospores produced galls on 11 (19%) seedlings of a susceptible family from 5 different haploid cultures. This is thought to be the first report of infection of pine seedling epicotyls with haploid cultures of C. quercuum f. sp. fusiforme. This method provides a potential means of studying host-pathogen gene interactions by challenging specific pine genotypes with single haploid isolates of the fungus.

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LATE-SEASON MORTALITY IN LONGLEAF PINE NURSERIES CAUSED BY FUSARIUM SUBGLUTINANS. W. A. Carey and W. D. Kelley, School of Forestry, Auburn University, AL 36849-5418.

Fusarium subglutinans (Wollenw. & Reink.) Nelson, Toussoun & Marasas was isolated consistently from diseased longleaf (Pinus palustris Mill.) seedlings both at a bareroot nursery and at a containerized nursery. Seedling mortality at the bareroot nursery was 26% in Feb., 1991; mortality at the containerized nursery was 47% in Nov., 1993. Mycelium, polyphialids, and microconidia typical of F.subglutinans grew abundantly on the symptomatic tissues of dissected seedlings from each nursery after 3 to 5 days incubation in moist chambers. Wound inoculations with hyphae and spores of isolates from longleaf seedlings produced similar symptoms in other longleaf seedlings and symptoms characteristic of F.subglutinans infections (pitch canker) in loblolly pine (P.taeda (L.)) seedlings. Pitch-soaked tissues characteristic of infected loblolly seedlings generally were absent in longleaf seedlings. Many of the nursery infections were associated with insect wounds.

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THE EFFECT OF PREFORMED TANNINS AND PROTEINS IN AMERICAN AND CHINESE CHESTNUT BARK ON A PURIFIED POLYGALACTURONASE FROM CRYPHONECTRIA PARASITICA. S. Gao and L. Shain, Dept. of Plant Pathology, Univ. of Kentucky, Lexington, Kentucky 40546-0091.

Tannins extracted from American and Chinese chestnut inhibited a purified endopolygalacturonase (PG) produced by the chestnut blight fungus. Bark tannin of susceptible American chestnut, however, was more inhibitory to this enzyme than bark tannin of resistant Chinese chestnut. Proteins extracted from Chinese chestnut bark, on the other hand, were at least fifteen times more inhibitory to this PG than similar extracts from American chestnut bark. PG activity in cankers of Chinese chestnut also was far less than that in cankers of American chestnut as detected by direct assay and Western blotting. This and other evidence suggest that PG may be a virulence factor for the blight fungus and that the PG inhibitor proteins may be a resistance factor for Chinese chestnut.

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RFLPs IN nuDNA DIFFERENTIATE ISOLATES OF CERATOCYSTIS FAGACEARUM IN A TEXAS OAK WILT CENTER. K.L. Ivors, P.A.I. Guthrie, B.A. McDonald, and D.N. Appel. Dept. of Plant Pathology and Microbiology, Texas A&M University, College Station, Tx 77843.

DNA from 40 isolates of Ceratocystis fagacearum originating from a single, asexually expanding disease center in Round Rock, TX was digested with 3 restriction enzymes. Southern blots were hybridized with 7 anonymous probes from a nuclear genomic library. These probes detected RFLPs in a previous screen of a geographically diverse group of isolates. Four polymorphic loci were found, with 2 to 3 alleles occurring at each locus. Allele frequencies at RFLP loci ranged from 3% to 97%. By combining data from all probes to create multi-locus haplotypes, 4 different genotypes were found among the 40 isolates. The number of RFLP loci having different alleles ranged from 1 to 3 when pairwise comparisons were made between the four haplotypes. These results indicate that more than one genotype can exist in a single, asexually expanding disease center.

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SEGREGATION AND PHYLOGENETIC DATA SUGGEST SPECIATION MAY BE OCCURRING WITHIN *UROMYCES APPENDICULATUS*. J. P. Martinez, J. V. Groth, and N. D. Young, Univ. of Minnesota, Dept. of Plant Pathology, St. Paul MN, 55108.

In F2 progeny derived from a cross between a telia producing (sexual) and a non-telia producing (asexual) isolate, a high frequency (21/44) of DNA markers

failed to fit a 3:1 or 1:2:1 segregation ratio. Moreover, biased segregation toward the sexual parent and nonrandom assortment of chromosomes was also observed. When 164 DNA markers were compared between the parental isolates, 91% were polymorphic. These results are comparable to those observed in analyses of parents and their progeny in some interspecific crosses of plants. In separate experiments, phylogenetic analysis with 74 DNA markers indicated that some telia and non-telia producing isolates are genetically distinct. Relationships among 18 *U. appendiculatus* isolates were determined using either distance or parsimony methods. The 18 isolates fell into two distinct groups, or into a third intermediate group. The sexual parent belonged to one of the groups composed mostly (9/12) of telia producing isolates. The asexual parent belonged to a second, distinct group composed entirely of non-telia and reluctant telia producing isolates. Reproductive isolation has apparently occurred long enough for the two groups to begin diverging into separate species.

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PHYLOGENETIC ANALYSIS OF COLLETOTRICHUM GLOEOSPORIOIDES BASED ON RIBOSOMAL DNA SEQUENCES. C.L. Trout and D.O. TeBeest, Department of Plant Pathology, University of Arkansas, Fayetteville, Arkansas 72701

Colletotrichum gloeosporioides causes diseases of a wide range of crop hosts and weeds. C. gloeosporioides is a species complex separate from other species of Colletotrichum based primarily upon morphological characters such as conidial size and shape, appressorial size and shape, and presence or absence of setae. However, these morphological characters are extremely variable among isolates of C. gloeosporioides. The present system of describing and identifying this taxon is inadequate because it reveals little information about relationships within this taxon. Comparative studies of the nucleotide sequences of the conserved ribosomal RNA (rRNA) genes may provide a means for analyzing variability and phylogenetic relationships within this taxon. Oligonucleotide primers were used to amplify the internal transcribed spacer 1 (ITS1) region, the 5.8S rRNA gene, and the ITS2 region of ribosomal DNA from several isolates of C. gloeosporioides from a diverse group of hosts. Direct sequencing of the PCR amplification products was performed. Analysis of the sequences and phylogenetic implications will be discussed.

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PHYLOGENIC COMPARISON OF *MAGNAPORTHE* SPP. USING ITS-1 SEQUENCE ANALYSIS
Bunting, T. E., Plumley, K. A., Clarke, B. B., and Hillman B. I.
Department of Plant Pathology, Rutgers University, New Brunswick, New Jersey 08903-0231.

Magnaporthe poae causes summer patch disease of bluegrasses and fine fescues. The fungus is a heterothallic ascomycete that does not readily sporulate in culture and has not been found to sporulate in nature. We have designed primers which amplify a 453 base pair product from *M. poae* DNA and may be used for rapid identification. A closely related homothallic fungus, *M. rhizophila* will also amplify a similar-sized product with the primers. Sequences from the first internal transcribed spacer region (ITS-1) of the nuclear ribosomal DNA from *Magnaporthe* spp., *Gaeumannomyces* spp., and *Colletotrichum* (*Glomerella*) sp. were analyzed using parsimony analysis. Interestingly *M. rhizophila* and the *M. poae* isolates all clustered out from the others with an 88% bootstrap frequency. The other fungi did not resolve except for two isolates of *Gaeumannomyces graminis* var *graminis* (83% bootstrap frequency). We are currently studying enzyme activities of M. poae that may be important for virulence such as ß-fructofuranosidase and lignin peroxidases.

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THE USE OF mtDNA RFLPS AND RAPDS TO DISTINGUISH AMONG GENOTYPES OF ACREMONIUM FROM TALL FESCUE. W. J. Ross, J. C. Correll, J. C. Guerber, and [1]C. P. West. Dept. of Plant Pathology and [1]Dept. of Agronomy, University of Arkansas, Fayetteville, AR 72701.

Fifteen isolates of Acremonium coenophialum and Acremonium sp., recovered from Festuca spp., were examined for mtDNA RFLPs and RAPDs. mtDNA RFLPs were examined using cloned mtDNA probes from Colletotrichum orbiculare. RAPDs were examined by initially screening 20 RAPD 10 mer primers and then selecting five for isolate comparisons. Twelve of the 15 isolates examined belonged to a single mtDNA RFLP group; several RFLPs were detected with certain enzymes (particularly Pst1) among this predominant mtDNA RFLP group. Each of the other three isolates examined belonged to a unique mtDNA RFLP group. RAPDs detected multiple genotypes among isolates in the predominant mtDNA RFLP group. For example, with one primer, 923CA, five RAPD genotypes were identified among the 12 isolates from the common mtDNA RFLP group. The various genotypes are being characterized for other traits including ergovaline production.

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VARIATION OF ITS1 AMONG ISOLATES OF COLLETOTRICHUM ORBICULARE, C. MAGNA, AND GLOMERELLA CINGULATA var. ORBICULARE. McCormick, T. W., Correll, J. C., and *Rhoads, D. D. Departments of Plant Pathology and *Biological Sciences, University of Arkansas, Fayetteville, AR 72701.

Colletotrichum sp. causes anthracnose diseases of crops from many plant families. In a previous study, various taxa including Colletotrichum orbiculare, Glomerella cingulata var. orbiculare (the putative teleomorph of C. orbiculare), and C. magna, recovered from cucurbit hosts were characterized for VCG, mtDNA haplotype, and RAPD haplotype. In an attempt to infer the phylogenic relationship within and between these taxa, the ITS1 region of selected isolates was amplified using the polymerase chain reaction (using primers ITS1 and ITS2), cloned, and sequenced. The sequence data for ITS1 from the various isolates were compared for percent similarity. The ITS1 region varied from 181-212 bp among the isolates examined. Based on ITS1 sequence similarity, isolates could be grouped into four ITS1 groups. Isolates of C. orbiculare pathogenic on cucurbits belonged to Group I and varied by less than 5% except for one pathogenic isolate (Group II) differed from Group I isolates by 5.0-9.0% The ITS1 sequence of G. cingulata var.orbiculare and C. magna differed from C. orbiculare by 9-15% and 30-36%, respectively. Differences in the level of ITS1 sequence diversity detected within and between these taxa in this study suggest caution should be used in phylogenic interpretation of ITS1 sequence data for some species of Colletotrichum.

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POLYMORPHIC ELECTROPHORETIC KARYOTYPES AMONG GEOGRAPHICALLY DIVERSE ISOLATES OF THE SUGARCANE SMUT USTILAGO SCITAMINEA. K. E. Damann and S. A. Lopes, Dept. of Plant Pathology & Crop Physiology, LSU Agricultural Ctr., Baton Rouge, LA 70803-1720.

Previous work with Louisiana isolates of U. scitaminea collected over a ten-year period failed to reveal any size or number polymorphisms in electrophoretic karyotypes. All had 17 bands estimated to total 13.4Mb of DNA per haploid nucleus (Damann & Navarre, Phytopathology 81:1161). That result was contrary to the unique karyotypes detected for representatives of each of 14 races of U. hordei (McCluskey & Mills, MPMI 3:366-373). Comparison of karyotypes of Louisiana isolates with those from Japan and Brazil revealed several polymorphisms which will be discussed. The ability to detect karyotypic polymorphisms and relate them to geographic areas may allow inferences regarding the origin of inoculum and spread of this disease.

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ELECTROPHORETIC KARYOTYPE ANALYSIS OF Pythium spp. AND MECHANISMS CONTRIBUTING TO INTRASPECIFIC POLYMORPHISMS. Frank N. Martin, Plant Pathology Department, University of Florida, Gainesville, FL 32611

The electrophoretic karyotypes were determined for 73 geographically separated isolates of 10 Pythium spp. While the general range in chromosome sizes was conserved within a species, intraspecific polymorphisms were such that with a few exceptions, no two isolates were identical. These polymorphisms were most commonly due to differences in chromosome numbers, although for several species chromosome-size differences also were observed. Chromosome numbers ranged from 7 to 20 and estimated minimum genome sizes from 19.9 to 41.5 Mb. Depending on the species, intraspecific variation of genome size was found to vary by as much as 37%. Three species were examined for the influence of meiosis on karyotype stability. With the exception of a putative supernumerary chromosome, no polymorphisms were detected for the homothallic species P. oligandrum. In contrast, while progeny of the homothallic species P. spinosum were similar to the parental isolate, approximately 50% were polymorphic for at least one chromosome-sized DNA. The most extensive variation was observed in progeny of the heterothallic species P. sylvaticum, in which 76% of the progeny chromosomes were polymorphic in size or location of coding regions compared to the parental isolates. The mechanisms responsible for generating this level of variation as well as aspects of species biology contributing to its occurrence will be discussed.

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INTRASPECIFIC VARIATION OF THE INTERGENIC SPACER (IGS) REGION OF THE RDNA IN FUSARIUM OXYSPORUM. D.J. Appel and T.R. Gordon.

Forty eight isolates of *Fusarium oxysporum*, including *F. o.* f. sp. *melonis* and nonpathogenic strains, were chosen from a larger collection to represent diversity in vegetative compatibility groups (VCG), mitochondrial DNA (mtDNA) haplotype, geographic distribution and virulence. Using PCR, a 2.6kb fragment including the intergenic spacer (IGS) region of the ribosomal DNA was amplified from each isolate. The enzymes *Eco* RI, *Sau* 3A, *Cfo* I and *Ava* II, cut this fragment differentially, revealing 5, 6, 6 and 7 patterns, respectively. Among the 48 isolates, a total of 13 unique IGS haplotypes was identified.

Among *F. oxysporum* f. sp. *melonis* isolates, IGS haplotype correlated with VCG and mtDNA haplotype. The IGS haplotype did not differentiate races and was conserved in each pathogen VCG, except for nonpathogens which shared the same VC phenotype. Overall, the IGS haplotype was much more variable among the nonpathogenic *F. oxysporum* VCGs which represented 12 of the 13 IGS haplotypes found. Nonpathogenic isolates that shared a common mtDNA haplotype, but were associated with different VCGs, often had different IGS haplotypes. Variability in IGS may show finer intraspecific differences than can be found using mtDNA haplotype data.

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Genetic Diversity of *Fusarium oxysporum* Isolates Pathogenic to Sugar Beets. G.A. Fisher and J.S. Gerik, Holly Sugar Co., P.O. Box 60, Tracy CA 95376.

Fifty *Fusarium oxysporum* isolates were collected from sugar beet fields and diseased sugar beets (*Beta vulgaris*) from California, Texas, Wyoming, Montana, and Oregon. After pathogenicity testing in the greenhouse, these isolates were analyzed using the RAPD PCR technique to determine their genetic diversity. A considerable amount of diversity was found, and it correlated well with the geographical origin of the isolates. The isolates could be divided into four sub-populations. The isolates from Montana, California, and Oregon all had unique banding patterns, while those from Texas and Wyoming were very similar to each other but different from the other states. Analysis of vegetative compatibility groups was done using the "nit" mutant method. This study is also the first report of beet-pathogenic *Fusarium oxysporum* in California.

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CHARACTERIZATION OF CELL WALL PROTEINS IN SEVERAL SPECIES OF *PYTHIUM*. S. Takenaka[1], M. Tojo[2], S. Kawasaki[3] and T. Ichitani[2]. [1]Hokuriku National Agricultural Experiment Station, Niigata, [2]University of Osaka Prefecture, Osaka, [3]National Institute of Agrobiological Resources, Ibaraki, Japan.

Cell wall proteins were extracted from 13 *Pythium* spp. and examined for intra- and interspecific variations. Each species had one or more major cell wall proteins in the MW range from 25 to 40 kDa, which were stable among isolates of the same species. Electrophoretic and N-terminal amino acid sequence analysis of these major cell wall proteins showed significant differences not only among morphologically distinct species, but also among morphologically similar species (except for *P.deliense* vs. *P.aphanidermatum*). However, these major cell wall proteins shared common epitope(s) because all of them reacted with an antiserum against *P.iwayamai* cell wall protein by western blot analysis. These results suggest that these species-specific cell wall proteins may have both distinctive and conserved sequence regions. Moreover, the amino acid analysis of four species revealed a common characteristic; the high content of alanine and the presence of a significant amount of hydroxyproline, which showed a similarity with arabinogalactan cell wall protein in higher plants.

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CHARACTERIZATION OF THE *NOR-1* GENE FROM *ASPERGILLUS FLAVUS*. *C. S. Brown-Jenco*, J. F. Brewer, and G. A. Payne. Dept. Plant Pathology, N. C. State Univ., Raleigh, 27695-7616

Aflatoxins are toxic and carcinogenic products that contaminate feed sources colonized by *Aspergillus flavus* and *A. parasiticus*. In order to develop strategies to control aflatoxin contamination, it is important to understand the regulation and biosynthesis of aflatoxins. One way to study aflatoxin biosynthesis is through the use of pathway mutants. We are studying a mutant of *A. flavus* partially blocked in aflatoxin biosynthesis that accumulates norsolorinic acid (NOR), the first stable pathway intermediate. It is important to know if this mutant is defective at the *nor-1* locus because alternate routes have been proposed for the conversion of NOR to aflatoxin. We previously reported isolation of *nor-1* from *A. flavus* and showed that two overlapping cosmids containing this gene complemented the mutation. Recently a 2.8-kb fragment that contains *nor-1* was subcloned and transformed into the mutant. Transformants were recovered that did not accumulate NOR and produced high concentrations of aflatoxin. The *nor-1* gene from *A. flavus* contains an open reading frame of 813 bp with 98% identity to the *nor-1* of *A. parasiticus* (the amino acid sequences share 96% identity). Thus the block in *A. flavus* appears to be within the same gene as in *A. parasiticus*. Furthermore, evidence suggests that *nor-1* is involved in aflatoxin biosynthesis. We have shown by transcript accumulation and promoter analysis that this gene is expressed during aflatoxin biosynthesis, and that its expression is governed by *aflR*, a pathway regulatory gene.

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GENE EXPRESSION OF THE LINEAR DNA PLASMIDS pRS64 IN *RHIZOCTONIA SOLANI*. T. Hashiba, M. Hongo, F. Suzuki, and A. Miyasaka, Faculty of Agriculture, Tohoku University, Sendai 981, Japan.

The plant pathogenic fungus, isolate RI-64 of anastomosis group 4 of *Rhizoctonia solani* possesses three linear DNA plasmids (pRS64-1, -2, and -3). Unique poly(A)$^-$RNA of 0.5 kb hybridizable with the pRS64 DNAs was found in mycelial cells of the isolate RI-64. The overall homology at the nucleotide level between pRS64-1, -2, and -3, and the cDNA prepared for the poly(A)$^-$RNA was 100%, 73%, and 84%, respectively. The open reading frames found in pRS64-1, -2, and -3 (ORF1-1, ORF2-1, and ORF3-1) were 68 amino acids long. The amino acid sequence showed no significant homology with known proteins. Extracts from *E. coli* cells expressing ORF1-1 gave a specific protein of 7 kDa. Antisera raised to the ORF1-1 product obtained from *E. coli* cells cross-reacted with the specific proteins found in the mycelia. The results indicate that the DNA plasmids found in *R. solani* contain a sequence for a specific protein, that may be involved in determination of pathogenicity to plants.

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PRODUCTION OF CAROTENOID-MINUS MUTANTS OF CERCOSPORA NICOTIANAE USING GENE DISRUPTION. M. Ehrenshaft, A. E. Jenns, and M. E. Daub. Dept. of Plant Path., NCSU, Raleigh, NC 27695.

Cercospora species produce a photoactivated, singlet oxygen-generating toxin, cercosporin, which has been shown to be required for infection of host plants. Nearly all other organisms tested, including bacteria, plants, and mice, are inhibited or killed by micromolar concentrations of cercosporin. *Cercospora* species, however, can accumulate millimolar concentrations without measurable toxic effect. Because carotenoids are known to be potent quenchers of singlet oxygen, we are testing the effect of their absence on the resistance of *Cercospora* to cercosporin. We isolated the *Cercospora nicotianae* gene for phytoene dehydrogenase, an enzyme that catalyzes the conversion of the carotenoid precursor phytoene to the colored carotenoid pigments. We have constructed versions of this gene which we are transforming into the wild-type strain. We have recovered several carotenoid-minus transformants and are analyzing them for cercosporin sensitivity and other changes in phenotype.

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IDENTIFICATION OF REGULATORY ELEMENTS FROM TWO GENES INVOLVED IN AFLATOXIN BIOSYNTHESIS. F. Trail, T.-S. Wu and J. E. Linz, Department of Food Sciences, Michigan State University, East Lansing, MI 48824.

The phytopathogenic fungus, Aspergillus parasiticus, produces the potent hepatocarcinogen, aflatoxin, resulting in contaminated food and feed crops worldwide. Aflatoxin is a secondary metabolite synthesized from acetate units through a complex series of reactions currently not well understood. Several genes associated with this pathway have now been cloned. Two of these genes, nor-1 and ver-1, are responsible for two of the intermediary modification steps in the pathway. Regulation of aflatoxin production has been characterized under a variety of conditions, but the underlying mechanisms of control have not been elucidated at this time. The transcription of nor-1 and ver-1 appears to be coordinately regulated. A transcript map of two overlapping cosmid clones encompassing

the nor-1 and ver-1 genes has revealed an additional 11 transcribed genes that are similarly regulated. To identify the cis-acting sites and trans-acting factors responsible for regulation of these genes, we have performed gel shift assays on nor-1 and ver-1 promoter regions. The function of putative regulatory elements identified in these assays is being investigated through promoter-reporter gene fusions with the B-glucuronidase gene. Promoter-gene fusions will be mutated within the proposed regulatory regions and assayed for function in the A. parasiticus genome.

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IDENTIFYING STEM ROT RESISTANT PEANUT GENOTYPES. F. M. Shokes, D. W. Gorbet, Univ.of Florida, No. Florida Research and Education Center, Rt. 3 Box 4370, Quincy, Fl 32351, Z. Weber, H. A. Pudelko, and M. Taczanowski, Poznan Agricultural Univ., Mazowiecka 45/46, 60-623 Poznan, Poland.

Six field tests were conducted over three years on peanut (*Arachis hypogaea* L.) to identify genotypes with promising levels of resistance to southern stem rot caused by *Sclerotium rolfsii* Sacc. Entries (54-56 in each test) were planted in 2.4 m length rows (10-12 plants/row) with an RCB design at two locations in 1991-1993. Alternate plants were flagged and hand inoculated using a 1-cm PDA agar plug with a germinated sclerotium and mycelium. The isolate used for inoculum (SR8) was pre-tested for pathogenicity. Plots were irrigated (1.25 cm) on the day of inoculation and for two days thereafter. Plants were assessed for stem rot 4-7 times using a 1-5 scale; 1 = healthy and 5 = >50% of stems dead or dying. The most resistant genotypes were selected for further testing in yield studies. Inoculated rows (160 ml/row of oat seed inoculum) were compared to paired, uninoculated rows for pod yields. Uninoculated rows were kept nearly disease free by using the fungicide thifluzamide. In four tests over two years at two locations, the two best genotypes (UF 81206-2 and 79x4-6-2-) lost only 23% and 24%,respectively, of their pod yield, compared to 64% for the cultivar Florunner.

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A NEW SOURCE OF RESISTANCE TO *PSEUDOCERCOSPORELLA HERPOTRICHOIDES* LOCATED ON CHROMOSOME 4V OF *DASYPYRUM VILLOSUM*. T. D. Murray, R. C. de la Peña, A. Yildirim, and S. S. Jones. Department of Plant Pathology, and Crop and Soil Sciences Department, and USDA-ARS, Washington State University, Pullman, WA 99164-6430.

Resistance to *P. herpotrichoides* in *D. villosum* accession W6 7283, Chinese Spring disomic addition lines of *D. villosum* chromosomes 1V, 2V, 4V, 5V, 6V, and 7V, and five wheat genotypes was determined by measuring disease progress in seedlings inoculated with a ß-glucuronidase-transformed strain of the pathogen. *D. villosum* and the chromosome 4V addition line were as resistant as wheat cultivars VPM-1 and Cappelle Desprez, each of which has a single resistance gene, but less resistant than Rendezvous, which has two resistance genes. Presence of chromosome 4V in the addition line and its homoeology with chromosome 4 in wheat was confirmed by Southern analysis of genomic DNA using wheat chromosome 4-specific clones. This locus is not homoeologous with other known resistance genes and thus represents a new source of resistance.

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INTROGRESSION OF *SOLANUM BREVIDENS* DNA INTO DISEASE RESISTANT POTATOES. J. P. Helgeson, S. M. Wielgus, C. E. Williams, G. T. Haberlach and J. M. McGrath. ARS/USDA. Plant Disease Resistance Research Unit, Dept.of Plant Path., Univ. of Wisconsin, Madison, WI 53706

By the use of somatic hybrids, wild species such as *Solanum brevidens* that are sexually incompatible with potato can provide new genes for resistances to early blight, soft rot and other potato diseases. However, since crossing of the somatic hybrids with potato breeding lines is required to obtain tubers of marketable quality, it is essential to determine if DNA from the sexually incompatible species can be introgressed into potato. We used *S. brevidens* chromosome-specific markers (RFLPs and RAPDs) to determine if we had somatic hybrids and then to follow the inheritance of *S. brevidens* DNA through 3 backcross generations. Recombination between non-homologous *S. brevidens* chromosomes as well as introgression of *S. brevidens* DNA into the potato genome occurred in these progeny. Soft rot and early blight resistances were also retained in some high yielding BC_3 progeny.

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FURTHER EVALUATION OF THE IMPORTANCE OF PTR-NECROSIS TOXIN PRODUCED BY *PYRENOPHORA TRITICI REPENTIS* IN PATHOGENESIS ON SUSCEPTIBLE WHEAT PLANT. N.P. Orolaza, L. Lamari, H. Otondo, G.M. Ballance and C.C. Bernier. Department of Plant Science, University of Manitoba, Winnipeg, MB., Canada, R3T, 2N2

Pyrenophora tritici repentis (*Ptr*) causes tan necrosis and/or extensive chlorosis in wheat. The development of each symptom results from specific interactions between individual isolates/pathotypes of the fungus and wheat genotypes. Necrosis inducing isolates (nec+) produce, *in vitro*, a high molecular weight cultivar specific toxin, designated as Ptr-necrosis toxin. There is also evidence of the presence of the Ptr-necrosis toxin in the intercellular washing fluids (IWF) of leaves infected by nec+, but not nec-, isolates of *Ptr*. Western blotting analysis was used to detect the presence of Ptr-necrosis toxin in the IWF. When toxin was added to the spore suspension of avirulent isolate 90-2 (nec-chl-) and chlorotic-only (nec-chl+) isolates D308 and Hy331-9, infection was successfully established and necrosis induced in susceptible cultivar Glenlea but not in the resistant lines Salamouni and Glenlea^6 x Salamouni. The toxin initiated and promoted infection of these non-pathogens in a concentration and time dependent manner. This suggests that the toxin is a constitutive "signal" from the pathogen to establish compatibility, thus supporting the hypothesis that the Ptr-necrosis toxin is an essential factor in the pathogenicity of this fungus to wheat.

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Genetic Analysis of Cultivar-Specific Avirulence/Virulence in a Field Isolate of *Magnaporthe grisea*. Kim, Yun-Sik and Albert H. Ellingboe. Plant Pathology Department, University of Wisconsin, Madison WI.

An isolate of *Magnaporthe grisea* (Tm4) obtained from a rice field in Texas was found to be avirulent on several rice cultivars. The genes involved in avirulence have been analyzed by crosses with highly fertile laboratory isolates that are virulent on these cultivars. The analyses have shown that 4 loci control avirulence/virulence on rice cultivar Katy. The data suggest that two pairs of genes (P genes and their corresponding S genes) determine avirulence/virulence on Katy. Crosses of these isolates with isolates identified in an earlier study (Phytopathology 83: 375-382) show that the genes controlling avirulence on Katy from Tm4 are different from P11, P12, S11, and S12 genes identified in the earlier study. The results suggest that 4 pairs of P and S genes control avirulence/virulence on cultivar Katy.

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INHERITANCE OF RESISTANCE TO SCAB IN BREAD WHEAT CULTIVAR FRONTANA. R.P. Singh, H. Ma, and S. Rajaram, CIMMYT, Lisboa 27, Apdo. postal 6-641, 06600, México, D.F.

Scab, or head blight (caused by *Fusarium graminearum* Schwabe) is a common wheat disease in the humid wheat growing regions. The Brazilian wheat (*Triticum aestivum* L.) cultivar Frontana is resistant to scab in México and various other countries. The number of genes involved in resistance to the disease was estimated by evaluating random inbred F_6 lines and their parents for scab resistance in the field. The lines were derived from the crosses of Frontana with susceptible, or moderately susceptible, cultivars Inia 66, Opata 85, and Pavon 76. Spikes, close to anthesis, were inoculated by placing a tiny tuft of cotton soaked with the inoculum in the middle spikelet between the glumes. Glassine bags were placed over the inoculated spikes. These spikes were harvested 45 days after inoculation. Scab severity was determined by recording the number of infected and healthy spikelets. The narrow sense heritability estimates for the crosses evaluated during 1991 and 1993 were 0.66 and 0.93, respectively. The resistance of Frontana is controlled by the additive interaction of a minimum of three minor genes. Transgressive segregants were identified in each cross and some F_6 lines had singnificantly better scab resistance than that of Frontana.

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VARIATION IN *FUSARIUM GRAMINEARUM* AND STABILITY OF RESISTANCE TO WHEAT SCAB. Bai, G. and G. Shaner Dept. Botany and Plant Pathology, Purdue University, West Lafayette, IN 47907-1155.

Six isolates of *Fusarium graminearum* from China and the U.S. were compared for variation in cultural characters and aggressiveness on nine wheat cultivars with various levels of resistance to scab. Chinese isolates caused more scab on average, but there was no cultivar-isolate specificity. Subculturing the fungus on PDA for eight generations did not reduce aggressiveness. Following inoculation of one floret in a central spikelet, the rate of invasion of uninoculated spikelets was a useful criterion of cultivar resistance. Ning 7840, Sumai #49, Fu 5114, and Sumai #3 were consistently resistant. Eight cultivars were tested 5 times over 3 years with an Indiana isolate of the fungus. The fungus invaded uninoculated spikelets of resistant cultivars in less than 20% of the plants, and this spread was not evident until 12 days after inoculation. All plants of the susceptible cultivar Clark showed spread of infection by 8 days after inoculation. Measurement of spread of scab within a spike is a stable and reliable estimate of resistance.

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THE SUPPRESSION OF PYCNIDIAL PRODUCTION ON WHEAT FOLLOWING CHALLENGE INOCULATION BY *Septoria tritici* ISOLATES. Tamar Stein, Silvia

Schuster, Aviah Zilberstein, Smadar Pnini-Cohen, and Zahir Eyal. Dept. of Botany, Tel-Aviv University, Tel Aviv 69978, Israel.

Inoculation of seedlings of Seri 82 with the avirulent *S. tritici* isolate ISR398 and challenged separately at 2, 5, 6, or 10 days later with the virulent isolate ISR8036 resulted in marked reductions in pycnidial coverage. Reductions were also recorded on the susceptible cultivar Shafir. Inconsistent reductions resulted from the reversal in inoculation order of the isolates. No reductions were observed when culture filtrates replaced the challenged isolate. Sub-isolates produced by reisolation from pycnidia on Seri 82 inoculated by ISR398 and challenged 2 or 5 days later by ISR8036, were ISR8036-like as verified by their virulence on Seri 82 and by probing with the *S. tritici* minisatellite probe ST398-3.7A. The majority of the sub-isolates resulting from the reversal in the order of the challenge (ISR8036/ISR398) on Seri 82 were ISR8036-like. Different ratios were obtained from reisolation of sub-isolates from Shafir. Colonization ratios of host tissue by the two isolates during various challenge regimes were estimated by measuring band intensity on Southern blots of infected wheat probed with ST398-3.7A.

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CECROPIN-MEDIATED DISEASE RESISTANCE IN TRANSGENIC TOBACCO PLANTS. Y. Huang[1], M. Di[1], L. Owens[2] and J. H. McBeath[1]. [1]University of Alaska, Plant and Animal Science, Fairbanks, AK 99775 and [2]USDA, Plant Molecular Biology Laboratory, Beltsville, MD 20705

Cecropin B encoded by an insect gene has been shown to confer antimicrobial activity. A chimeric gene, consisting of plant promoter-secretory sequence-cecropin gene coding region, was constructed and transformed into tobacco. Infiltration of untransformed plant leaves with tobacco wildfire pathogen *Pseudomonas syringae* pv. *tabaci* at levels of 10^2 10^3 10^4 10^5 and 10^6 CFU/ml resulted in necrosis at all inoculum levels. With cecropin-transgenic plants, however, necrosis was observed only in leaf areas infiltrated with the two highest dilutions. No necrosis was evident in the areas infiltrated with bacterial dilutions 10^4 CFU/ml or less. Bacterial multiplication in cecropin-transgenic plants was suppressed. Expression of the cecropin gene product in these transgenic plants was confirmed by western blot analysis.

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β-Aminobytyric acid induces resistance against fungal diseases in crop plants. Yigal Cohen, Dept. of Life Sciences, Bar-Ilan University, Ramat-Gan, Israel

β-aminobutyric acid (BABA), but not α- or γ-aminobutyric acids protects (>90%) tomato plants against the late blight fungal agent *Phytophthora infestans* (Y. Cohen *et al.*, Plant Physiology 104: 59-66, 1994). BABA also protects tobacco against blue mold incited by *Peronospora tabacina*, and other crop plants against Oomycetes and Ascomycetes fungi. BABA had no antifungal activity *in vitro* and does not serve as a precursor for the synthesis of fungicidal compounds by the plant. It acts postinfectiously against mycelial growth in plant tissue probably by altering plant structure and/or metabolism. In tomato BABA induced enhanced accumulation of pathogenesis-related (PR) proteins but not in stem-injected tobacco plants in which BABA protected against disease. ^{14}C-BABA was not metabolized by either tomato or tobacco but a small fraction of it was covalently bound to cell walls. We currently study the possibility that cell wall structure or behavior are altered by BABA so to make them resistant against fungal invasion.

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SYSTEMIC INDUCED CROSS-RESISTANCE BY PLANT PATHOGENS AND THE PROOXIDANT HERBICIDE PARAQUAT. N. Strobel and J. Kuć. Dept. of Plant Pathology, University of Kentucky, Lexington, KY 40546.

The superoxide anion-generating herbicide paraquat was employed as a model prooxidant to investigate the relationship of oxidative stress to the induction and expression of systemic disease resistance in cucumber and tobacco plants. Treatment of lower leaves of greenhouse-grown plants with dilute paraquat (40-160 μM ai) resulted in lipid peroxidation, necrosis, and induced systemic resistance to the fungal pathogens Colletotrichum lagenarium (cucumber) and Peronospora tabacina (tobacco). Treatment of plants with agents known to induce systemic disease resistance in cucumber (C. lagenarium, K_2HPO_4, paraquat) and tobacco (local lesion-forming strain of TMV) also reduced the extent of lipid peroxidation and necrosis incited by paraquat applied as a challenge agent to upper leaves. Local treatments with sodium salicylate or the cytokinin 6-benzylaminopurine also reduced paraquat damage. We speculate that promotion of oxidative stress in treated (inducer) leaves can induce a systemic enhancement of plant antioxidant mechanisms that may contribute to the expression of induced resistance to pathogens and prooxidant abiotic stresses.

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MOLECULAR MAPPING OF A GENE FOR RESISTANCE TO TOMATO SPOTTED WILT VIRUS IN TOMATO. D. K. Heiny, M. R. Stevens[1], and D. D. Rhoads, Dept. of Biological Sciences, University of Arkansas, Fayetteville, AR 72701, and [1]Gulf Coast Research & Education Center, Bradenton, FL 34203.

We have been characterizing a locus, Sw-5, introgressed from Lycopersicon peruvianum that expresses dominant resistance to tomato spotted wilt virus (TSWV) in L. esculentum lines. We developed a pair of near-isogenic lines (NILs) and an F_2 L. esculentum × L. pennellii population segregating for Sw-5 resistance to map the location and identify linked molecular markers. We have identified one primer that produces a ca. 2200 bp random amplified polymorphic DNA (RAPD) band linked to Sw-5. Cosegregation of resistance and restriction fragment length polymorphisms (RFLPs) in the F_2 interspecific population positions Sw-5 between the CT71 and CT220 markers on chromosome 9 (Tanksley et al., 1992, Genetics 132:1141-1160). We are now refining our map data using long range restriction mapping of the cloned molecular markers from chromosome 9. Data from these experiments will be presented.

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TISSUE CULTURE REGENERATION OF VIRUS RESISTANT CAPSICUM GENOTYPES. Benigno Villalón. Texas Agricultural Experiment Station, 2415 East Highway 83, Weslaco, TX 78596.

A reproducible plant regeneration system from tissue culture of advanced pepper genotypes was achieved, making possible the incorporation of plant transformation by direct gene transfer research at the Texas Agricultural Experiment Station's pepper breeding program at Weslaco. Multiple virus resistant pepper cultivars TAM Veracruz - hot jalapeño, Jaloro-yellow hot jalapeño, tobacco etch virus susceptible New Mexico-6 Chile (NM-6) and McIlhenny Tabasco (MT) were successfully regenerated to whole plants from cotyledon explants. Shoot bud initiation was achieved in all genotypes when the explants were placed on Murashige and Skoog (MS) medium supplemented with 6-benzylaminopurine at 5 mg/l and indole-3-acetic acid at 3 mg/l. Shoot buds were elongated on MS medium supplemented with gibberellic acid at 3 mg/l and BA at 3 mg/l. Rooting occurred in MS medium containing indole-3-butyric acid at 1 mg/l and naphthaleneacetic at 1 mg/l. Seeds were saved from harvested pepper fruit of NM-6 and MT matured plants. Preliminary transformation experiments with NM-6 chile utilizing *Agrobacterium tumefaciens* has shown promising results. Transient GUS expression was observed using histochemistry on treated cotyledon explants exhibiting kanamycin resistance.

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INTERRELATIONSHIPS BETWEEN *DIAPORTHE PHASEOLORUM* VAR. *CAULIVORA*, *RHIZOCTONIA SOLANI*, AND *ROTYLENCHULUS RENIFORMIS* ON DAVIS SOYBEAN. S.R. Erwin, E.C. McGawley, and J.S. Russin. Department of Plant Pathology and Crop Physiology, Louisiana State University Agricultural Center, Baton Rouge, LA 70803.

A 90-day greenhouse experiment was conducted to evaluate interrelationships between *Diaporthe phaseolorum* var. *caulivora* (*Dpc*), *Rhizoctonia solani* AG-1 IA (*Rs*), and *Rotylenchulus reniformis* (*Rr*) on Davis soybean. Treatments were *Dpc* (stem inoculated with an infested or noninfested toothpick section), *Rs* (foliage sprayed with 10.5 x 10^6 mycelial fragments per plant or sterile distilled water), and *Rr* (0, 500, or 1000 vermiform individuals/pot) in a factorial arrangement. Numbers of juveniles and total *Rr* in soil were greater on plants colonized by Dpc, but only at the highest nematode infestation level. Nematode egg production was not influenced by either fungus at the highest *Rr* infestation level and declined significantly at the lower (500/pot) level when plants were colonized by *Dpc*. At the highest *Rr* infestation level, egg hatch was increased significantly on plants inoculated with either *Dpc* or *Rs*. When *Dpc* and *Rs* colonized the same plant, egg hatch did not differ from that observed when *Rr* was alone. Stem canker lesion length was not affected by either *Rs* or *Rr*. Aerial blight severity was not affected by either *Dpc* or *Rr*. Significant reductions in plant weight were observed only on plants colonized by *Dpc*.

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SOYBEAN SDS YIELD LOSSES IN THE MIDWEST. S. Abney[1], J. Melgar[2], T. Richards[1], J. Young[2], and M. Booker[2]. [1]USDA, ARS Crop Production and Pathology, and [2]Department Botany and Plant Pathology, Purdue University, West Lafayette, IN 47907-1155

Sudden death syndrome, caused by a highly virulent form of *Fusarium solani*, has become a major disease of soybeans in the Midwest. Abundant moisture during the early reproductive stages of the host enhanced SDS damage in the Midwest during the 1990's. Root infections are common and occur early, but seldom advance into the taproot prior to the flowering stage. Delayed planting and management practices that improve plant health during the flowering period help reduce disease losses. Results of yield loss and foliar symptom studies with diverse soybean germplasm sources [90 public & private maturity Group III-IV cultivars commonly grown in the Midwest]

indicate that the disease can be very destructive. Disease evaluations of selected exotic germplasm lines (i.e. sources of resistance to Phythophthora root rot & soybean cyst nematode) identified P.I. 437654 (resistant to all known SCN races) as highly susceptible to SDS. Only a few cultivars have SDS resistance similar to the cultivar Ripley.

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INCIDENCE OF BROWN STEM ROT AS INFLUENCED BY SOYBEAN CYST NEMATODE. K.M. Tubajika, G.L. Tylka, H. Tachibana, and X.B. Yang. Department of Plant Pathology, Iowa State University, Ames, Iowa 50011.

Greenhouse and field experiments were conducted to investigate the influence of soybean cyst nematode, *Heterodera glycines*, on the incidence of brown stem rot (BSR) of soybean, caused by *Phialophora gregata*. Selected soybean varieties representing the four possible combinations of resistance or susceptibility to the two pathogens were used, and a consistent association of increased BSR incidence in the presence of *H. glycines* was noted. In a greenhouse experiment, the average BSR incidence for all four varieties after 87 days was 50% greater when grown in soil naturally infested only with *P. gregata* than when grown in soil infested with *P. gregata* and *H. glycines*. When grown in soil infested with both pathogens, Kenwood, the soybean variety susceptible to both pathogens, exhibited 55% BSR incidence whereas Newton, which is reportedly resistant only to *H. glycines*, exhibited only 8% BSR incidence. In similarly infested soil, BSR incidence for IA 2008, which is resistant only to *P. gregata*, was 28%, and no symptoms of BSR were observed with Jack, which is resistant to both pathogens. In fields infested with both pathogens in 1993, BSR incidence was 91% for Kenwood, 79% for IA 2008, 38% for Newton, and 28% for Jack whereas BSR incidence for all varieties ranged from 49 to 64% in a field infested with *P. gregata*.

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INTERACTION OF FIVE PEANUT GENOTYPES WITH 10 ISOLATES OF *Sclerotium rolfsii*. F.D. Smith, T.B. Brenneman, and B.G. Mullinix, Univ. of Georgia, Coastal Plain Expt. Station, Tifton, GA 31793; F.M. Shokes and D.W. Gorbet, Univ. of Florida, North Florida Res. & Educ. Cen., Marianna, FL 32446.

One peanut (*Arachis hypogaea*) plant from each of five genotypes was transplanted into 88 field microplots. The cultivars, Florunner, Southern Runner and Georgia Browne, and breeding lines, F-1025 and 79x4-6-2-1-1-b3-Z1-b2-B, were managed according to standard production techniques. Plants were individually inoculated with one of 10 isolates of *Sclerotium rolfsii*. The isolates varied from high to low virulence. In two weeks, a resistant reaction (healthy or small lesion) was observed on 63% of F-1025, 45% of 79x4-, 40% of Georgia Browne, 22% of Southern Runner, and 18% of Florunner. F-1025 was significantly more resistant than all other genotypes. Average yield (g/plant) was 75 g for F-1025, 99 g for 79x4-, 45 g for Georgia Browne, 31 g for Southern Runner, and 36 g for Florunner. 79x4- yielded significantly better than other genotypes. No interaction was detected between fungal isolates and peanut genotypes. Both F-1025 and 79x4- have a high level of resistance to a wide range of *S. rolfsii* isolates. Georgia Browne was more resistant than the other runner cultivars, Florunner and Southern Runner.

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SELECTIVE IDENTIFICATION OF PHYMATOTRICHUM OMNIVORUM FROM OTHER FUNGI ON TRADITIONAL AND NON-TRADITIONAL HOSTS. Jennifer L. Riggs, Department of Entomology, Plant Pathology and Weed Science, New Mexico State University, Las Cruces, NM 88003 and S. D. Lyda, Texas A&M University, College Station, TX 77843.

A plasmid containing an approximately 3-kb fragment of *Phymatotrichum omnivorum* mtDNA, pPO2, has potential as a molecular diagnostic tool to selectively identify *P. omnivorum*. Homology exists between pPO2 and mycelial, conidial and sclerotial chromosomal DNA of *P. omnivorum*, but not with DNA from ten other fungi, some of which are ecologically and physiologically similar to *P. omnivorum*. A PCR 15-mer pair, from the forward sequence of the first 650 nucleotides of the insert in pPO2, reveals selective annealing to and amplification of DNA of *P. omnivorum*. Homology studies with DNA isolated from greenhouse-inoculated and non-inoculated cotton roots confirmed selective hybridization with DNA from *P. omnivorum* infected plants. Positive reactions were also obtained with DNA from roots of inoculated barley, corn, sorghum and wheat. *P. omnivorum* was confirmed by plating on selective media. Crop rotations with monocotyledonous plants have long been suggested as a cultural control practice to reduce inoculum levels of the pathogen. These results indicate potential fungal colonization of these grasses in the field. Therefore, a decrease in inoculum level cannot be assumed in crop rotations utilizing monocotyledonous plants.

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SITES OF INFECTION IN RICE SEEDS AND SEEDLINGS BY *PYTHIUM* SP. S-C. Chun and R.W. Schneider, Dept. of Plant Pathology and Crop Physiology, Louisiana Agricultural Experiment Station, Louisiana State University Agricultural Center, Baton Rouge, LA 70803.

Pythium sp. causes seedling stand loss of rice (*Oryza sativa* L.). The etiology of this disease is largely unknown. Rates of recovery of the pathogen from embryos, endosperms, shoots, and roots were recorded for 5 days following planting for seeds that had been pregerminated and seeds that were dry at planting. Endosperm infection rates after 3 and 5 days were 10% and 30%, respectively, while those for embryos were 28% and 80%, respectively. Infection rates of pregerminated seeds (100% for shoots, 90% for roots, 98% for embryos, and 36% for endosperms after 3 days) were higher than for seeds not pregerminated (46% for shoots, 28% for embryos, and 10% for endosperms after 3 days), indicating that *Pythium* infects embryos when or after rice seeds germinate. The development of roots from dry planted seeds (4%) was significantly reduced by *Pythium* compared to that of roots from noninoculated controls (88%) after 5 days.

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INFECTION OF CANOLA BY MULTIPLE GENOTYPES OF *SCLEROTINIA SCLEROTIORUM* IN CENTRAL MISSOURI. A. D. Maltby and J. D. Mihail, Department of Plant Pathology, University of Missouri, Columbia MO, 65211

Plant populations are frequently exposed to many different genotypes of a pathogen, and often individual plants are colonized by many different genotypes of a given pathogen. Canola (*Brassica napus*) is a host of *Sclerotinia sclerotiorum*, which is disseminated by aerially dispersed spores. In 1993, more than 90 genotypes of the pathogen, as determined by vegetative compatibility (VC) analysis, were recovered from petals and stems of canola in two small plots in central Missouri. Of 80 diseased plants examined, 73 were colonized by a single genotype, and more than 85% of the 73 infecting genotypes were unique. This phenomenon is being studied further in the current growing season by examining all sclerotia within diseased plants using VC analysis. Mechanisms of this apparent competition among fungal genotypes within a host plant are being evaluated in a controlled field study with inoculated plants.

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DETERMINATION OF THE PRESENCE OF VIRULENCE GENES IN *BLUMERIA GRAMINIS* f.sp. *TRITICI* ON WINTER WHEAT IN THE EASTERN UNITED STATES. Amy L. Sowell and Steven Leath, Department of Plant Pathology and USDA-ARS, North Carolina State University, Raleigh, North Carolina 27695.

Samples of perithecia of *Blumeria graminis* f.sp. *tritici* from senescing wheat leaves were collected by cooperators from 18 states. Ascospores were discharged from the perithecia and single-spore isolates were characterized for virulence genes using a differential series. A total of 250 isolates from 45 locations in 17 states were characterized. Virulence presence and associations among virulence genes varied across regions. For example, isolates found within Indiana, Kentucky, North Carolina, and Virginia were virulent to 10 resistance genes whereas isolates from Kansas were virulent to five resistance genes. The data were analyzed for associations among sets of virulence genes, and the geographical distribution of phenotypes was examined.

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EFFECTS OF COMMON ROOT ROT ON WHEAT CULTIVARS AND BREEDING LINES CLOSELY RELATED TO TAM 107. K. M. Vaughn, C. M. Rush, and M. D. Lazar. Texas Agric. Exp. Stn., P.O. Drawer 10, Bushland, TX 79012.

Common root rot caused by *Bipolaris sorokiniana* (Sacc.) Shoemaker is a disease of wheat (*Triticum aestivum* L.) associated with plant stress. It occurs in many areas of the United States, the Prairie Provinces of Canada, and Australia. A dryland field study was conducted to evaluate whether observed drought tolerance of three cultivars (Siouxland, TAM 200, and TAM 107) and eight breeding lines closely related to TAM 107 was associated with disease susceptibility to common root rot. Seed of all entries were treated with imazalil, which controls common root rot, or left untreated. Wheat was planted September 20 in soil naturally infested with *B. sorokiniana*. In March, plants were evaluated for disease incidence and severity. There were no cultivar/breeding line X seed treatment interactions, and minimum variation existed among entries. Seed treated with imazalil had a significantly lower disease index (DI) than nontreated seed treatments. There were few significant differences ($P=0.05$) in disease incidence and severity among entries. Siouxland (resistant control) not treated with fungicide had a significantly lower DI than TAM 200 (susceptible control) nontreated; and TXGH10440 treated with imazalil had a significantly lower DI than TAM 200 treated.

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Incidence of Barley Diseases in Tunisia, with Emphasis on Virulence types of *Erysiphe graminis f. sp. hordei*. Amor Yahyaoui (E.S.A.Kef, Tunisia), M.R. Reinold (M.S.U. Montana USA), and M. Harrabi (I.N.A.T., Tunisia).

Annual disease surveys were conducted in Tunisia. An average incidence rate of 40% for powdery mildew has been established based on its occurrence in the barley fields surveyed. Over a three year period, more than 200 barley fields were surveyed. There was a tendency of specific disease prevalence for each barley growing area. Net blotch appeared to be more prevalent in the northeastern and coastal regions, Barley stripe had a high incidence in the central region. Scald was more predominant in the northwestern and central regions. Powdery mildew had a high incidence level across

the major barley growing areas of Tunisia. The virulence pool of the *Erysiphe graminis f.sp. hordei* population within each collection site was determined. Virulence types associated with these populations differed within and between regions. Highly virulent types were found in the barley growing areas of central Tunisia. Barley cultivars recommended for this region should have the resistance genes "Mla7, " and "Ml-a9,-" in combinations or the effective resistance genes "ml-o" and "ML-at". The observed variability in virulence types of this pathogen should be carefully monitored.

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FUNGAL BROWN SPOT OF CULTIVATED WILD RICE IS TWO DIFFERENT DISEASES. R. F. Nyvall, J. A. Percich, and J. R. Brantner. University of Minnesota, North Central Experiment Station, 1861 Hwy 169 East, Grand Rapids, MN 55744 and 495 Borlaug Hall, 1991 Upper Buford Circle, St. Paul, MN 55108.

Since the first disease report in 1961, fungal brown spot of cultivated wild rice (*Zizania palustris*) has been considered as one disease. However, the disease is caused by two related fungi, *Bipolaris oryzae* and *B. sorokiniana* that produce different disease symptoms. *Bipolaris oryzae* produces the toxin ophiobolin but *B. sorokiniana* does not. *Bipolaris sorokiniana* survives in grasses and residue and causes disease earlier in the growing season while *B. oryzae* is thought to survive only in residue but is a better parasite and causes disease later in the season. Under optimum conditions for disease, *B.oryzae* predominates but *B. sorokiniana* is the dominant pathogen under less optimum conditions. We suggest the name fungal brown spot be retained for the disease caused by *B. oryzae* and spot blotch be used for the disease caused by *B. sorokiniana*.

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SCLEROTINIA ROT OF PEARS IN OREGON. Robert A. Spotts and Tara Chand-Goyal, Mid-Columbia Research and Extension Center, Oregon State University, 3005 Experiment Station Drive, Hood River, OR 97031.

Record rainfall occurred in Hood River valley, Oregon during spring, 1993. In the late spring, lesions upto 2 cm. diam. were observed on d'Anjou pear (*Pyrus communis* , L.) fruitlets on calyx end or where senescent flower parts adhered to the fruits. Lesions were brown, sometimes with darker boarder. *Sclerotinia sclerotiorum* was consistently isolated from infected tissue. Pathogenicity of the fungus was tested in the field and laboratory, and symptoms identical to those seen in commercial orchards were produced on pear fruits. *Sclerotinia sclerotiorum* was isolated from these inoculated fruits. Mycelial growth of the pathogen was studied on potato dextrose agar at 0 to 30 C. At the optimum temperature of 20 C, the average growth rate was 10 mm per day. The susceptibility of 5 pear cultivars to *S. sclerotiorum* was tested, and cultivars ranked in order of increasing resistance as follows: d'Anjou, Bosc, Red Colunbian, Bartlett, and Comice. The efficacy of several fungicides was tested by drenching fruits, and then inoculating with blocks of colonized agar. Dodine, fenarimol, morestan, triadimefon, ziram, mancozeb, and thiabendazole were relatively ineffective, but iprodione gave good control.

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THE INFLUENCE OF FRUIT CONTACT ON THE SUSCEPTIBILITY OF FRENCH PRUNE TO *MONILINIA FRUCTICOLA*. Themis J. Michailides, and David P. Morgan, University of California, Kearney Agricultural Center, Parlier 93648.

In prune (*Prunus domestica* 'French'), more than 50% of the brown rot infections caused by *Monilinia fructicola* and/or *M. laxa* initiated from fruit contact surfaces. Contact surfaces had microcracks (730-2,255 μm long) and lacked or had less epicuticular wax. About 87% of fruit in clusters retained water drops mainly on contact surfaces while only 12% of the single fruit had water droplets 4 h after spraying with water. Eighty-eight and 92% of the *M. fructicola* conidia placed on contact surfaces and 75 and 77% of those placed on non-contact surfaces germinated after 3 and 5 h, respectively. After spray-inoculation of mature fruit with $1.2X10^5$ conidia/ml of *M. fructicola*, significantly more (75 to 94%) fruit were infected when placed in groups of 4 to 5 fruits than (16 to 42%) single fruit after 4 days at 23 C and >97% relative humidity. Contact surfaces of mature fruit had more mycoflora (27-98 cfu, including *M. fructicola*/cm^2) than non-contact surfaces (only 7-29 fungal cfu/cm^2). Protection of fruit before it comes in contact should result in successful control of fruit brown rot in the orchard.

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ECONOMIC ANALYSIS OF THE AU-PNUTS ADVISORY FOR CONTROL OF PEANUT DISEASES. J. C. Jacobi and P. A. Backman, Department of Plant Pathology, Auburn University, AL 36849-5409.

Field experiments were conducted over three years, 1991-1993, to evaluate the effects of fungicide schedule and treatment on peanut yield and value. Fungicide schedule and treatment combinations were: untreated control, chlorothalonil (1.26 kg a.i./ha) applied on both a 14-day and AU-Pnuts advisory schedules, and a tank-mix of chlorothalonil (0.62 kg a.i./ha) and cyproconazole (0.01 kg a.i./ha) applied on the AU-Pnuts advisory schedule. The advisory averaged one less fungicide application in each experiment compared to the 14-day schedule. Average increases in yield and net return for the advisory in comparison to the 14-day schedule for chlorothalonil alone were 101 kg/ha and $137.84/ha. Yield and net return were highest in each experiment for the tank-mix of chlorothalonil and cyproconazole. Increases in net return for advisory treatments were attributed to increased yields, and reduced disease control costs.

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EFFECTS OF PLANTING DATE AND ROW SPACING ON PEANUT MICROCLIMATE, LESSER CORNSTALK BORERS, AND AFLATOXIGENIC FUNGI. S.D. Stewart, K.L. Bowen, T.P. Mack, and J.W. Kloepper, Depts. of Entomology and Plant Pathology, 209 Life Sciences Bldg, Auburn, AL 36849.

The lesser cornstalk borer (LCB) and aflatoxigenic fungi (*Aspergillus flavus* and *A. parasiticus*) are two of the most important pests of dryland peanut in the Southeast U.S. LCB larvae will vector fungi that produce aflatoxins, and both pests are favored by hot dry conditions. Irrigation is currently the only effective means of controlling aflatoxigenic fungal invasion of peanut seed. In 1993, a study was initiated to determine the effects of planting date (mid- and late-May) and row spacing (normal, wide, and twin) on the peanut microclimate, LCB populations and aflatoxigenic fungi. Soil temperatures were 1.7C higher in late-planted peanuts. Late-planted peanuts had consistently greater numbers of LCB due to fewer predators, higher aflatoxin concentrations, and lower yields. Soil temperatures under the canopy of peanut grown on twin-row spacing were hotter than in others, and natural enemies of LCB were less abundant. However, no differences were detected in LCB abundance, aflatoxin contamination or yields due to row spacing.

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CONSERVATION TILLAGE AND SEEDLING DISEASES IN COTTON AND SOYBEAN DOUBLE-CROPPED WITH TRITICALE. D. R. Sumner, C. C. Dowler, A. W. Johnson, and S. H. Baker, University of Georgia and USDA/ARS, Coastal Plain Experiment Station, Tifton, GA 31793-0748.

Triticale-cotton and triticale-soybean double-crops were grown alternately for 4 yr with conservation tillage (no-till, row-till, or ridge-plant) or conventional tillage (mold-board plowing) under sprinkler center-pivot irrigation. Tillage treatments were implemented before planting cotton or soybean, and triticale was planted no-till. Triticale stubble was cut short (20 cm) or tall (60 cm) or burned before planting. Populations of Rhizoctonia solani AG-4 in soil after planting cotton or soybean were low and not influenced by tillage or triticale residue management treatments. Root and hypocotyl disease levels were low to moderate and were increased by conservation tillage in soybean but not in cotton. Burning triticale residues reduced disease levels slightly compared with nonburning. Fusarium oxysporium, F. solani, R. solani AG-4, Fusarium spp. and Rhizoctonia spp. (in order) were isolated most frequently from diseased cotton and soybean tissues.

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SOLARIZATION TO REDUCE INOCULUM DENSITY OF *RHIZOCTONIA SOLANI* AND BELLY ROT ON PICKLING CUCUMBER. A. P. Keinath, Dept. Plant Pathology & Physiology, Clemson Univ., Charleston, SC 29414-5341.

Belly rot of cucumber, caused by *Rhizoctonia solani* AG-4, is considered by processors to be the most important disease on pickling cucumber. Nonsterile sandy loam soil was infested (1% v/v) with *R. solani* and either heated to 35 C or held at 25 C for 1 wk. Then, 'Calypso' cucumber seed were planted in the soils in two greenhouse tests with four replications each. After 10 days, pre- and post-emergence damping-off were lower ($P<0.01$) in heated (12% and 2.4%) than in control soil (45% and 44%). Two field experiments were conducted with 15-m, single-row plots in 6 randomized, complete blocks. Plots were solarized under a single layer of 0.03-mm thick clear polyethylene from 17 Jul to 31 Aug 1992 and 9 Jul to 12 Aug 1993, or not solarized. 'Calypso' cucumber seed was planted in each plot after solarization. Max. soil temperatures in solarized plots (10-cm depth) were 44 C in 1992 and 45 C in 1993. After solarization, percentage organic matter colonized by *R. solani* was 0.9% and 7% in solarized soil compared with 23% and 41% in nonsolarized soil in 1992 and 1993, respectively. In 1992, percentage fruit surface area rotted was less ($P=0.05$) in solarized plots (14%) than in nonsolarized plots (32%). Solarization may be a useful component of integrated management to reduce belly rot on fall-grown pickling cucumber.

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THE EFFECT OF MULCHES, GYPSUM AND FUNGICIDES ON THE PERFORMANCE OF AVOCADO PLANTED IN SOIL WITH *PHYTOPHTHORA CINNAMOMI* AND *PHYTOPHTHORA CITRICOLA*. J. A. Menge, H. D. Ohr, E. L. V. Johnson, S. Campbell, F. Guillemet, N. Grech, and E. Pond. University of California, Riverside, CA 92521.

Nursery-grown avocado"Hass" on "Duke 7" rootstock were planted in 1990 in a field infested with *Phytophthora cinnamomi* and *P. citricola*. Alfalfa hay, dairy manure, gypsum and a porous plastic tarp were applied alone or in combinations under the tree canopy as mulches. Metalaxyl and fosetyl-Al were also applied alone or in combination with the mulches. None of the mulches except gypsum significantly improved avocado yield or growth when applied alone, but combinations of alfalfa hay, tarp or gypsum resulted in the best avocado growth or yield. Some of these mulch combinations were as beneficial as fosetyl-Al, the best fungicide treatment. Gypsum significantly improved root growth and reduced populations of *P. citricola*.

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FUSARIUM WILT AND ROOT-KNOT NEMATODE INFECTION OF COTTON IN CONVENTIONAL AND MINIMAL TILLAGE PRODUCTION SYSTEMS FOLLOWING DIFFERENT COVER CROPS. P.D. Colyer[1], T.L. Kirkpatrick[2], and P.R. Vernon[1]. [1]Louisiana Agricultural Experiment Station, Bossier City, LA 71113 and [2]University of Arkansas, Hope, AR 71801.

Fusarium wilt (Fusarium oxysporum f.sp. vasinfectum) and root-knot nematode (Meloidogyne incognita) infection of cotton planted using either minimal or conventional tillage following winter fallow, hairy vetch or common vetch cover crops were evaluated. Soil populations of M. incognita and root galling were higher in minimal tillage plots at harvest. Populations of F. oxysporum were not significantly different among cover crops or between tillage treatments at harvest, but the severity of Fusarium wilt was higher in the minimal tillage plots. The incidence of wilt was positively correlated with the severity of root galling caused by M. incognita.

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DAMAGE THRESHOLDS AND ROTATION SYSTEMS FOR MANAGING THE RENIFORM NEMATODE ON COTTON. S. R. Koenning and K. R. Barker, Department of Plant Pathology, North Carolina State University, Box 7616, Raleigh, NC 27695-7616.

The damage and reproductive potentials of the reniform nematode, Rotylenchulus reniformis, on cotton were investigated during the 1992 and 1993 growing seasons at two locations in North Carolina. The effects of 1-yr rotation with corn or soybean cultivars resistant or susceptible to this pathogen on final population densities (Pf) of reniform nematode and cotton yield were evaluated at these sites. The Pf of reniform nematode was greater ($P = 0.05$) in plots planted to cotton than in those planted to corn or resistant soybean. Nematode reproduction rates were lowest on resistant soybean cultivar Centennial. Cotton-lint yield was increased by 5 to 15 % following rotation with either of these crops compared to continuous cotton. Significant cotton-yield suppression was incurred when the preplant density of reniform nematode exceeded 400 per 500 cm^3 soil. The damage potential was greatest in the coarse-textured soil at one location, however. Raising the damage threshold from the current North Carolina threshold of 10 reniform nematodes per 500 cm^3 soil to 400 to 1600 may result in less pesticide usage and enhance profits for cotton growers.

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INTEGRATED MANAGEMENT OF CAVITY SPOT ON CARROTS GROWN ON ORGANIC SOIL. M.R. McDonald and J.C. Sutton, Muck Research Station, Kettleby, ON, L0G 1J0 and University of Guelph, Guelph, ON, N1G 2W1.

Field trials were conducted on Pythium-infested organic soil from 1986-1993. Carrot cultivars that had partial resistance to cavity spot (i.e. Six Pak) had lower disease incidence compared to susceptible cv.'s Chanton, Huron and Red Core Chantaney. Metalaxyl suppressed disease when applied as a granular formulation (0.5 kg ai/ha) at seeding or as a drench (2.0 kg ai/ha) within 6 weeks of seeding. Metalaxyl was more effective on susceptible than on partially-resistant cultivars and on carrots harvested late in the season (Oct. and Nov.) Metalaxyl (3.6 kg ai/ha) and fosetyl-Al (4.8 kg ai/ha) applied 12 or 17 weeks after seeding also suppressed incidence, but not as effectively as early application of metalaxyl. Disease management involved estimating disease risk, selecting resistant cultivars and using metalaxyl when risk was high. There is a potential to predict disease increase and apply fungicide only when conditions favour development.

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REDUCTION OF FUSARIUM CROWN AND ROOT ROT IN FIELD-GROWN TOMATO BY SOIL FUMIGANTS AND A BIOCONTROL AGENT. R.J. McGovern[1], L.E. Datnoff[2], C.S. Vavrina[1], and T.A. Obreza[1]. University of Florida, [1]SWFREC, Immokalee, FL 33934, [2]EREC, Belle Glade, FL 33430.

A commercial tomato field in southwest Florida, naturally infested with *Fusarium oxysporum* f.sp. *radicis-lycopersici*, was used to test the ability of the soil-injected fumigants methyl bromide:chloropicrin, 67%:33% (MBC, 336 kg/ha), 1,3-dichloropropene:chloropicrin, 78%:17% (1,3-DCPC, 327.5 l/ha), and chloropicrin, 96.5% (CPC, 172.5 kg/ha), and a municipal solid waste product (MSW, 62.7 t/ha), alone and in combination with the biocontrol agents *Bacillus subtilis* ($1.0x10^7$ CFU/cm^3), *Streptomyces lydicus* ($2.0x10^5$ CFU/cm^3), and *Trichoderma harzianum* ($1.0x10^4$ conidia/cm^3), in reducing crown and root rot in the tomato cv. Sunny. The MSW was applied prior to bed formation, and fumigants were injected 21.6 cm deep at bed formation. Biocontrol agents were incorporated into the transplant medium at seeding. Controls consisted of nonfumigated soil, and transplants grown in the nonammended medium. A randomized complete block (61x1.5 m bed) design was used for each soil treatment; blocks were further subdivided to test biocontrols. Significant ($p=0.05$) decreases were observed in crown rot incidence and severity, respectively, with MBC (-57%, -66%), 1,3-DCPC (-52%, -67%), and CPC (-36%, -52%). Disease incidence was significantly decreased by *S. lydicus* (-20%), and severity increased by the MSW (+35%). Yield was significantly increased by 1,3-DCPC (+13%) and CPC (+14%), and decreased by the MSW (-23%).

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EFFECTS OF IRRIGATION METHODS ON DISEASE MANAGEMENT IN LETTUCE. K. V. Subbarao, J. C. Hubbard, and K. F. Schulbach. Dept. of Plant Pathology, University of California, Davis, c/o U. S. Agricultural Research Station, Salinas, CA 93905.

Subsurface drip and furrow irrigation were evaluated on cultivar 'Salinas' for their effects on yield, and incidence and severity of three important diseases in spring and winter crops of lettuce in the Salinas Valley. The diseases examined included lettuce drop (LD) (*Sclerotinia minor*), downy mildew (DM) (*Bremia lactucae*) and corky root (CR) (*Rhizomonas suberifaciens*). Replicated plots of subsurface drip and furrow irrigation were arranged in a randomized block design and irrigation treatments were begun immediately after thinning. The furrow plots were irrigated once a wk and the drip plots twice a wk. Plants in all plots were inoculated with *S. minor* by placing a mixture of sclerotia and oat infested with mycelium in the 'competence zone' (*sensu* Grogan). Plots were not inoculated for DM and CR. Plots were evaluated for LD and DM at weekly

intervals until maturity, and yield and CR were determined at maturity. Lettuce drop incidence and CR severity were significantly lower, and yields higher in plots under subsurface drip irrigation compared with furrow irrigation. Incidence and severity of DM were not significantly different between the two irrigations. The differential moisture levels in the soil profile created by the two irrigation methods resulted in the observed effects. The differential ecoclimates created by the two irrigation treatments did not affect DM infection because the macroclimate is usually favorable in the Salinas Valley. Subsurface drip irrigation has the potential to be a viable, long-term strategy for soilborne disease management in lettuce.

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TRANSPLANTING FOR CONTROL OF *STRIGA HERMONTHICA* IN AFRICA. D. K. Berner and F. O. Ikie. International Institute of Tropical Agriculture, PMB 5320, Oyo Road, Ibadan, Nigeria.

Striga hermonthica **(Del.) Benth. is one of the most serious parasites of cereals in Africa. Time of infection is critical to parasite reproduction; yield losses can be minimized if the host is protected from parasitism for a period of 4–6 weeks after planting. This study was undertaken to test transplanting as one possible means of host protection. Experiments were conducted on the soil floor of a screenhouse at IITA, Ibadan, Nigeria and in a field at Abuja, Nigeria. All trials were infested with *S. hermonthica* seeds at time of first planting. Screenhouse and field infestation rates were 5,000 and 10,000 germinable seeds hill^{-1}, respectively. Transplant ages were 0 (direct seeded) or 1–7 weeks old. Weekly *S. hermonthica* emergence counts, sorghum dry stover weight and grain yield were recorded. Transplanting 4- to 7-week-old seedlings significantly, and consistently, reduced *S. hermonthica* emergence, and generally resulted in increased sorghum grain yields. Regular use of transplanting can inexorably reduce *S. hermonthica* infestations, and trials in farmers' fields have shown that transplanting can be an acceptable control option in some locations in Africa.**

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INFLUENCE OF PLANTING DATE ON *STRIGA* INFESTATION AND YIELD OF PEARL MILLET. D.E. Hess and J.H. Williams, ICRISAT Sahelian Center, B.P. 12404, Niamey, Niger.

In southern Niger, pearl millet (*Pennisetum glaucum*) is sown at the first rainfall and farmers may continue sowing with subsequent rainfall for up to 8-10 weeks. A trial was conducted during the 1992 and 1993 crop seasons to investigate the effect of delayed sowing and density of the early millet landrace (partially photosensitive HKB) and the late millet landrace (photosensitive Somno) on infestation by *Striga hermonthica* and on millet yield. Delayed sowing (5-7 weeks) greatly reduced *Striga* infestation from 40 to 7 plants m^{-2} and millet biomass production from 3.7 to 0.9 t ha^{-1}. HKB yielded more grain with early sowing than did Somno (0.86 vs. 0.75 t ha^{-1}), but Somno maintained a more stable yield over the two sowing dates. Crop growth rate (C) and reproductive growth rate (R) for both varieties were highest at the first sowing date, but delayed sowing caused less reduction for Somno. Increased plant density from traditional and recommended 10,000 plants ha^{-1} to 25,000 plants ha^{-1} largely compensated for yield reduction in late-sown Somno through increased C and R. HKB did not have this response to increased plant population. Since C was the basis for decreased yield resulting from delayed sowing, addition of NPK fertilizer should allow further compensation for late sowing by promoting leaf expansion. Thus *Striga* management techniques in the Sudano-Sahelian zone could include late July plantings of photosensitive pearl millet genotypes such as landrace Somno, especially at high plant populations and with NPK fertilization.

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DRIP IRRIGATION AS A POTENTIAL POTATO DISEASE MANAGEMENT TOOL IN CALIFORNIA. G.T. Browne, W.R. Detar[1], and B.M. Sanden; Univ. of Calif. Coop. Ext., Bakersfield, 93307; and [1]USDA-ARS, Shafter, CA 93263.

Subsurface drip irrigation (SSDI) and conventional sprinkler irrigation (CSI) were compared for effects on selected potato diseases and soilborne pathogens in a field trial. During a 2-mo period, CSI accumulated 670 h of canopy leaf wetness; SSDI accumulated 176 h and 314 h leaf wetness when drip lines were centered under conventional single-row beds (line spacing 81 cm) and double-row beds (line spacing 162 cm), respectively. By season's end, mean severity of late blight was significantly less with SSDI (5-10% foliage blighted) than with CSI (46% foliage blighted). Preplant applications of metham by CSI eradicated inoculum of *Phytophthora erythroseptica* and *Sclerotium rolfsii* more effectively under furrows than within plant beds; the reverse was true for metham applied by SSDI with conventional beds. Metham application through SSDI under double-row beds was ineffective. Compared to potato yields in CSI, yields with SSDI were increased significantly in conventional beds and were equivalent or reduced significantly in double-row beds. SSDI is worthy of continued evaluation for use in potato disease management.

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INTEGRATED CONTROL OF FOLIAR DISEASES OF TOMATO. M.L. Gullino and A. Garibaldi, DI.Va.P.R.A., Via Giuria 15, 10126 Torino, Italy.

Several foliar pathogens, such as Botrytis cinerea, Fulvia fulva, Alternaria solani, Phytophthora infestans, Erysiphe sp., cause significant losses in tomato production. During the last years, experimental trials have been carried out on tomato grown under greenhouse, by integrating cultural, chemical and biological measures in order to effectively manage such pathogens. The combination of balanced fertilization, ventilation, heating, and use of chemicals and/or Trichoderma sp. strongly reduced grey mould incidence. The mixture dicarboximide+thiram controlled resistant populations of B. cinerea, reduced the severity of F. fulva but did not satisfactorily control late blight. The environmental conditions unfavourable to grey mould and late blight were not adverse to Erysiphe spp.. Potassium-monobasic phosphate, used at weekly intervals, controlled powdery mildew.

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DETECTION OF DNA COMPONENTS OF TWO STRAINS OF SQUASH LEAF CURL VIRUS USING POLYMERASE CHAIN REACTION. K.R Wendt[1], S.D. Wyatt[2] and J.K. Brown[1]. [1]Dept. of Plant Sciences, University of Arizona, Tucson, AZ 85721. [2]Dept. of Plant Pathology, Washington State University, Pullman, WA 99163.

Two biologically distinct strains of squash leaf curl virus (SLCV), a whitefly transmitted geminivirus, have been reported as causal agents of squash leaf curl disease. The "extended" (SLCV-E) and the "restricted" (SLCV-R) host range strains frequently occur in mixed infection in the field. Polymerase chain reaction (PCR) primers were designed and used to detect the cloned DNA components of SLCV-E and SLCV-R. PCR amplification of A and B clones of SLCV-E yields fragments of 2.3 and 0.45 kb, respectively, while A and B clones of SLCV-R yield fragments of 2.1 and 1.4 kb. Using these same primers, PCR amplified fragments which were diagnostic for the SLCV DNA components were detected in SLCV infected leaf samples. PCR provides a level of sensitivity which allows for re-evaluation of the SLCV strain complex in infected plants and in the whitefly vector.

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CHARACTERIZATION AND NUCLEIC ACID SEQUENCING OF COTTON LEAF CURL VIRUS DNA. A. Nadeem, M. R. Nelson and Z. Xiong. Department of Plant Pathology, University of Arizona, Tucson AZ 85721.

Cotton leaf curl geminivirus (CLCuV) causes a major disease of cotton in Pakistan. Leaves of infected cotton curl upward and bear leaf-like enations on the underside. Plants infected early in the season are stunted and yield is reduced drastically. In order to understand the nature and the relationship of this virus with other geminiviruses, the nucleic acid sequence and genome organization of CLCuV are being determined. The DNA A of CLCuV was amplified by the polymerase chain reaction (PCR) as two fragments using primers specific for the DNA A of all whitefly transmitted geminiviruses. Two DNA fragments of about 1.2 kb and 1.5 kb were amplified. These fragments were subsequently cloned and are now being sequenced. Partial sequences in the coding regions of AL1 gene and coat protein of CLCuV indicate that CLCuV is closely related to tomato yellow leaf curl virus, cassava latent virus, and Indian cassava mosaic virus from the old world. The complete nucleic acid sequence and its analysis will be presented. The existence of the DNA B of CLCuV is being determined by PCR using primers specific for the sequences in the intergenic region.

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MOLECULAR CHARACTERIZATION OF TOMATO LEAF CRUMPLE GEMINIVIRUS: A NEW SAP-TRANSMISSIBLE BIPARTITE GEMINIVIRUS FROM SINOLOA, MEXICO. Y. M. Hou[1], E. P. Paplomatas[2], V. P. Patel[3], A. Noueiry[1], and R. L. Gilbertson[1], [1]Dept. of Plant Pathology, University of California, Davis, CA 95616; [2]Benaki Phytopathological Institute, Greece; [3]Dept. of Immunology, University of California, Los Angeles, CA 90024.

Tomatoes grown in the Culiacan Valley of northwestern Mexico (Sinoloa State) may be infected by one or more whitefly-transmitted geminiviruses. A sap-transmissible bipartite geminivirus was isolated from tomato leaves showing leaf crumpling and yellow mottle symptoms. The virus was readily sap-transmitted to bean and *N. benthamiana* and, with difficulty, to tomato. Geminiviral DNA-A and DNA-B components were cloned from infected bean, and were infectious in bean, *N. benthamiana*, and tomato, inducing the same symptoms as the sap-transmissible geminivirus. Nucleotide sequence comparisons of the common region, coat protein, and BR1 open reading frames indicated that this geminivirus is different from previously characterized geminiviruses and the name tomato leaf crumple (TLCrV) is proposed. TLCrV is most closely related to tomato mottle, abutilon mosaic, and bean dwarf mosaic geminiviruses. The role of TLCrV in the geminivirus disease complex in the Culiacan Valley and cloning of a related but biologically distinct DNA-B component associated with TLCrV will be discussed.

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CLONING AND SEQUENCING THE 5' TERMINAL TWO-THIRDS OF THE CITRUS TRISTEZA VIRUS GENOME. A.V. Karasev[1,2], V.P. Boyko[2], S. Gowda[2], O.V. Nikolaeva[1,2], D.J. Gumpf[1], S.M. Garnsey[3], R.F. Lee[2], and W.O. Dawson[2]. [1]*Dept. of Plant Pathology, University of California, Riverside, CA 92521;* [2]*University of Florida, CREC, Lake Alfred, FL 33850;* [3]*USDA-ARS, Horticultural Res. Lab., Orlando, FL 32803.*

The 3' terminal 7,292 nt of the CTV genome have been recently cloned and sequenced (Pappu *et al.*, 1994, *Virology* **199**, 35-46). Here we present data on cloning and sequencing the remaining two-thirds of the CTV genome. The 5' proximal open reading frame 1a (ORF1a) of CTV encodes a large polyprotein of more than 300 kDa. Three conserved domains, i.e. a papain-like protease, a methyltransferase, and a helicase, were confidently identified within this polyprotein. The downstream ORF1b encodes the (putative) 55 kDa CTV polymerase and is suggested to be expressed by a translational frame-shift. The ORF1b is followed by ORFs 2 and 3 encoding proteins of 33 kDa and 6 kDa, respectively; both these ORFs are presumably expressed via formation of subgenomic RNAs. Protein products encoded by ORFs 1a, 1b, and 3 demonstrate various degrees of similarity to analogously encoded proteins of beet yellows closterovirus (BYV). However, ORF 2 of CTV has no counterparts in the BYV genome. Phylogenetic analysis suggests close relationships between CTV and BYV, despite obvious differences in their genome organizations.

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OPTIMIZING TISSUE SAMPLING FOR DETECTION OF CTV IN FIELD GROWN CITRUS TREES. J. Ghazanfari[1] and J. A. Dodds[2]. [1]California State Dept. Food and Agric., Tulare, CA 93274, and [2]Dept. Plant Pathology, University of California, Riverside, CA 92521.

In order to improve citrus tristeza virus (CTV) sample quality for ELISA testing of sweet orange in the suppressive area in Tulare, Kern and Fresno counties, CA, test samples have been collected from different parts of infected field trees including quadrants of trees (north, south, east and west) and different types of tissue (old and new shoots, leaf lamina and petiole). Results indicate that CTV is not uniformly distributed in all parts of trees. When data from 520 infected trees was analyzed, significant differences were detected between the four quadrants and the highest titer was found on the north side of the canopy. Petioles of newly formed leaves had the highest titer, followed in decreasing order by old twigs (a leafless shoot including bark and wood), new twigs, old petioles, new leaf lamina, and old leaf lamina. These results should help optimize tissue sampling in the future.

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IDENTIFICATION OF THE ANTIGENIC DETERMINANT RECOGNIZED BY THE CITRUS TRISTEZA CLOSTEROVIRUS-SPECIFIC MONOCLONAL ANTIBODY 3DF1. H.R. Pappu, S.S. Pappu, R.F. Lee*, and C.L. Niblett. Plant Pathology Department, University of Florida, Gainesville, FL 32611, *CREC, Lake Alfred, FL 33850.

The citrus tristeza virus (CTV)-specific monoclonal antibody 3DF1 reacts with over 98% of the known CTV isolates from different parts of the world. On Western blots, it reacts only with the intact capsid protein (CP) of 25 kDa and not with its two smaller hydrolysis products of 24 and 21 kDa, whereas a majority of the mono- and polyclonal antibodies detect all three CP forms. In order to precisely map the epitope recognized by 3DF1, the CP genes of two 3DF1-nonreactive isolates, B188 and B215, were cloned as cDNA and sequenced. When compared with the CP sequences of several 3DF1-reactive isolates, the CPs of B188 and B215 revealed differences at three positions in their amino termini. The amino acids Asp^2, Lys^{13}, and Phe^{28} were conserved in all the 3DF1-reactive isolates, but they were replaced by Gly, Thr, and Tyr, respectively, in the non-reactive CPs of B188 and B215. Individual point mutations were introduced into the cloned CP genes of the wild type (wt) B215, and a 3DF1-reactive isolate, T36. The serological reactivities of the wt and mutant CPs of B215 and T36, expressed as recombinant fusion proteins in *E. coli* were evaluated by Western blot. A point mutation (A→G) resulting in an Asp→Gly change in the second position of the T36 CP abolished the reactivity with 3DF1, whereas a reverse mutation (Gly→Asp) conferred reactivity on the CP of the non-reactive B215 isolate.

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CLONING AND SEQUENCING OF PEA STREAK CARLAVIRUS 3'-TERMINUS. T.D. Cavileer and P.H. Berger. PSES, AgSci 242, University of Idaho, Moscow, ID 83844-2339.

Oligo d(T) primed cDNA, representing the 3' terminus (1.8 kb) of pea streak carlavirus (ATCC PV87), was generated, cloned and sequenced. Sequence analysis revealed, 5' to 3', one partial and three complete open reading frames, an 87nt non-translated region followed by a poly (A) tail. Genomic organization is similar to other published carlaviruses with the exception of the 3' ORF, which is not present. Although the genomic arrangement is reminiscent of potexviruses, SDS-PAGE and western blot analysis of coat protein and formaldehyde gel analysis of genomic RNA place pea streak in the carlavirus group. The first partial ORF (8.5kD), ORF2 (11.3kD) and ORF3 (7.3kD) overlap to form a 'triple block' motif found in all carlaviruses sequenced to date. Protein comparisons of the conserved carboxy terminus of the coat protein region with carlavirus or potexvirus coat proteins averaged 60% and 50% respectively. The relationships and homology of PSV with the carla- and potexviruses will be discussed.

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IDENTIFICATION OF A NEW VIRAL AGENT ASSOCIATED WITH CALIFORNIA CARROT MOTLEY DWARF. M.T. Watson and B.W. Falk. Department of Plant Pathology, University of California, Davis, CA. 95616.

Carrot motley dwarf disease (CMD) has been described from various parts of the world and is caused by a complex of two viruses, carrot red leaf luteovirus (CRLV) and carrot mottle umbravirus (CMoV). Virions were purified from CMD-affected plants and cDNAs were constructed to virion RNAs. Hybridization analyses detected the expected CRLV and CMoV RNAs, and a new virus agent referred to here as CRLV-associated RNA (CRLV-aRNA). Analyses of field and greenhouse samples showed the CRLV-aRNA to be consistently associated with plants infected with CRLV and CMoV. The CRLV-aRNA is approximately 3kb and is dependent upon CRLV for transmission by the willow-carrot aphid, *Cavariella aegopodii*. The CRLV-aRNA alone did not systemically infect plants and sap transmission from CMD-affected plants resulted in plants systemically infected only with CMoV. The complete nucleotide sequence of CRLV-aRNA will be presented along with comparisons to other plant viral RNAs.

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MOLECULAR CHARACTERIZATION AND DETECTION OF BANANA BUNCHY TOP VIRUS IN HAWAII. W. S. Xie, J. S. Hu, and D. Sether, Department of Plant Pathology, University of Hawaii, Honolulu, HI 96822.

Banana bunchy top virus (BBTV) has a genome consisting of at least six single-stranded, circular DNA components about 1 kb each. A Hawaiian BBTV isolate was purified from 4 kg BBTV-infected banana leaf tissue. Viral DNA was isolated from purified virus and cloned. Two cDNA clones representing the full-length of two of BBTV genome components were sequenced. One of the two components encodes a putative replicase gene, which shares 98% sequence identity with the Australia BBTV isolate. The cDNA clones were used in dot blot analysis for the detection of BBTV using both radioactive and non-radioactive labelled probes. PCR was developed for the detection of BBTV in both banana plants and aphids. The sensitivities of ELISA and dot blot were similar, but PCR was 8,000 times more sensitive, allowing detection of BBTV from single viruliferous aphids in PCR.

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NUCLEOTIDE SEQUENCE AND EXPRESSION OF THE COAT PROTEIN (CP) GENE OF A DASHEEN MOSAIC VIRUS ISOLATE (DsMV-Ch) FROM *CALADIUM*. R. H. Li, F. W. Zettler, and E. Hiebert. Plant Pathology Dept., Univ. Florida, Gainesville 32611

The 3' terminal 1186-nucleotide sequence of DsMV-Ch was determined. It consisted of a 939-nucleotide CP gene and a 247-nucleotide polyadenylated noncoding region. The CP consists of 313 amino acid residues with a calculated MW of 35 kd. The DsMV-Ch CP gene was determined to have 86-88 and 56-65% nucleotide similarities, respectively, to DsMV isolates from *Colocasia* (Pappu et al. 1994. J. Gen. Virol. 75:239) and to other potyviruses. Since DsMV is difficult to purify from infected plants, the CP gene of DsMV-Ch was amplified by PCR, cloned into a pETh-3 vector, and expressed in *E. coli*. A 40 kd product resulted, which included an N-terminal fusion protein consisting of 15 amino acids. In immunodiffusion tests, homologous precipitin lines of expressed DsMV-Ch CP fused without spur formation with lines representing DsMV antigens in plant extracts. Based on Western blot studies, (Li et al. 1992. Phytopathology 82:1090), CP MWs of seven DsMV isolates were 38-46 kd, including that of DsMV-Ch which was 44 kd. The disparity between the calculated MW of 35 kd and the spuriously high values noted in immunoblots may be due to the conformation of DsMV CP in SDS-PAGE.

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MOLECULAR CLONING AND GENOME ORGANIZATION OF SAGUARO CACTUS CARMOVIRUS. Z. Weng, and Z. Xiong. Department of Plant Pathology, University of Arizona, Tucson, AZ. 85721.

Saguaro cactus carmovirus (SCV) causes symptomless infection in saguaro cactus. The isometric virus consists of a single stranded RNA genome about 4.0 kb and a major capsid protein about 39 kd. SCV is easily transmitted mechanically and is suspected to be transmitted by pollen in nature. The genomic RNA was extracted from the purified virus particles. The cDNA was synthesized from poly A tailed genomic RNA and the dsDNAs were cloned into pBR322 vector. The relationships between clones were analyzed by hybridization using probes synthesized by random primer. The cDNA clones covering the entire genome were subcloned into pBS (+) vector and sequenced from exoIII-generated nested subclones. A genome organization typical of carmoviruses was predicted from SCV nucleotide sequence.

The genomic nucleotide sequence shows the highest degree of homology with that of carnation mottle virus and various degrees of homology with those of other carmoviruses, dianthoviruses, and tombusviruses. Similar to other carmovirus, two subgenomic RNAs were detected from virion-associated viral RNA. Both hybridized with cDNA clones representing the 3' portion of the genomic RNA, but not with the 5' cDNA clones.

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Turnip crinkle carmovirus-specific binding of erythrocytes *in vitro* is further evidence of saccharide binding by icosahedral plant virus particles. **M. H. Walter, L. A. Heaton, Department of Plant Pathology, Kansas State University, Manhattan, KS 66506-5502.**

The structural relatedness of turnip crinkle carmovirus (TCV) and tomato bushy stunt tombusvirus (TBSV) to the jack-bean seed lectin concanavalin-A suggests that the viruses may bind carbohydrates *in vivo* (Argos, P., Tsukihara, T. and Rossmann, M.G. 1980). Previous results from hemagglutination assays (HAs) using swine erythrocytes (RBCs) suggested saccharide binding by tomato bushy stunt virus (TBSV) and turnip crinkle carmovirus (TCV). In other binding assays (Parrish, Aquadro & Carmichael, 1988), virus was incubated with glutaraldehyde-treated RBCs, resuspended in PBS (pH 6.8) or was pre-incubated with an inhibitor. Following washing, RBCs were suspended in 50-100 ul of 0.01M sodium acetate, pH 5.5 and loaded onto microtitre plates for serological assay. TCV binding affinity to RBCs was approximately 1/3 that of TBSV, but three times above background levels with much lower variability than with TBSV. Pre-incubation of RBCs with neuraminidase (from *Salmonella sp.*, 0.25U/ml) decreased TBSV:RBC binding by 50%. Western blot analysis revealed TBSV coat protein associated with RBCs. Binding of TBSV to RBCs was optimal at pH 6.8 in PBS.

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EXPLOITATION OF THE PLUM POX VIRUS 3' NON-CODING REGION FOR ACCURATE VIRAL IDENTIFICATION AND PRELIMINARY STRAIN DIFFERENTIATION. L. Levy and A. Hadidi. National Germplasm Resources Laboratory, USDA ARS, Beltsville, MD 20705.

The plum pox potyvirus (PPV) is the most economically destructive virus disease of stone fruits. Recently, a closely related virus, prunus latent potyvirus (PLV) was isolated from *Prunus spp.* PLV reacts positively in ELISA with PPV antiserum from France, but negatively with PPV DNA primers specific for the 3' non-coding region (NCR) of PPV in RT-PCR assays. PCR of the PPV-NAT clone using DNA primers for the 3' NCR generated a 220 bp product which was cloned and sequenced. This clone identified several strains of PPV from France, Spain, Italy, Hungary, Romania, Germany and Egypt in dot blot assays using cRNA probes. To date, PPV-3' NCR clone is PPV-specific and fails to detect several other potyviruses. Gel electrophoretic analysis of 3' NCR clones of geographically distinct isolates of PPV revealed a heterogeneity in the size of the 3' NCR. Sequence comparison and preliminary relationships within PPV isolates are described.

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A PLANT RESISTANCE MECHANISM ASSOCIATED WITH THE GENERATION OF RESISTANCE BREAKING ISOLATES OF POTATO VIRUS Y. R. Acosta-Leal, and Z. Xiong, Dept. of Plant Pathology, University of Arizona, Tucson, AZ. 85721.

'Virgin A Mutant' (VAM) tobacco breeding line contains a single recessive gene conditioning resistance to potato virus Y (PVY). NC745 is an anther-doubled line derived from a cross between VAM and Coker 86 and exhibits similar PVY resistance. Mechanical inoculation of PVY strain NN on VAM and NC745 produced 0/993 and 31/873 infected plants, respectively. Infectious virus and viral RNA were not detected from the asymptomatic plants by back inoculation to susceptible Burley tobacco and by Northern hybridization. Virus progeny recovered from the infected NC745 plants were able to overcome the resistance in both VAM and NC745 plants and resulted in 100% infection when mechanically inoculated. Replication of PVY-NN RNA in transfected VAM protoplasts was not detectable by Northern hybridization. A low level of PVY-NN RNA replication was detected in transfected NC745 protoplasts. These data suggest that the replication of PVY-NN was blocked in resistant VAM and NC745 tobacco lines. This resistance mechanism may also affect the replication fidelity of PVY-NN in NC745, leading to a high mutation rate and consequently generating resistance breaking isolates.

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ARMILLARIA ROOT DISEASE IN PLANTED PONDEROSA PINE IN NORTHERN NEW MEXICO. Charles G. Shaw III and Mark Schultz. USDA Forest Service, Rocky Mountain Research Station, Fort Collins CO 80526.

In 1968, 69 of 277 Pinus ponderosa trees (25%) examined on a 1/2 acre trend plot in the Los Conchas plantation (established in 1962) were infected with or already killed by Armillaria root disease. In 1972, 128 of the 277 trees remained, only 109 of which were healthy. When reestablished in 1988, only 38 trees remained on the plot, 28 of which were healthy. In 1972, fifty two 1/100 acre plots were established across the 26 acre plantation on a 1 x 5 chain grid. Ten percent of the 309 trees examined were dead or declining primarily due to Armillaria root disease. In 1992, only 110 healthy trees remained in a similar 52 plot sample. Based on grid plots, stocking was still adequate to meet timber objectives, unless diseased-caused mortality markedly increases. Based on the 1/2 acre trend plot, however, remaining stocking is well below the minimal acceptable level for growing timber, indicating the need for foresters to evaluate spatial activity of this root disease in their management decisions.

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Effects of thinning and defoliation on Armillaria and a potential antagonist. E.A. Burrill, J.J. Worrall & P.M. Wargo*, State University of New York College of Env. Sci. & Forestry, Syracuse, NY 13210 and *USDA For. Serv., Hamden, CT 06514.

Defoliation by gypsy moth and subsequent Armillaria root rot are major problems in oak forests. Thinning prior to defoliation may increase tree vigor and the ability to withstand defoliation stress. Alternatively, thinning may increase pathogen inoculum potential by providing a substrate base, the residual stumps. *Tricholomopsis platyphylla* is a cord-forming saprobe that may act as a biocontrol agent through preemption of the substrate. A 3x4 factorial, with 3 levels of thinning and 4 levels of defoliation intensity, was used to examine the effects of thinning and defoliation on the two fungi. Percent *T. platyphylla* and percent *Armillaria* were visually estimated on stumps, debris and snags in each of 6 plots in 28 stands. Analyses suggest that thinning mitigates the effects of defoliation on *Armillaria* colonization. Stands thinned and defoliated had significantly less *Armillaria* colonization than unthinned, defoliated stands. Colonization by *T. platyphylla* was significantly higher on debris than on other substrates; colonization by *Armillaria* was significantly higher on stumps than on other substrates.

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DISTRIBUTION OF *ARMILLARIA OSTOYAE* GENETS IN A *PINUS RESINOSA-P. BANKSIANA* FOREST. D.M. Rizzo[1], G. May[2], and R.A. Blanchette[1], Departments of [1]Plant Pathology and [2]Plant Biology, University of Minnesota, St. Paul, MN 55108.

Diploid isolates of *Armillaria ostoyae* were obtained from three 50 x 25 m plots (two clearcut and one uncut) in a *Pinus resinosa-P. banksiana* stand in northern Minnesota. Based on pairings among 439 isolates, 16 somatic incompatibility groups (SIGs) of *A. ostoyae* were identified. Molecular analysis of 95 isolates representing the 16 SIGs delineated 16 nuclear and 8 mitochondrial haplotypes. All isolates tested within a SIG were identical for the molecular markers; one SIG exception consisted of 3 different nuclear haplotypes. Each method alone did not delineate all of the potential genets on the site; however, the combination of cultural and molecular data indicates at least 18 genets. The genets consisted of large (up to 140 m diam), continuous genets intermixed with small, recently established genets and older, fragmented genets. The spatial distribution of *A. ostoyae* was influenced by a number of genetic and other interacting factors.

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ARMILLARIA SPECIES FROM FORESTS OF CENTRAL MEXICO. D. Alvarado-Rosales and R.A. Blanchette. Department of Plant Pathology, University of Minnesota, St. Paul, MN 55108.

A collection of 76 isolates of *Armillaria* spp. from Mexico were paired with known haploid tester strains from the USA. The isolates were collected in 4 forested regions of Central Mexico, from basidiospores, rhizomorphs or mycelial fans. Compatible and incompatible reactions of diploid-haploid or haploid-haploid pairings were used to identify the different species of *Armillaria*. *A. gallica* was identified in eastern Mexico state from *Pinus hartwegii* (located at an altitude of 3810 m), and in northeastern Morelos from *Quercus* (2600 m elevation). *A. mellea* was identified from a mixed *Quercus-Pinus* forest located in northwest Veracruz (2700 m elevation). Isolates from the fourth area (eastern Mexico state) showed incompatibility reactions with the tester strains used. This is the first extensive study to identify *Armillaria* species from Mexico and the first report of *Armillaria gallica* in pine growing at one of the highest elevations in Mexico.

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EFFECTS OF RESOURCE LIMITATIONS AND ELEVATED ATMOSPHERIC CO_2 ON ECTOMYCORRHIZAE OF LONGLEAF PINE. G.B. Runion, R.J. Mitchell,

H.H. Rogers, and S.A. Prior. School of Forestry, Auburn University, AL, Joseph W. Jones Ecological Research Center, Newton, GA, and USDA National Soil Dynamics Lab, Auburn, AL.

Longleaf pine (*Pinus palustris* Mill.) seedlings were exposed to two concentrations of atmospheric CO_2 (365 or 720 μmol mol^{-1}) and to two levels of N fertility (40 or 400 kg N ha^{-1} yr^{-1}) within open top chambers from March through November, 1993. In August, two levels of water stress (-0.5 or -1.5 MPa xylem pressure potential) were also implemented. Fine root samples were collected in July and November, 1993. Numbers of ectomycorrhizal short roots per cm of fine root were quantified and percentage of mycorrhizal short roots was calculated. Elevated CO_2 increased the total number of ectomycorrhizae and the percentage of mycorrhizal short roots at both harvests. Numbers of ectomycorrhizae and percentage of mycorrhizal short roots were decreased by the high N treatment at both harvests and by water stress at the second harvest. There were few significant interactions among the main treatment variables at either harvest. Due to increases in total fine root length and mycorrhizal colonization, elevated CO_2 resulted in a doubling of the number of ectomycorrhizae per longleaf pine seedling.

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COMPARATIVE GROWTH OF LOBLOLLY PINES WITH DIFFERENT LEVELS OF RESISTANCE TO FUSIFORM RUST. **C. H. Walkinshaw. USDA Forest Service, Box 5500, Pineville, LA 71361-5500.**

During 1980-90, approximately one billion loblolly pines (Pinus taeda L.) were planted annually in the southern United States. Fusiform rust in these plantings ranged from 0 to 100%. In Alabama, Georgia, Louisiana and Mississippi rust infection was generally less than 50%. These millions of infected trees serve as spore sources when conditions favor disease spread. The potential for high disease incidence has stimulated research in breeding for resistance. This study evaluated tree growth and response to the rust in a variety of families. Results of evaluations indicate that significant reduction in diameter growth is associated with highly-resistant families. At age 15, the most resistant family was 4-5 cm smaller in dbh than the susceptible check. Many moderately resistant families are good growers. Rejection of a susceptibe family that grows rapidly may be invalid. Such a family remains ideal for many areas with low rust incidence.

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INFECTION OF *PINUS ELLIOTTII* SEED BY *LASIODIPLODIA THEOBROMAE*. A. J. Cilliers[1], W. J. Swart[2], and M. J. Wingfield[3], [1,3]Department of Microbiology and Biochemistry and [2]Department of Plant Pathology, University of the Orange Free State, Bloemfontein 9300, South Africa.

Clonal seed orchards of *Pinus elliottii* in the Eastern Transvaal province of South Africa consistently yield relatively large numbers of seed that are discolored and have reduced viability. The fungus, *Lasiodiplodia theobromae* has constantly been associated with the phenomenon. Although the mode of seed infection is still unclear, location of fungal mycelium within discolored seeds indicates the possibility of infection occurring during pollination. Preliminary observations have also suggested that the intensity of the disease is related to cone harvesting and storage procedures. A trial was therefore initiated to investigate the effect of cone maturity as well as cone storage under various environmental conditions on the incidence of *L. theobromae*. Isolations from immature seeds inside one-year-old cones yielded low percentages of *L. theobromae*. Seeds obtained from mature cones stored in closed hessian bags generally showed a higher percentage of *L. theobromae* than from cones stored on open trays. Both batches of mature cones showed a general increase in the incidence of *L. theobromae* isolated from seeds for the first two weeks after harvest followed by a significant decrease from four to six weeks after harvest. These results suggest that the environmental conditions under which *P. elliottii* cones are stored strongly influence the incidence of *L. theobromae* on seeds.

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NATURAL COLONIZATION OF OAK WILT FUNGUS MATS BY OPHIOSTOMA PICEAE. J. Juzwik and J. Meyer, USDA Forest Service, 1992 Folwell Ave., St. Paul, MN, and D.W. French, Dept. of Plant Pathology, Univ. of Minnesota, St. Paul 55108.

The Graphium anamorph of Ophiostoma piceae (OP) commonly colonizes sporulating mats of Ceratocystis fagacearum (CF) and reduces the probability of insect transmission of the latter. Frequency and extent of OP colonization of CF mats at different developmental stages are being evaluated during spring and fall in oak wilt areas near Minneapolis and St. Paul, MN. Forty mats that had sufficiently cracked the bark of 15 northern pin oaks to allow insect access were sampled between 15 September and 29 October 1993. Synnemata of OP were observed microscopically on only aging (4 of 17) and declining (2 of 5) mats. However, OP or both OP and CF were recovered through serial dilution plating of subsamples from 10 aging and 3 declining mats, while CF only was isolated from 7 aging mats and 1 declining mat. Of 18 immature and mature mats, CF was isolated from 10 mats, both OP and CF were isolated from 8, and no OP was isolated alone. Colonization of fall CF mats by OP was therefore greatest (59% incidence) in post-mature mats. If similar results are found in spring evaluations, augmentation sprays using OP could potentially enhance mat colonization and reduce overland disease spread.

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BIOCONTROL OF BLUE STAIN FUNGI: INTERACTIONS IN WOOD AMONG A COLORLESS STRAIN OF *OPHIOSTOMA PILIFERUM* AND OTHER FUNGI. C.J. Behrendt[1], R.A. Blanchette[1] and R.L. Farrell[2]. [1]Department of Plant Pathology, University of Minnesota, St. Paul 55108, [2]Sandoz Chemicals Biotech Research Corp., Lexington, MA 02173.

Successful control of blue stain fungi (*Ophiostoma* sp.) in cut wood following treatment with a colorless strain of *Ophiostoma piliferum* (commercially available as Cartapip 97®, Sandoz Chemicals, Charlotte, N.C.) was shown in the laboratory and field. Inoculation of *Pinus resinosa* by the colorless strain 2 wks prior to inoculation with wild type *O. piliferum*, *O. piceae*, *Phanerochaete gigantea*, or *Trichoderma* sp. resulted in sapwood colonization percentages for wild type fungi of 0, 0, 0, and 61, respectively. Sapwood colonization by the colorless strain ranged from 58-68% among treatments. Inoculation of *O. piliferum*, *O. piceae*, *P. gigantea*, or *Trichoderma* sp. 2 wks prior to the colorless strain resulted in 56, 55, 51, and 64 % colonization, respectively; while colonization by the colorless strain was 0, 6, 5, and 45%, respectively. Effective biological control of wild type blue stain fungi and *P. gigantea* was observed in logs that received prior treatment with the colorless strain. *Trichoderma* sp. was not inhibited and did not appear to affect the biocontrol potential of colorless *O. piliferum*.

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DISEASE-CAUSED CANOPY GAPS AND MEXICAN SPOTTED OWL PREY. **J.E. Lundquist, B.W. Geils and J.P. Ward. Rocky Mountain Research Station, USDA Forest Service, Fort Collins, CO 80526.**

The Mexican spotted owl (Strix occidentalis lucida) is a canyon dwelling raptor found in portions of the southwestern United States and northern Mexico. This owl has recently been listed as a threatened species in the US. In this study we describe how root diseases, dwarf mistletoe, stem diseases and other disturbance agents interact to alter habitat for primary prey of the Mexican spotted owl. Plots were established in pinyon pine/juniper, mixed conifer, and ponderosa pine forests in the Sacramento Mountains of New Mexico. In this region, the most common prey of the Mexican spotted owl are brush mice (P. boylii), deer mice (Peromyscus maniculatus), long-tailed voles (Microtus longicaudus), Mexican voles (M. mexicanus) and Mexican woodrats (Neotoma mexicana). Spatial distributions of each prey species and canopy gap cause were overlaid to determine their relationship. The most common disturbances involved interactions among root diseases, bark beetles, wildfire, and tree cutting. Habitat use patterns of different species corresponded to disturbance patterns.

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TWO NOVEL TOBACCO CHITINASES WITH *IN VITRO* ANTIFUNGAL ACTIVITY: A CHITIN BINDING CLASS I PR-4 PROTEIN AND A CLASS V CHITINASE, HOMOLOGOUS TO BACTERIAL EXO-CHITINASES

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Tobacco plants respond to stress by the synthesis of a large number of pathogenesis related (PR) proteins subdivided into five distinct groups. Each group consists of basic, vacuolarly targeted, class I proteins and acidic, extracellularly targeted, class II proteins. Recently, two new PR-proteins induced by TMV infection and wounding were identified in our lab. Analysis of one of the proteins (CBP20) and corresponding cDNAs revealed that it contains a N-terminal chitin-binding domain and a C-terminal domain showing homology to class II PR-4 proteins from tobacco (PR-4a,4b) and tomato (P2) and the putative WIN1 and WIN2 proteins of potato. CBP is an endo-chitinase and localized intracellularly. The second protein (Chi-V) isolated in our lab belongs to a new class of endo-chitinases showing no sequence similarity to the previously identified chitinases (class I - IV) but share homology with some bacterial exo-chitinases. However, Chi-V proteins lack exo-chitinase activity. *In vitro*, CBP and the class V chitinases are antifungal against *Trichoderma viride* and *Fusarium solani*, both alone and in synergy with tobacco class I β-1,3-glucanase. CBP20 also showed synergy with tobacco class I chitinase.

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INDUCTION OF β-1,3-GLUCANASES AND CHITINASES IN TOBACCO BY SEED TREATMENT WITH SELECT STRAINS OF PLANT GROWTH PROMOTING RHIZOBACTERIA. R.J. Sayler, G. Wei, J.W. Kloepper, and S. Tuzun, Department of Plant Pathology, Auburn University, AL 36849.

β-1,3-glucanases and chitinases, hydrolytic enzymes produced by plants in response to pathogenesis, are implicated in induced systemic disease resistance. Multiple plant growth promoting rhizobacteria (PGPR) strains, which are known inducers of systemic resistance in cucumber (Wei *et al.*, Phytopathology 81:1508-1512), were examined for their ability to induce accumulation β-1,3-glucanases and chitinases in tobacco. Tobacco cultivar Kentucky 14 was seeded and drenched with bacterial suspensions. At two months after planting (one month after transplanting to individual containers), leaf samples were taken and analyzed for hydrolytic enzyme accumulation by Western blot analyses. Three bacterial strains (*Serratia*

marcescens, CG-90-166; *Pseudomonas putida*, 89B61; and an unidentified isolate, INR-11) produced strong induction of at least one isozyme of each of these hydrolytic enzymes. Two other strains (*Bacillus megaterium*, INR-15; and *B. pabuli*, INR-16) produced intermediate induction, whereas several other strains (*B. coagulans*, INR-1; *Flavimonas oryzihabitans*, INR-5; and *B. pumilus*, INR-7) produced no induction compared to controls. Since only specific β-1,3-glucanase and chitinase isozymes are induced by seed drench with bacteria, the induction of these isozymes maybe used as a marker for selecting bacterial strains which induce host defense responses.

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COMPARISON OF PECTIC ZYMOGRAMS PRODUCED BY DIFFERENT CLONES OF CANOLA STEM ROT PATHOGEN SCLEROTINIA SCLEROTIORUM IN CULTURE. D. Errampalli and L. M. Kohn. Department of Botany, University of Toronto, Erindale College, Mississauga, Ontario L5L 1C6 Canada.

The isozymes of polygalacturonase (PG) and pectinmethylesterase (PME), produced in vitro by 36 strains belonging to 14 clones of *Sclerotinia sclerotiorum*, were detected by isoelectric focusing on polyacrylamide gels and activity staining of agarose overlays containing appropriate substrate. Analysis of the PG isozyme (zymogram) banding patterns identified two distinct *S. sclerotiorum* zymogram groups (SSZG), SSZG-1 and SSZG-2. Seventy-one percent of the clones belonged to SSZG-1 and 29% of the clones belonged to SSZG-II. Isozyme analysis demonstrated that many different clonal genotypes were included within each PG isozyme group. Two isolates, one from cultivated sunflower and one from a wild plant, *Ranunculus ficaria*, showed isozyme patterns different from the isolates obtained from agricultural fields. Comparison of zymograms showed that there are fewer zymogram groups than clones, with groups of clones belonging to the same zymogram group. One frequently sampled clone with wide geographical distribution comprised some isolates in SSZG-1 and some in SSZG-2. There is no correspondence between the isozyme pattern and aggressiveness on canola or geographic origin of clones.

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PARTIAL RESTORATION OF ELICITOR COMPETENCY IN SOYBEAN WITH HETEROLOGOUS WOUND FACTORS. P. A. Abbasi and T. L. Graham, Department of Plant Pathology, The Ohio State University, Columbus, OH 43210.

It was recently found that wound-associated elicitation competency factors (CFs) are required for the proximal cell responses (glyceollin accumulation and phenolic polymer deposition) of soybean tissues to the *Phytophthora sojae* (PS) wall glucan elicitor. The glucan elicitors from PS elicit a broad range of responses in a cultivar non-specific manner in soybeans and also in a host non-specific manner in other plants. To determine whether the CFs themselves are host-specific or they occur in a wide range of plants, a number of plants from different families were tested to see if they can provide CFs for glyceollin accumulation in soybean. Wound exudates from other legumes (bean, pea, chickpea) and non-legumes (potato, carrot, turnip) were co-applied with elicitor preparations from PS in various soybean cotyledon elicitation assays to determine their ability to confer elicitor competency. Treated tissues were harvested and analysed for isoflavone metabolite accumulation by HPLC. All the wound juices tested caused a substantially higher accumulation of glyceollin with elicitor as compared to wall glucan or water alone. Thus, plants other than legumes possess wound factors which can at least partially restore competency to non-wounded soybean cells. This suggests that some elements of the competency phenomenon may be conserved across a wide range of plant species.

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Pectic isoenzymes of *Stenocarpella maydis* (*Diplodia maydis*).
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Stenocarpella maydis causes seedling blight, crown rot, stalk rot, and ear rot of maize and is endemic in many maize growing regions. Isozyme pattern analysis was initiated to assess relatedness among geographically diverse strains. Extracellular pectolytic enzymes were analyzed for single spore isolates of eight strains. Cultures were grown from mycelial plugs placed in a mineral medium containing 5% polygalacturonic acid (PGA) and amended with micronutrients. Peak enzyme activity occurred in 10-12 d. Culture fluids, concentrated 3- to 6-fold by ultrafiltration (10kDa exclusion), were isoelectrically focused (IEF) on acrylamide. A detection agarose overlay (pH 5.5) with PGA and EDTA was incubated on the IEF gel. After staining with ruthenium red, clear bands in the overlay indicated pectolytic activities focused at pIs 4.4, 4.6, and 8.2. Transeliminative cleavage of PGA at pH 5.5 and 8.0 in the presence of EDTA, measured at A_{232nm}, indicated calcium independent pectate lyases focused at each pI point. Pectate lyases, especially calcium independent enzymes, have not been reported often from plant pathogenic fungi. Their role in pathogenesis is being evaluated.

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DIFFERENTIAL ACTIVATION OF THE BEAN CHALCONE SYNTHASE GENE IN TRANSGENIC TOBACCO BY COMPATIBLE AND INCOMPATIBLE STRAINS OF *PSEUDOMONAS SOLANACEARUM*. Y. Huang and J. H. McBeath. University of Alaska, Plant and Animal Science, Fairbanks, Alaska 99775

Transgenic tobacco plants containing the bean chalcone synthase (CHS8)-ß-glucuronidase (GUS) gene fusion were inoculated with compatible (K60) and incompatible (B1) strains of *P. solanacearum* to study the functional properties of the CHS8 gene during wilt symptom development and the hypersensitive reaction in tobacco. The CHS8 promoter was rapidly but differentially activated by K60 and B1 in transgenic tobacco. The temporal induction pattern established by B1 showed a faster increase rate and higher magnitude of GUS activity than that elicited by K60. Spatial pattern analysis indicated that induction of the CHS8 gene by both K60 and B1 is localized to the bacterial infiltrated area. These results suggest that operation of the bean CHS8 gene in response to microbial infection is conserved in tobacco.

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ISOZYME AND ACTIVITY PATTERNS OF CHITINASES AND β-1,3-GLUCANASES DURING PATHOGENESIS IN TOBACCO LINES RESISTANT AND SUSCEPTIBLE TO BLUE MOLD. T. L. Robertson and S. Tuzun, Department of Plant Pathology, Auburn University, AL 36849-5409.

Tobacco lines resistant (NC-BMR 42, NC-BMR 90, Ovens 62, DH 113, and Chemical Mutant) and susceptible (KY 14) to *Peronospora tabacina*, the causal agent of Blue Mold, differentially accumulate chitinase (CHL) and β-1,3-glucanase (BGL) isozymes in response to pathogenesis. Greenhouse grown plants were sprayed with a $5.4x10^5$ sporangiospore suspension of *P. tabacina* at the 4 - 5 leaf stage. Foliar samples were collected at zero, two, four and seven days after infection (DAI). SDS-PAGE and Western blot analyses showed earlier induction and accumulation of four CHL isozymes and three BGL isozymes in those tobacco lines resistant to *P. tabacina*. The CHL isozymes were constitutively present at low levels in the resistant lines and their levels increased with time, whereas the BGL isozymes accumulated at two and four DAI in the resistant lines. CHL and BGL did not accumulate in the susceptible line until symptom development (7 DAI). Resistant lines had a greater basal level of chitinase activity. The susceptible line, however, did not have increased levels of chitinase activity until seven DAI. The accumulation patterns of CHL and BGL, as well as their enzyme activities, correlate with resistance to *P. tabacina*.

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DIFFERENTIAL REGULATION OF ACIDIC AND BASIC CHITINASE AND β-1,3-GLUCANASE GENES IN COMPATIBLE AND INCOMPATIBLE INTERACTIONS OF TOMATO WITH *ALTERNARIA SOLANI*. C.B. Lawrence and S. Tuzun. Dept. of Plant Pathology, Auburn University, Alabama 36849.

Induction patterns of pathogenesis-related (PR) proteins were investigated during compatible and incompatible interactions of tomato with *Alternaria solani*. Three resistant (R) lines (NC EBR-1, NC EBR-2, and 71B2) and one susceptible (S) variety (Piedmont) were mist inoculated with a conidial suspension of *A. solani*. The induction of at least 12 proteins was observed in both R and S tomato varieties during infection, however, these proteins accumulated earlier in R varieties. Northern blots of total RNA isolated from *A. solani* infected Piedmont or NC EBR-2 plants were examined for the expression of the genes encoding the 26 kD acidic extracellular and 30 kD basic vacuolar tomato chitinases. The genes encoding these isozymes were induced in both R and S varieties but had markedly different expression patterns. In NC EBR-2, the gene encoding the acidic isozyme was induced one day after inoculation and reached maximal induction at two days, whereas in Piedmont, maximal transcript accumulation did not occur until six days post-inoculation and never reached the levels detected in NC EBR-2. The gene encoding the basic isozyme was strongly induced at one day following inoculation, reaching a maximum at two days in NC EBR-2. A similar pattern was observed in Piedmont but the induction level was dramatically reduced when compared to that exhibited by NC EBR-2. The expression pattern of the genes encoding the 33 kD basic vacuolar and 35 kD acidic extracellular β-1,3-glucanases was identical to those observed with the acidic and basic chitinase genes. We suggest that the early and increased expression of basic PR-genes may be a genetically inherited mechanism of early blight resistance.

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A CHITINASE/LYSOZYME mRNA IS INDUCED IN BEAN NODULES FOLLOWING EXPOSURE TO ACETYLENE GAS. A.T. Trese and M. Kassim, Envir. & Plant Biology, Ohio University, Athens, Ohio 45701.

Through the action of nitrogenase, exposure of nitrogen fixing nodules to acetylene gas leads to the production of ethylene. The ethylene produced may initiate a plant defense reaction and induce senescence. We have monitored the level of mRNA encoding a basic chitinase-lysozyme in nodules exposed to 5% acetylene in a flow through gas chamber. In nodules, induction occurs within 3 hours of acetylene treatment, and in leaves within 2 days. Nodules become green (senescent) between 1 and 2 days after treatment. These nodules do not recover when the acetylene gas is removed, in contrast to the reversible, dark induced senescence reported in soybean nodules. We are studying how other defense genes and nodulins respond.

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INDUCTION PATTERNS OF PEROXIDASES IN CRUCIFERS DURING COMPATIBLE AND INCOMPATIBLE INTERACTIONS WITH *XANTHOMONAS CAMPESTRIS* PV. *CAMPESTRIS*. P.A. Gay, K.M. Dodson, T.L. Robertson, C.B. Lawrence, and S. Tuzun, Department of Plant Pathology, Auburn University, Alabama 36849.

Peroxidase isozyme patterns have been studied in two cabbage varieties, Hancock and Perfect Ball, and in *Arabidopsis thaliana* ecotypes, Columbia and Landsberg *erecta*, which are resistant and susceptible to *Xanthomonas campestris* pv. *campestris* (Xcc), respectively. Isoelectric focusing of proteins extracted from cabbage indicated the presence of six peroxidase isozymes, however only one (pI 4.0) is unique to the resistant variety. We detected only one acidic and one basic peroxidase isozyme in *A. thaliana*, whereas other plant species, including cabbage, have multiple isozymes. This may indicate that each peroxidase isozyme in *A. thaliana* has the ability to perform multiple physiological processes, in contrast to greater specificity of different isozymes found in other plant species. We are currently investigating the expression patterns of anionic peroxidases in cabbage and *A. thaliana* upon Xcc infection utilizing Northern blot analyses of mRNA isolated at various times after inoculation.

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VIRULENCE AND ISOZYME VARIATION AMONG HC-TOXIN PRODUCING ISOLATES OF *Bipolaris zeicola*. E. J. Traut, H. L. Warren, and A. W. Way. Dept. of Plant Pathology, Physiology and Weed Science, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061-0331.

Disease reaction on 14 maize inbred lines and isozyme polymorphism of 10 isolates were used to study variability of *Bipolaris zeicola* race 1. Typical symptoms of HC-toxin production on the inbred line Pr were incited by all isolates and observed 2 days after inoculation; however, seven of 10 isolates also induced grayish, irregular, susceptible-type lesions on the inbred line H95, 9 days after inoculation. Aspartate aminotransferase, one of 21 enzymes, differentiated between virulent and avirulent isolates to H95 in the ten isolates examined. These results indicate that two physiological races of *B. zeicola* that produce HC-toxin can be distinguished by differential hosts or electrophoretic phenotypes.

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EXPRESSION OF HMG-CoA REDUCTASE GENES AND ACTIVATION OF LIPOXYGENASE IN POTATO IN RESPONSE TO FUNGAL INFECTION, METHYL JASMONATE, OR THE ELICITOR ARACHIDONIC ACID. Ana L. Fidantsef and Richard M. Bostock, Dept. of Plant Pathology, University of California, Davis, CA 95616.

The expression of different HMG-CoA reductase genes (*hmg*1, *hmg*2, & *hmg*3) and lipoxygenase (LOX) activity was examined in potato leaves following infection by *Phytophthora infestans*, or treatment with the fungal elicitor arachidonic acid (AA) or methyl jasmonate (MJ). Similar to their expression in potato tuber, *hmg*2 and *hmg*3 are induced by AA and *P. infestans*, and the accumulation of transcripts for these genes appears to be more rapid in the incompatible interaction. MJ partially suppressed *hmg*2 expression in leaves, similar to its effect in tuber discs. Unlike the case in tubers, we did not detect expression of any HMGR gene in wounded leaves, nor did we detect transcripts for *hmg*1 following any treatment. LOX activity was induced in leaves following treatment with AA or MJ, but the response to AA was more rapid and stronger. LOX activity also increased within 9-12 hr after inoculation with *P. infestans*, the time shown in a previous study to be required for release of AA from spores into leaf cells. We will discuss the participation of LOX in the signal transduction pathway leading from elicitor treatment to altered isoprenoid metabolism, and report on our progress in the isolation and characterization of potato LOX genes.

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INCORPORATION OF SULFUR FROM CYSTEINE INTO CAMALEXIN, A PHYTOALEXIN FROM *ARABIDOPSIS THALIANA*. Michael Zook[1], Shauna Somerville[2], and Raymond Hammerschmidt[1], [1]Dept. of Botany and Plant Pathology, Michigan State University, East Lansing, MI 48824, [2]Carnegie Institute, Stanford University, Palo Alto, CA 94305.

Inoculation of leaves of *Arabidopsis thaliana* with *Cochlibolus carbonum* elicits the accumulation of camalexin (3-thiazol-2'-yl-indole). It has been hypothesized that some or all of the thiazol ring of camalexin is derived from cysteine. To test this hypothesis, [^{35}S]cysteine or [^{35}S]methionine were fed through petioles of fungal inoculated or noninoculated detached *Arabidopsis* leaves. There was no difference in the incorporation of radioactivity from methionine between inoculated and noninoculated leaves, whereas there was a 4-fold greater incorporation of radioactivity from cysteine into HPLC-purified camalexin from inoculated leaves as compared to noninoculated leaves. Further experiments are in progress to determine whether other portions of the cysteine molecule are incorporated into camalexin.

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ERWINIA CAROTOVORA SUBSP. *CAROTOVORA*: PROTEASE IN SOFT ROT. Verlyn K. Stromberg, George H. Lacy, and Sirkka Kyöstiö. Department of Plant Pathology, Physiology, and Weed Science, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061-0330.

Soft-rot pathogens produce cell-degrading enzymes including proteases. Functions for erwinia proteases in pathogenesis remain controversial. Some workers report that protease damages cell membranes; others find no effect on rot. Potato tubers (*Solanum tuberosum* cv Russett Burbank) inoculated with protease-deficient site replacement mutants of *Erwinia carotovora* subsp. *carotovora* rot less (18-70%, $P \leq 0.01$-0.05) than tubers challenged with the wildtype pathogen. For six experiments, each tuber was inoculated to a depth of 1 cm at 10 sites with the eye of a sewing needle (#7) filled with inoculum scraped from agar. Tubers (18-20/treatment) were incubated for 72 h at 28C in humidified chambers aerated with air bubbled through water (> 100 cm^3/min). Rot was estimated by loss of mass after removal of macerated tissue. Protease production was restored in the protease-deficient mutant by conjugal acquisition of plasmid pSK23, which contains a cloned erwinia gene. Restored mutants were intermediate in rotting ability compared to the protease-deficient mutant and the wildtype. The differences were not consistently significantly different from either the wildtype or the protease-deficient mutant.

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Isolation of mutants of *Erwinia carotovora* subsp. *carotovora* strain 71 (Ecc71) that produce pectate lyase (Pel), polygalacturonase (Peh) and cellulase (Cel) in the absence of plant signals and the quorum sensing signal, N-(3-oxohexanoyl) homoserine lactone (HSL). A. Chatterjee, Y. Liu and A. K. Chatterjee, Univ. of Missouri, Columbia, MO 65211.

We have determined that HSL as well as plant signals are needed for the activation of exoenzyme production in Ecc71. To further analyze the system integrating these signals, we have isolated by ethylmethane sulfonate (EMS) mutagenesis of Ecc71, AepX$^-$ mutants that overproduce Pel, Peh, and Cel in the absence of plant signals. Transcript assays and the use of a *pel-lacZ* operon fusion indicated that the derepression was due to a high basal level of expression of the exoenzyme genes. An AepX$^-$ strain was rendered HSL$^-$ by marker exchanging with *hsl::MudIlacZ*. HSL-deficiency did not affect the production of Pel, Peh and Cel and tissue maceration. Starting with an HSL$^-$ Ecc strain deficient in exoenzymes, we also obtained AepX$^-$-like mutants by EMS mutagenesis. Thus, the AepX$^-$ mutants can function in the absence of HSL as well as plant signals.

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Production of N-(3-oxohexanoyl) homoserine lactone (HSL) by soft-rot *Erwinia* and cloning of *hsl*$^+$ DNA segments of *E. chrysanthemi* (EC16) and *E. carotovora* subsp. *carotovora* strain 71 (Ecc71). A. Chatterjee, Y. Liu and A. K. Chatterjee, Univ. of Missouri, Columbia, MO 65211.

The quorum sensing signal, HSL, has been reported to control exoenzyme production in two Ecc strains. We detected the presence of HSL in cultures of most strains of *E. chrysanthemi and E. carotovora* subspecies. These findings indicate that HSL may be required for the production of exoenzymes in these bacteria as well. From Ecc71 and EC16 we cloned hsl$^+$ DNA segments. The Ecc71 DNA specified HSL production in *E. carotovora* subspecies as well as in *E. amylovora, E. rhapontici, E. coli* and *Pseudomonas fluorescens* that otherwise do not produce HSL. In an HSL$^-$ strain derived from Ecc71 by marker exchange, pectate lyase, cellulase, polygalacturonase, and protease production remained noninducible. The HSL$^-$ strain also did not macerate plant tissues. However, HSL-deficiency had no effect on the activation of pectin lyase and bacteriocin production by DNA-damaging agents.

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IDENTIFICATION OF A GENOMIC REGION THAT AFFECTS BOTH ANTIBIOTIC AND PROTEASE PRODUCTION OF *ERWINIA CAROTOVORA* SUBSP. *BETAVASCULORUM*. J.M. Costa, and J.E. Loper. USDA-ARS HCRL, 3420 NW Orchard Ave., Corvallis, Oregon 97330.

Erwinia carotovora subsp. *betavasculorum* strain Ecb168 causes vascular necrosis and root rot of sugarbeet as well as soft rot of potato. Strain Ecb168 also produces an antibiotic(s) that inhibits other phytopathogenic *Erwinia* spp. Pleiotropic mutants of Ecb168 which do not produce protease or an antibiotic and do not cause soft rot have been described (Rella *et al.* 1989. Appl. Environ. Microbiol. 55:934-939). From a genomic library of Ecb168, we identified four cosmids that shared a common 7-kb *Eco*RI fragment. These cosmids restored the pleiotropic mutants of Ecb168 to antibiotic and protease production. Current research focuses on exploring differential regulation of genes required for antibiotic and enzyme production as well as virulence in *E. c. betavasculorum*.

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Molecular characterization of *rdg* genes required for the activation of pectin lyase (Pnl) production by DNA-damaging agents in *Erwinia carotovora* subsp. *carotovora* strain 71 (Ecc71). Y. Liu, A. Chatterjee, and A. K. Chatterjee, Univ. of Missouri, Columbia, MO 65211.

In Ecc71, production of Pnl is activated by mitomycin C, requiring the functions of *recA* and *rdg* genes (*rdg* = regulator of damage-inducible gene). The *rdg* region contains *rdgA* and *rdgB* as separate transcriptional units. The presence of helix-turn-helix motifs in both RdgA and RdgB indicates that they probably can bind DNA. While RdgA shares significant homology with phage repressors, RdgB has high homology with activators of Mu phage. The putative operators of *rdgA* and *rdgB* share similarity with each other as well as with the consensus operator sequences of phage repressor and bacterial damage-inducible genes. By replacing *rdgB* promoter with the IPTG-inducible *tac* promoter, we have determined that RdgB by itself can activate Pnl production in *E. coli*. However, when driven by their native promoters, functions of both *rdgA* and *rdgB* were required for the activation of Pnl production. We postulate that RdgA modulates the expression of *rdgB*.

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HIGH FREQUENCY TRANSFORMATION OF CLAVIBACTER MICHIGANENSE SUBSP. SEPEDONICUM OBTAINED BY ELECTROPORATION. M. Laine[1], R. Eichenlaub[2] and M. Metzler[1]. [1]Dept. of Biology, U. of Turku, FIN-20500 Turku, Finland; [2]Gentechnologie/Mikrobiologie, Universität Bielefeld, D-4800 Bielefeld 1, Germany.

Clavibacter michiganense subsp. *sepedonicum* (*Cms*), the causal agent of potato ring rot, is poorly characterized on molecular level, and therefore the factors affecting pathogenicity have remained unclear. In order to improve our understanding of how *Cms* colonizes and causes disease symptoms in potato, we have developed an efficient method to transform *Cms* with several cloning vectors, which have been prepared for *C. m.* subsp. *michiganense*. A number of factors are important in determining the efficiency of transformation obtained. Cells grown on plates in the presence of BSA and glucose and harvested in the early phase of growth were easiest to transform. Furthermore, different *Cms* strains had a greatly different transformation efficiency, which suggests the presence of transformation limiting system in some strains. Also the electroporation conditions as well as recovery medium had a significant effect on the efficiency of transformation obtained.

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CHARACTERIZATION OF THE GALACTURONATE PERMEASE GENE (*exuT*) OF *ERWINIA CHRYSANTHEMI* EC16-GENE PRODUCTS AND RELEVANCE. T. Freeman, C. McMullen, M. Melkus and M. San Francisco. Department of Biological Sciences, Texas Tech University, Lubbock TX 79409.

Erwinia chrysanthemi produces enzymes that breach the pectinaceous component of the plant cell wall. The action of these enzymes releases monomers, saturated and unsaturated dimers and oligomers of galacturonic acid as well as 2-keto-3-deoxy gluconate. These compounds are taken up into the bacterium where they are catabolized to yield energy as well as compounds for the induction of pectin degrading enzymes. A 3.4 kb *EcoRV* fragment of EC16 DNA capable of complementing an *exuT* mutant was cloned into pUC19. Transposon Tn*phoA* mutagenesis revealed the existence of two possible transcripts, both of which were required for full complementation of the mutation. At least one Tn*phoA-exuT* fusion yielded a hybrid protein that was localized to the membrane fraction as indicated by immunoblot analysis. Identification of the gene products encoded by the 3.4 kb *EcoRV* fragment was also made using the T-7 RNA Polymerase expression system. The relevance of the galacturonate permease to the disease-causing potential of *E. chrysanthemi* was also studied.

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UPTAKE OF DIGALACTURONIC ACID IN *ERWINIA CHRYSANTHEMI*. M. San Francisco and Z-x. Xiang. Department of Biological Sciences, Texas Tech University, Lubbock, TX 79409.

One of the major products formed as a result of pectinase action on the plant cell wall is digalacturonic acid. We have studied the uptake system for this molecule in *Erwinia chrysanthemi* using [^{3}H]digalacturonic acid. Uptake was inducible with growth on galacturonic acid, digalacturonic acid and a mixture of pectin plus polygalacturonic acid, induction by the monomer being approximately 2.5-fold less compared to the dimer and polymers as substrates. Glycerol-grown cells possessed a basal uptake activity several-fold lower than cells grown on pectin or its derivatives. Uptake of the molecule showed saturation kinetics and was found to be an active, energy-requiring process, being sensitive to dissipators of the proton motive force. Uptake of [^{3}H]digalacturonic acid was competitvely inhibited by unsaturated digalacturonic acid but not galacturonic acid. This suggests that seperate systems exist for the uptake of galacturonic acid and digalacturonic acid.

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PHYLOGENETIC AND PATHOTYPIC ANALYSIS OF BACTERIAL BLIGHT RACE 3. M.R. Finckh, V.M. Luman-ag, and R.J. Nelson. International Rice Research Institute, Manila, Philippines.

To assess the phylogenetic and phenotypic diversity of one race of the bacterial blight pathogen *Xanthomonas oryzae* pv. *oryzae* in the Philippines, 120 strains of Xoo previously classified as race 3 (i.e. strains were virulent on resistance genes *Xa-4, Xa-10,* and *Xa-14,* but avirulent on *xa-5*) were subjected to DNA fingerprinting and virulence analysis. Based on three RFLP probes, 37 distinct haplotypes separating into three distinct phylogenetic lineages (at the 75% similarity level) were identified. Lineages were highly robust based on bootstrap analysis. Two haplotypes were represented by 56 isolates and 23 haplotypes were unique. Within each lineage there was great variability in aggressiveness to the differential cultivars. There were also significant differences in pathogenicity among lineages with respect to the resistance gene *Xa-7*, with members of one lineage pathogenic, one lineage non-pathogenic, and the third lineage showing mixed reactions. The aggressiveness of two of the three lineages was significantly affected by the testing date, suggesting differential host*isolate*environment interactions.

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A SIMPLE AND RELIABLE METHOD TO TEST THE PATHOGENICITY OF XANTHOMONAS ORYZAE IN RICE SEEDLINGS. N. W. Schaad, Z. K. Wang, G. L. Peterson, and M. R. Bonde. USDA, ARS, Frederick, MD 21702.

Rice plants and seeds often contain numerous yellow-pigmented xanthomonas-like bacteria. During pathogenicity testing of known strains by the standard leaf clipping technique with 10^{8-9} cfu/ml (Kauffman et al., 1970, Pl. Dis. Rept. 57:537-541), we observed inconsistent results. Normally a hypersensitive-like reaction preceded a general yellowing or browning of the tissue. The pathogen was seldom recovered from such lesions. We never observed water-soaked (ws) lesions, even in a lighted dew chamber (100% RH). Using a modified Hagbord infiltration method (Hagbord, 1970, Can. J. Bot. 48:1135-1136) and an inoculum of 10^{6-7} cfu/ml, ws lesions developed on leaves of 4- to 5-leaf stage plants after 7-10 days in the dew chamber (22 C night, 29 C day). Exudate was usually evident. Four strains of X. oryzae pv oryzae (XOO) and one X. o. pv oryzicola from China, three XOO strains (X1-5, X1-8, and X37-2) from Texas, and two yellow bacteria isolated from California rice seeds were tested by both methods. All produced general yellow-brown lesions

by clipping. All strains from China produced ws lesions by infiltration whereas the Texas and yellow bacteria did not. This simple infiltration method should be useful for determination of the pathogenicity of X. oryzae.

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DEOXYRIBONUCLEIC ACID RELATEDNESS AMONG SEVERAL RICE XANTHOMONADS. Z. K. Wang, G. L. Peterson, M. R. Bonde, and N. W. Schaad, USDA, ARS, Frederick, MD 21702.

DNA-DNA hybridization (hydroxyapatite method of Brenner et al., 1982, J. Clin. Microbiol. 15:1133-1140) was used to determine relatedness among several strains of Xanthomonas oryzae pv oryzae (XOO) and X. leersia (XL) from China, XOO from Texas, and two yellow pigmented bacteria from rice seed. XOO strain OS-13 from China was labeled with (^{32}P) dCTP by nick translation. Hybridization reactions were performed at 65 C for 21 hr. All reactions were done three times. Divergence (D) values were calculated according to Brenner. OS-13 was 73, 88, and 94% related to XOO strains HN84-31, NX-19, and OS-86; and 87 and 88% to X. leesia strains 89-84 and 88-84, respectively. The D values were 1, 2, 1, 2, and 2, respectively. XOO strains X1-8, X1-5, and X37-2 from Texas were 76, 24, and 60% related to OS-13 and had D values of 1, 4, and 9, respectively. Yellow bacteria 6-3 and 5-3 were 36 and 21% related to OS-13 with D values of 17 and 30. All strains were pathogenic using leaf infiltration except the yellow bacteria and XOO strains from Texas. These data suggest that the strains of XOO and XL from China are a single species whereas the Texas strains are in one or more species.

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VARIATION AMONG EIGHT MARYLAND ISOLATES OF *CLAVIBACTER XYLI* SUBSP. *CYNODONTIS*. R. H. Ford and A. B. Swain. Biology Dept., Washington College, Chestertown, MD 21620.

Isolates of *Clavibacter xyli* subsp. *cynodontis* (CxC), an endophyte of bermudagrass *(Cynodon dactylon)*, were collected from three counties on Maryland's Eastern Shore. Colonized plants from golf greens and roadsides were identified by culturing the bacterium on an enriched medium since no characteristic symptoms are produced. The isolates varied in the shape of the cell, the mean length of the rod, and the presence of the 50-kilobase cryptic plasmid. One isolate was predominantly a coccus with an attached rod. Three groups were identified based on bacteriocin activity.

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CHARACTERIZATION OF *XANTHOMONAS FRAGARIAE* BY FATTY ACID METHYL ESTER AND RESTRICTION FRAGMENT LENGTH POLYMORPHISM ANALYSES. P. D. Roberts, N. C. Hodge, J. B. Jones, R. E. Stall, and R. D. Berger. Plant Pathology Department, University of Florida, Gainesville, 32611.

Strains of *Xanthomonas fragariae* (Xf), casual agent of angular leaf spot of strawberry, were examined for relatedness using fatty acid methyl ester (FAME) and restriction fragment length polymorphism (RFLP) analyses. A dendrogram based on FAME profiles of 67 strains from North America divided the population into at least five quantitatively different clusters. One cluster contained the type strain and three closely related strains that were qualitatively differentiated by the absence of one acid, 16:1 ω7c. Xf could be distinguished from *Xanthomonas campestris* pathovars by the high 15:0 iso and the low 15:0 anteiso percentages in Xf FAME profiles. Genetic diversity of 47 strains was examined using infrequent cutting restriction endonuclease analysis of total genomic DNA. At least four distinct RFLP patterns (groups A-D) were identified and similarity coefficients were calculated by scoring for the presence/absence of bands. Groups A, B, C, and D contained 2, 80, 13, and 5 % of strains, respectively. Based upon these results, there appears to be considerable diversity within the species.

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SELECTIVE ENHANCEMENT OF BACTERIAL POPULATION SIZE IN THE PHYLLOSPHERE USING THE CARBON SOURCE SALICYLATE. M. Wilson[1], and S. E. Lindow[2]. Dept. of Plant Pathology, Auburn University, Auburn AL 36849[1]. Dept. of Environmental Science, Policy and Management, University of California, Berkeley, CA 94720[2].

Epiphytic bacterial populations in the phyllosphere are limited by carbon availability. The plasmid NAH7 confers the ability to catabolize salicylate, a carbon source which is utilized infrequently by epiphytic bacterial populations. The epiphytic population size of inoculated *Pseudomonas putida* R20(pNAH7) [Colbert *et al.* Appl. Environ. Microbiol. 59:2071-2076.] was increased by up to 11-fold through the exogenous application of 1.2 g/l salicylate to bean leaves. The growth rate of *P. putida* R20(pNAH7) was not significantly increased by the addition of salicylate. The plasmid NAH7 was mobilized into *Pseudomonas fluorescens* strain A506, a biocontrol agent of frost injury, fire blight and some post-harvest diseases. The epiphytic population size of inoculated *P. fluorescens* A506(pNAH7) was increased by more than 6-fold through the exogenous application of salicylate to bean leaves. Exogenous application of the carbon source salicylate, which increased the carrying capacity of the leaf for the salicylate-catabolizing strains by provision of a novel catabolic niche, may be a useful way to selectively enhance the population size of epiphytic biocontrol agents.

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ANALYSIS OF *XANTHOMONAS CAMPESTRIS* PV. *VESICATORIA* STRAINS FROM THE CARIBBEAN BASIN. H. Bouzar, J. B. Jones, R. E. Stall, G. C. Somodi, R. O. Kelly, N. Daouzli, F. J. Louws, F. J. de Bruijn, and M. Schneider. University of Florida, 5007 60th St. E., Bradenton 34203.

Strains of *X. c. vesicatoria* (Xcv) were isolated from tomato and pepper plants grown in production fields of the Barbados, Costa Rica, Guadeloupe, Guatemala, Nicaragua, Puerto Rico, and the U.S. Virgin Islands. Of 120 strains, 104 were affiliated to Xcv group A (i.e., 32 kDa α protein band, A serovars, *cis*-aconitate positive, nonamylolytic and nonpectolytic). Most of the A strains were pathogenic on both pepper and tomato. Five strains (1 from Costa Rica and 4 from Guatemala) were typed to Xcv group B (i.e., 27 kDa β protein band, B serovars, *cis*-aconitate negative, amylolytic and pectolytic). Two of the B strains were pathogenic on tomato but not on pepper, and three were not pathogenic on the plants used for pathogenicity testing. Eleven strains could not be assigned to either Xcv group. Four of these strains, found throughout a pepper field in the Barbados, were similar to group A strains in their antigenic make-up and their ability to utilize *cis*-aconitate, but were amylolytic and pectolytic like group B strains. The reverse was true for 7 strains recovered from two tomato fields in Costa Rica. DNA profiles obtained by PCR amplification using primers to reptitive elements (REP, ERIC, BOX) corroborated the phenotypic analysis.

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DROUGHT EFFECTS ON BACTERIAL RING ROT OF POTATO. K.L. Crabtree and M.L. Powelson, Oregon State University, Corvallis.

Population size of Clavibacter michiganensis subsp. sepedonicus in potato cv Russet Burbank and plant response as affected by drought were assessed in a greenhouse experiment. Treatments of drought and no drought, and inoculum densities of 0 or 2 X 10^7 cfu C. m. sepedonicus/seed-piece were arranged factorially. Compared to the non-inoculated control, inoculum reduced aerial biomass from 18 to 58% and tuber yield from 36 to 126% in samples taken four times post-drought. Drought reduced these same variables from 8 to 53% and 43 to 186%, respectively compared to the non-stressed control. Both number of tubers with symptoms of bacterial ring rot and stem populations of C. m. sepedonicus were significantly higher in the no drought treatment compared to the drought treatment. Effects of drought and inoculum on tuber yield and aerial biomass of potato are similar.

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ISOLATION, IDENTIFICATION AND CHARACTERIZATION OF OHIO STRAINS OF *XANTHOMONAS CAMPESTRIS PV. VESICATORIA (Xcv)*, CAUSUAL AGENT OF BACTERIAL SPOT OF PEPPER. F. Sahin and S. A. Miller, Dept. of Plant Path., OARDC, Ohio State University, Wooster, OH 44691.

In 1993, 70 samples from pepper (*Capsicum annuum L.*) plants exhibiting symptoms of bacterial spot were collected from commercial fields and home gardens throughout Ohio. Bacteria were isolated on semi selective medium from infected plants and identified by a combination of biochemical tests and gas

chromatographic analysis of phospholipid fatty acids. Thirty eight strains caused a hypersensitive reaction on tobacco (*Nicotiana tabacum cv. Samsun*) and were virulent on the Xcv-susceptible pepper variety, Marengo. Strains were identified to race based on the response to infection of a set of near-isogenic pepper lines derived from and including Early Calwonder (ECW): ECW-10R, ECW-20R, and ECW-30R containing the resistance genes bs1, Bs1, Bs2 and Bs3, respectively. The majority of the strains (55%) were identified as race 3, whereas 15% were race 1, 16% were race 2, and the remaining 14% could not be identified to race using these differential pepper lines.

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GREATER EPIDEMIOLOGICAL FITNESS OF *XANTHOMONAS CAMPESTRIS* PV. *CAMPESTRIS* BLIGHT STRAINS IN THE SEEDBED. T. Shigaki, S.C. Nelson, and A.M. Alvarez. Department of Plant Pathology, University of Hawaii at Manoa, 3190 Maile Way, Honolulu, HI 96822.

Previous data suggested that *Xanthomonas campestris* pv. *campestris* typical black rot (BR) and blight (BL) strains differ in attributes of epidemiological fitness (Phytopathology 83:1405). To assess this conclusion, additional strains from BR and BL groups were compared for latent spread from introduced disease foci in two runs of an experiment in misted seedbeds. Samples from leaf wash collected from each plant were incubated on a selective medium in separate wells of microtiter plates and examined by ELISA with monoclonal antibodies that distinguished strains of the pathogen. In run one (avg. temp. 29.3°C), incidence of BL strains 14 days after inoculation was higher (19.1, 9.8, and 8.7% for strains G2-17, A3263, and G2-12, respectively) than for BR strain (6.0, 3.3, and 1.1% for GAC17, A249, and GAC137). In run two (avg. temp. 26.4°C), incidence was 14.7, 12.0, 10.9, 14.7, 7.6, and 8.7% (in the same order as in run one). Spatial analysis of the final incidence indicated that BL strains spread farther than BR strains. The data suggest greater epidemiological fitness of BL strains.

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ULTRASTRUCTURE OF *IN-VITRO* CULTURED POTATO TUBERS INOCULATED WITH THAXTOMIN A. B. Stein[1], R. King[2], and R. Hammerschmidt[1], [1]Dept. of Botany and Plant Pathology, Michigan State University, East Lansing, MI 48824, [2]Research Station, Agriculture Canada, P.O Box 20280, Fredericton, NB, Canada E3B 4Z7.

Streptomyces scabies is the cause of common scab on potato tubers. Symptoms of common scab, such as formation of brown lesions, were found to occur in advance of the actual colonization of tissue. These observations led to the isolation of the toxin thaxtomin (Lawrence *et al.* 1990, Phytopathology 80:606). Thaxtomin was shown to reproduce the visible symptoms of infection by *S. scabies* on *in vitro* cultured potato mini-tubers which are grown aseptically on White's media supplemented with 8% sucrose with 0.4% agar. Cells in inoculated tissue were collapsed and very similar in appearance to the cells of tissue infected with *S. scabies*. These findings suggest that *S. scabies* multiplies in tissue which was killed by thaxtomin before infection had occurred.

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MOLECULAR MARKERS FOR DEFENSE RESPONSE GENES IN SORGHUM. Yunxing Cui, Clint Magill, Jane Magill and Richard Frederiksen. Texas A&M University, College Station, TX 77843-2132.

Defense related genes have been cloned from a variety of plant species. By comparing sequences from distantly related species, we identified short, highly conserved sequences that flank less conserved regions of several of these genes. Primers specific for these regions were used with PCR to amplify segments of sorghum DNA that were expected to code for PAL, chitinase (CHT), glucanase (GLU), HMGCoA reductase (HMG), chalcone synthase (CHS), glutamine synthetase (GS) and dihydrofolate reductase (DFR). From amplified fragments inserted into pUC18, we have obtained four putative cloned PAL fragments, three CHS, two CHT, two GS and one HMG. Two (500 bp PAL and 600 bp CHS) of the six clones that have been sequenced were confirmed by showing >70% sequence homology within the coding region to the equivalent genes from other species. The other clones show <30% homology so may include introns or originate from pseudogenes. The confirmed PAL and CHS clones have been used as probes to locate the respective genes on a sorghum RFLP map.

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

The antimicrobial activities of the alfalfa (*Medicago sativa* L.) phytoalexin (-) medicarpin, its stereoisomer (+) medicarpin from peanut (*Arachis hypogaea*), (-)6a-hydroxymedicarpin, and 3-O-methyl, 6a-hydroxymedicarpin ((-) homopisatin) from lentil (*Lens culinaris*) were tested against a variety of phytopathogenic fungi using an agar plate assay. (+) Medicarpin and (-) homopisatin inhibit the linear mycelial growth of a variety of alfalfa fungal pathogens as well or better than the naturally occurring alfalfa phytoalexin (-) medicarpin. These experimental results will be useful in determining future goals of our program involving the genetic manipulation of the alfalfa phytoalexin pathway.

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ACCUMULATION OF CAMALEXIN IN SPORE DIFFUSATES ON LEAVES OF *ARABIDOPSIS THALIANA* INOCULATED WITH *COCHLIOBOLUS CARBONUM*. Michael Zook[1], Samantha Teplitzky[1], Shauna Somerville[2], and Raymond Hammerschmidt[1], [1]Dept. of Botany and Plant Pathology, Michigan State University, East Lansing, MI 48824, [2]Carnegie Institute, Stanford University, Palo Alto, CA 94305.

Arabidopsis thaliana produces the indole-derivative phytoalexin camalexin (3-thiazol-2'-yl-indole). Inoculation of leaves of *Arabidopsis* with *Cochliobolus carbonum*, *Pseudomonas syringae* pv. *syringae*, or treatment with silver nitrate elicits camalexin accumulation. Of these three treatments, inoculation with *C. carbonum* elicits the highest levels of camalexin accumulation. Camalexin also was found to accumulate in spore droplets of *C. carbonum* on leaves of *Arabidopsis*. The use of spore diffusates has greatly simplified the quantitation of camalexin accumulation in *Arabidopsis*.

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PR PROTEIN ACTIVITY IN CITRUS ROOT XYLEM FLUID CONTAINING FUSARIUM SOLANI NAPHTHAZARIN TOXINS. S. Nemec, USDA-ARS, SAA, U.S. Horticultural Research Laboratory, 2120 Camden Rd., Orlando, FL 32803.

Xylem fluid of scaffold roots from apparently healthy and blight-diseased citrus trees was analyzed for F. solani naphthazarin toxins by ELISA and for chitinase and β-1, 3-glucanase using antibody probes of Western blots. β-1,3-glucanase was present in 44% and 38% of healthy and blight tree roots, respectively, containing 0-200 ng · ml^{-1} toxin; and 79% to 94% of healthy and blight tree roots, respectively, containing 7,500 ng · ml^{-1} toxin. Chitinases were present in 75% of healthy and blight tree roots containing 0-200 ng · ml^{-1} toxin; and 85% of healthy and blight roots containing 7,500 ng · ml^{-1} toxin. Up to 4 bands per root were β-1, 3-glucanase positive and up to 5 per root were chitinase positive; some specificity occurred between toxin conc. but not between tree health. Three chitosanases, identified by activity staining, had kDs of ~ 55, 45, and 32 where chitinases were detected and thus may possess both activities. The most frequent chitosanase of ~ 8-12 kD was associated more with high toxin-containing roots, but was not the same 12 kD protein unique to blight roots (Plant Dis. 74:168-170). PR proteins account for some of the increase in proteins in blighted trees because they contain more toxin.

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DEVELOPMENT OF DELIVERY SYSTEMS FOR INTRODUCING ENDOPHYTIC BACTERIA INTO COTTON. G. Musson and J. W. Kloepper. Dept. of Plant Pathology, Biological Control Institute, Alabama Agric. Exp. Sta., Auburn University, AL 36849-5409.

As new bacterial strains with plant growth-promoting or biological control activity are identified or genetically constructed, the need for methods to deliver these strains to plants will increase. Because some of these strains colonize plants internally, methods to deliver

endophytic bacteria will be necessary. Rifampicin-resistant mutants (100 μ/ml) of 15 endophytic bacteria, 6 of which were shown to reduce symptom expression of Fusarium wilt in a biocontrol screen, were applied to cotton using 5 methods. These methods, chosen from preliminary testing, were: seed treatment with bacteria suspended in 2% methyl cellulose (MC), in-furrow application of vermiculite granules coated with bacteria in MC, a 2-hr seed-soak in bacteria suspended in 0.2% silwet, a foliar spray after emergence of bacteria suspended in buffer with 0.2% silwet, and stab-inoculation into stems of 7-day old cotton. Samples were taken from internal tissues of stem and root after 2 wks and were processed in 24-well microtiter plates filled with sterilant (clorox), buffer (for rinsing), tryptic soy broth (TSB) (as a sterility check), and rif-TSB (for detecting internal colonists). Stab-inoculation delivered most bacterial strains into plants. Using the other methods, analysis of variance indicated significant effects of strains (P=0.0001) and method*strain (P=0.0557) but not method. These results suggest that the optimum delivery system for endophytes will be strain-specific.

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BIOLOGICAL CONTROL OF ANTIBIOTIC-RESISTANT MUTANTS OF *STREPTOMYCES SCABIES* THAT CAUSE POTATO SCAB. E.C. Eckwall[1], J.L. Schottel[1] and N.A. Anderson[2], [1]Dept. of Biochemistry and [2]Dept. of Plant Pathology, Univ. of Minn., St. Paul, MN 55108.

Control of potato scab disease has been demonstrated in greenhouse and field tests by suppressive *Streptomyces* isolates PonSSII and PonR. These strains produce antibiotics against pathogenic *S. scabies* in *in vitro* assays. Antibiotic production assays on two different media (R2YE, NMM) suggested that strain PonR produced one antibiotic and PonSSII produced at least two inhibitory compounds. To study the role of antibiotics in scab suppression, several spontaneous mutants of *S. scabies* resistant to the inhibitory compounds produced by the suppressive strains were isolated. Two mutant *S. scabies* strains, M4 and M9, were resistant to the antibiotic produced by suppressive strain PonR and to one of the antibiotics produced by PonSSII. These mutants retained their pathogenicity and antibiotic resistance after inoculation and reisolation from lesions on potato minitubers cv Pontiac. Coinoculations of Pontiac minitubers were made with one of the suppressive strains (PonR or PonSSII) and with one of the pathogenic strains (RB4, M4, or M9). Significant biocontrol of the antibiotic-resistant pathogens and the wild type pathogenic strain was observed. These results suggest that either more than one antibiotic is involved in suppression or that competitive interactions are also important in biological control of potato scab.

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A NON-FLUORESCENT PSEUDOMONAS SP. ANTAGONISTIC TO BROWN PATCH. L. J. Giesler, and G. Y. Yuen. Department of Plant Pathology, University of Nebraska-Lincoln, Lincoln, NE 68583-0722.

Bacterial antagonists effective against brown patch disease caused by *Rhizoctonia solani* have not been reported previously. We identified a non-fluorescent *Pseudomonas* sp. (C3) as a potential bacterial antagonist of *R. solani* for use in turfgrass. In two bioassays, one involving grass blades in petri dishes and the other involving seedlings in a growth chamber, C3 exhibited the highest level of antagonism to *R. solani* out of 160 bacterial isolates. In a 1-yr.-old sward of tall fescue 'Fawn', C3 consistently reduced brown patch lesion development (P=0.05). In 2-yr.-old swards of tall fescue 'Fawn', 'El Dorado', and 'Emperor', C3 consistently inhibited *R. solani* in 'El Dorado' (P=0.05), but did not suppress disease in 'Emperor' or 'Fawn'. Disease ratings in 'El Dorado' C3-treated plots ranged from 2.8 to 4.7 on a 0-10 scale as compared to ratings of 3.8 to 5.2 in *R. solani* inoculated control plots. C3 maintained stable foliage populations of 6 log cfu/g tissue throughout field experiments; however, on grass blades in the laboratory, 9 log cfu/g was needed for antagonism, thus explaining the low level of field efficacy. Results show the need for a screening system which identifies bacterial antagonist effective at field levels.

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BIOLOGICAL CONTROL OF PYTHIUM GRAMINICOLA AND OTHER SOIL-BORNE PATHOGENS OF TURFGRASS WITH ACTINOMYCETES FROM COMPOSTS. C.A.Stockwell, E.B.Nelson, and C.M.Craft, Dept. of Plant Pathology, Cornell University, Ithaca, NY 14853

Several composts, which were included in both bioassays and field trials, were found to be suppressive to Pythium graminicola on creeping bentgrass (Agrostis palustris). This study was started to determine the the role of the actinomycetes present in these composts in this suppression. Of 104 strains of actinomycetes isolated from a variety of composts, 85% were found to suppress seed rot and damping-off caused by Pythium graminicola in bioassays. Eight consistently-suppressive strains were included in a field trial in which actinomycete-infested compost-amended sand was applied as a monthly top-dressing. No strain gave significant control of Pythium root rot or Brown patch, while 5 strains were shown to significantly control Dollar spot. These results illustrate both the potential and the limitations of using compost-inhabiting actinomycetes for biocontrol of soil-borne diseases of turfgrass.

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EFFICACY OF Pythium oligandrum IN FIELD EVALUATIONS FOR CONTROLLING DAMPING-OFF OF TOMATO TRANSPLANTS. **Frank N. Martin and C. R. Semer IV, Plant Pathology Department, University of Florida, Gainesville, FL 32611**

The efficacy of isolates of P. oligandrum in protecting tomato transplants from damping-off caused by P. aphanidermatum, P. irregulare, and P. ultimum was evaluated in field trials. When disease pressure ranged from 37 to 48% death, some isolates provided identical levels of disease control for six planting seasons as obtained with metalaxyl treatments. This was equivalent to a reduction of disease incidence of between 97 to 60%. Not all isolates were efficacious, with several providing control inconsistently or for a specific season. Control of damping-off was not evident with either fungicide or biocontrol treatment when the disease pressure was 85% transplant death. Aspects of the dynamics of root colonization by the biocontrol agent and its influence on host growth parameters will be discussed.

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ANALYSIS OF INFECTION COMPONENTS ON SEVERAL MUTANT AND WILD TYPE ISOLATES OF *Colletotrichum gloeosporiodes* f. sp. *aeschynomene*. **Y.Luo & D.O.TeBeest, Department of Plant Pathology, University of Arkansas, Fayetteville. AR 72701**

Infection components including spore germination, latent period, number of lesions, lesion expansion rate and sporulation of *Colletotrichum gloeosporiodes* f. sp. *aeschynomene*, causing anthracnose of northern jointvetch (NJV), *Aeschynomene virginica* (L.,) BSP., were measured in experiments conducted in growth chambers. Eight mutant isolates, four Benlate resistant isolates (B13, B15, B18 and B21) and four nitrate-nonutilization isolates (Na, Nr, Nl and Nt), were compared with two wild type isolates (CLA and 3.1.3). There was no significant difference between wild types and mutant isolates in the proportion of appresoria 24 hr. after inoculation. Wild types had shorter latent periods compared with all Benlate resistant isolates, but there was no difference between wild types and all Nit isolates except Nr. The average latent period for Benlate resistant isolates was 5.2 days compared with 4.3 days for wild types. Lesion expansion rates for all four Benlate resistant isolates are slower than those on wild types. The range of lesion expansion rate of Benlate resistant isolates was 0.9 - 2.9 mm^2/day compared with 6.8 - 8.0 mm^2/day for wild types and 3.2 -5.8 mm^2/day for Nit isolates. The number of lesions produced by all Nit isolates were significantly fewer than those of wild types but there was no difference between Benlate resistance and wild type isolates. High deviations for sporulation existed for all isolates.

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THE USE OF MEDIA TO ENHANCE BIOPROTECTANT ABILITY TO REDUCE FUNGAL STAINING OF WOOD PRODUCTS. **R. K. Velicheti and J. J. Morrell, Department of Forest Products, Oregon State University, Corvallis, OR 97331-7402.**

Bioprotectants, Bacillus subtilis 733a (Bs) and Pseudomonas fluorescens Pf5 (Pf5) inhibited growth of selected sapstain fungi on potato dextrose agar. The use of these rhizosphere bacteria to control fungal staining of wood is limited due to their poor growth on wood-products. The effect of adding nutrients with inocula of Bs and Pf5 to wood wafers to enhance bacterial growth and control sapstain fungi was evaluated. Bs and Pf5 isolates were grown in defined minimal salts media containing 5 level glucose (0 - 0.4 % for Bs) or glycerol (0 - 12.4 % for Pf5) at 4 pH levels (4, 6, 8, 10). Sapstain reduction was evaluated on ponderosa pine sapwood wafers (30 x 15 x 5 mm) dipped in non-inoculated media or 2- or 7-day-old cultures of Bs or Pf5. Wafers were then sprayed with spores and/or mycelial fragments of sapstain fungi and incubated in moist chambers at 24 +/- 2 C till the control wafers were completely stained. Colonization of bacteria

were recorded at different intervals. Growth media had a profound effect on the performance of bioprotectants, suggesting that careful selection of media can be useful in overcoming the difficulty of using non-wood inhabiting bacteria as bioprotectants on wood.

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EFFECTS OF SOIL SOLARIZATION, CHICKEN LITTER, RIVAL SEED TREATMENT, AND *BACILLUS SUBTILIS* ON STAND ESTABLISHMENT AND SEEDLING DISEASES OF LUPIN. D. J. Collins[1,3], C. Stevens[2], V. Khan[2], and S. Nightengale[3]. [1]Dept. of Plant Pathology, [3]Ala. Agric. Exp. Sta., Auburn University, AL 36849, and [2]GWC Agric. Exp. Sta., Tuskegee University, AL 36088.

White Lupin (*Lupinus albus* L.) are being evaluated as an alternative winter grain/ forage crop for Alabama. Disease surveys have shown Lupin seedling emergence and stands to be reduced by root and hypocotyl rots. Fungi isolated from diseased tissue have been *Rhizoctonia solani, Fusarium* spp., *Pleiochateta setosa*, and *Macrophomina phaseolina*. We evaluated soil solarization alone or in combination with chicken litter, Rival® seed treatment (Captan, PCNB, and Thiabendazole), *Bacillus subtilis* (Gustafson Incorporated, Plano, Texas) for stand establishment and control of seedling diseases of lupin. Final plant stands were significantly higher in all solarized (S) plots compared to the nonsolarized (NS) plots (i.e. 96 plants for S plots vs 69 for NS control plots). The *B. subtilis* treatments had significantly lower root and hypocotyl rot ratings when used alone or in combination with the Rival® seed treatment in both S and NS plots. Chicken litter produced lower disease ratings only in S plots.

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BIOCONTROL OF POTATO SCAB IN FIELD TESTS. D. Liu, N.A. Anderson, L.L. Kinkel. Department of Plant Pathology, University of Minnesota, St. Paul, MN 55108, USA.

Two nonpathogenic strains of *Streptomyces* (PonR, PonSSII) that produced bacteriocin-like reactions against virulent strains of *S. scabies* in-vitro, and that showed biocontrol potential in 4-yr field-pot tests were inoculated into a scab-conducive field (0.8 ha). Inoculum of the suppressive strains was grown on vermiculite plus oatmeal broth. In 1991, PonR and PonSSII were introduced singly or in combination at 15 or 45 cc per seed tuber or as a tuber dip treatment. In 1992, PonR and PonSSII were reinoculated into half of the plots receiving 15 or 45 cc inoculum in 1991, and three additional suppressive strains of *Streptomyces* (15, 32, 93) were inoculated at 45 cc per seed tuber. Also sweet corn and soybean rotation treatments were added to the experiment. In 1991, PonSSII (45cc) significantly reduced the number of scab lesions per tuber. In 1992, disease was significantly reduced by the 2-yr inoculation of PonR (15 or 45 cc), PonSSII (15 or 45 cc) and 1-yr inoculation of PonSSII (45 cc) and strains 15 and 93 (45 cc). In 1993, disease was significantly reduced by the 2-yr inoculation of PonSSII (15 or 45 cc), the 1-yr inoculation of PonSSII and strain 93 (45 cc), and the sweet corn and soybean treatments. Application of suppressive *Streptomyces* strains and rotation crop treatments contributed to scab decline in field tests.

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INFLUENCE OF INOCULUM PREPARATION ON ESTABLISHMENT OF BACTERIAL ANTAGONISTS ON PEAR AND APPLE BLOSSOMS. V. O. Stockwell[1], K. B. Johnson[2], and J. E. Loper[1], [1] USDA-ARS, Horticultural Crops Research Lab., and [2] Dept. of Botany and Plant Pathology, Oregon State Univ., Corvallis, OR, 97331.

Establishment of bacterial antagonists, *Pseudomonas fluorescens* A506 and *Erwinia herbicola* C9-1, on pear blossoms reduced incidence of fire blight by 50-80% in field trials in Oregon. Inocula of these antagonists were produced on nutrient agar containing 1% glycerol. Aqueous suspensions (10^8 cfu/ml) of A506 or C9-1 were made from freshly harvested or freeze-dried cells and sprayed on Bosc pear, Rome apple, and Golden Delicious apple trees in 50% bloom. Populations and incidence of recovery of A506 and C9-1 on stigmas of individual blossoms were estimated over several days. Fluorescent microspheres (1 μm) were added to sprays to confirm that lack of detection of antagonists in blossoms was due to their inability to establish rather than the escape of some blossoms from treatment. Fresh and freeze-dried cells of A506 and C9-1 achieved populations of 10^5 cfu/blossom. The percentage of blossoms with detectable populations of C9-1 (>60% on pear and 100% on apples) was similar with the two preparations. On pear and Rome apple, A506 was recovered from a significantly greater proportion of blossoms when applied as freeze-dried cells. The use of freeze-dried cells may decrease variability in establishment of bacterial antagonists on blossoms and thus result in more uniform disease control.

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PERFORMANCE OF ANTAGONIST MIXTURES AND CONCENTRATION EFFECTS OF BACTERIAL STRAINS ACTIVE AGAINST *FUSARIUM SAMBUCINUM*. D.A. Schisler, P.J. Slininger, R.J. Bothast. USDA-ARS, National Center for Agricultural Utilization Research, Peoria, IL, USA 61604.

Fusarium dry rot control in potato storages has become more burdensome with the emergence of thiabendazole-resistant strains of *Fusarium*. Biological control of dry rot incited by *F. sambucinum* (teleomorph=*Gibberella pulicaris*) is feasible using gram negative bacteria (Schisler and Slininger, 1994, Plt. Dis. 78:251-255). Eighteen bacterial strains were individually assayed against *F. sambucinum* ($5x10^5$ conidia/ml) by coinoculating antagonist and pathogen into wounds in Russet Burbank potatoes. All antagonist concentrations (10^8, 10^7 and 10^6 cfu/ml) decreased disease (30-85% vs control, $P<0.01$). When 4 strains were assayed at 11 concentrations (range: 10^5-10^8 cfu/ml), dry rot severity decreased as antagonist concentration increased (log diseased tissue(mm)=2.3-0.18*(log antagonist cfu/ml); $P<0.01$, $R^2=0.17$). All possible pairings within two sets of 10 antagonist strains (5 x 10^5 cfu/ml of each strain) resulted in 16 of 90 pairs controlling disease better than predicted from averaging the performance of individual strains making up the pair ($P\leq0.10$). Two pairs performed worse than predicted. Successful pairs reduced disease by 70% vs controls. Strain genus or soil of origin were not useful in predicting successful pairings.

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ASSAY CONDITIONS AND RHIZOSPHERE EFFECTS ON THE ISOLATION OF ANTAGONISTIC BACTERIA FROM SOILS. R. G. Linderman, J. L. Marlow, and E. A. Davis. USDA-ARS Horticultural Crops Research Laboratory, Corvallis, OR 97330.

The effect of time of incubation of soil dilution plates in an antagonism assay on the number and percentage of bacteria that inhibited several test root pathogens, including *Thielaviopsis basicola, Cylindrocladium scoparium, Pythium irregulare, Phytophthora cinnamomi*, and *Sclerotium cepivorum* was evaluated. Isolations were made on 10% TSA dilution plates from rhizosphere/rhizoplane soils from snapdragon or onion roots, compared to non-rhizosphere soil. All colonies were removed from dilution plates as they appeared at 24, 48, 72, 96, or 120 hrs of incubation at 20 C. Then all colonies were tested for antagonism against each pathogen at 20 C on a nutrient-weak soil-extract agar amended with 4 g ground dry snapdragon root/L (SESDA). The greatest percentage of antagonists occurred at the 72 hr isolation. A greater proportion of antagonists was recovered from rhizosphere soil than from non-rhizosphere soil, and many of those bacteria were no longer antagonistic when tested at 10 C on SESDA. Bacteria from onion rhizosphere soil, as a group, were more antagonistic against the onion pathogen *Sclerotium cepivorum* than those from snapdragon rhizosphere soil. These data indicate that roots selectively enrich for antagonists from the soil, and that early-appearing bacteria are much less antagonistic than those that appear later.

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INFLUENCE OF TEMPERATURE ON PRODUCTION OF HYDROGEN CYANIDE AND 2,4-DIACETYLPHLOROGLUCINOL, AND ON INHIBITION OF *GAEUMANNOMYCES GRAMINIS* VAR. *TRITICI* BY *PSEUDOMONAS AUREOFACIENS* Q2-87. R.B. Reeder and B.H. Ownley. Entomology and Plant Pathology Dept., The University of Tennessee, Knoxville, TN 37996.

Pseudomonas aureofaciens Q2-87 produces 2,4-diacetylphloroglucinol (Phl) and hydrogen cyanide (HCN). Strain Q2-87, applied as a seed treatment, suppresses take-all of wheat caused by *Gaeumannomyces graminis* var. *tritici* (*Ggt*) primarily by production of Phl. The role of HCN has not yet been determined. To optimize the efficacy of Q2-87, environmental factors that influence its suppressiveness must be characterized. The purpose of this study was to determine the effect of temperature on production of Phl and HCN, and on *in vitro* inhibition of *Ggt* by Q2-87 (Phl^+HCN^+) and a Phl^-HCN^+ mutant, Q2-87::Tn*5*. Production of HCN and Phl, and *in vitro* inhibition by the strains were determined after incubation on King Medium B + 0.44% glycine (KMBg) or Nutrient Broth Yeast Agar (NBY) at 5, 10, 15, 20, 25, and 30 C for 96 h. HCN production by both strains was significantly greater at 30 C, moderate at 20 and 25 C, and slight to undetectable at 5, 10, and 15 C. Strain Q2-87::Tn*5* did not inhibit growth of *Ggt* at any temperature. Inhibition of *Ggt* by Q2-87 was evident at all temperatures tested and was greatest at 25 C. Phl production was greatest at 25 and 30 C, moderate at 15 and 20 C, and not visibly detectable at 5 and 10 C.

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SOIL SOLARIZATION AND *GLIOCLADIUM VIRENS* REDUCE THE INCIDENCE OF SOUTHERN BLIGHT IN BELL PEPPER IN THE FIELD. J. B. Ristaino, K. B. Perry, and R. D. Lumsden. Dept. of Plant Pathology, Dept. of Horticulture, North Carolina State University, Raleigh, and Biocontrol of Plant Diseases Laboratory, USDA, ARS, Beltsville, MD.

Timing of solarization in relation to planting of pepper and timing of soil amendment with bran prill of *G. virens* in relation to solarization was evaluated for effects on incidence of southern blight and survival of buried sclerotia of *Sclerotium rolfsii* at 10, 20, and 30 cm depths and locations within plant rows, between plant rows, and at the edge of double row beds. Solarization during crop growth increased the incidence of disease and *G. virens* was not effective in these plots. In contrast, in both years *G. virens* reduced ($P = 0.05$) disease in nonsolarized soils and solarization for 6 weeks prior to crop growth reduced ($P = 0.05$) disease. *G. virens* reduced ($P = 0.05$) sclerotia survival at all depths and all locations in both years. Survival of sclerotia in soil without *G. virens* was lower in the plant row than between or at the edge of the bed in one year and lower at 10 cm than other depths in the second year. A model to estimate soil temperature in solarized and bare plots based on meteorological inputs and soil physical factors was developed.

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INHIBITION OF *PYTHIUM* SPP. BY TAXANES EXTRACTED FROM ORNAMENTAL YEWS. W. H. Elmer, M. J. I. Mattina, and G. J. MacEachern. The CT Agr. Exp. Sta., Box 1106, New Haven, CT 06504.

Taxanes were extracted from ornamental yew needles with methanol followed by solid phase extraction clean-up. The taxanes were eluted with 80 % methanol/20% water; dried, reconstituted in 95% ETOH, and analyzed for paclitaxel (Taxol®), cephalomannine, and baccatin III by HPLC with photodiode array detection. These taxanes were found in 10:5:1 concentration ratio, respectively along with several other unidentified taxanes. The ethanol extracts were amended into PDA to yield paclitaxel concentrations of 0.05-2.0 µg/ml. Plates amended with pure paclitaxel, cephalomannine, and baccatin III were similarly prepared. The EC_{50} (µg/ml) values were derived from radial growths from colonized agar plugs. The taxane extracts were most toxic to *P. aphanidermatum* (PA) followed by *P. myriotylum* (PM), and *P. irregulare* (PI). The EC_{50} for PA were 0.08, 0.09, 0.90, and >2.00, respectively, for the taxane extract, authentic paclitaxel, cephalomannine, and baccatin III. The EC_{50} for PM were 0.67, 2.00, >2.00, >2.00, respectively. PI was more sensitive to pure paclitaxel than the taxane extract, and the EC_{50} were 1.30, 0.37, 1.00, and >2.00, respectively. Compared to the authentic taxanes combined in a 10:5:1 ratio, the taxane extract was more toxic to PA and to PM, than to PI.

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EFFECTS OF THREE CONCENTRATIONS OF RIDOMIL 2E ON THE INCIDENCE OF TARO LEAF BLIGHT (PHYTOPHTHORA COLOCASIAE) ON TARO (COLOCASIA ESCULENTA) IN AMERICAN SAMOA. D.R. Greenough, S. Fa'aumu, R. Tilialo. American Samoa Community College, Pago Pago.

Taro leaf blight, now the prime limiting factor in the production of the staple food, taro, was first reported in American Samoa in June, 1993. Within weeks, an epiphytotic situation existed and a Territorial Diaster was declared by the Governor. Chemical and sanitation strategies were used to provide immediate protection with varying results. Concentrations of Ridomil 2E at 3, 5, and 7 fl. oz./2 gal. H_2O were applied as soil drench by a 2 gal. backsprayer delivering 3 fl. oz. of chemical/plant in the root zone of taro growing in a new field under natural inoculation conditions. Applications of Ridomil were applied at 2 months and 4 months after planting. Leaves showing blight symptoms were removed weekly and leaf counts taken. At harvest, corm length, diameter, and weights were measured. Plants treated with the highest concentration of Ridomil had the lowest incidence of blight, the greatest number of leaves/plant, and corms from these plants had the largest size and greatest yield. Slight phytotoxicity was observed after spraying at the highest concentration, but without permanent damage to taro.

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CHEMICAL CONTROL OF ALGAL CRUSTS ON GOLF GREENS. P. F. Colbaugh and S. P. Metz. Texas Agric. Experiment. Station. Dallas, TX 75252.

Control of algal crusts on golf course greens is an important problem in temperate climates throughout the world. Algae crusts or mats can be 0.3 cm thick and range from a few meters in diameter to larger areas that encompass an entire green. Investigations were undertaken to determine the effectiveness of chemical treatments to eliminate algal crusts from golf greens. Seven chemical products were screened in the laboratory for their ability to kill algal mixtures of Phormidium, Hormidium and Cosmarium sp. growing in a weak nutrient solution. The most effective treatments were Consan 20, sodium hypochlorite, formalin and Lysol. Greenhouse tests were conducted with replicate algae-infested soil cores treated with drenches of selected chemical treatments. Results demonstrated that some disinfectants alone were effective in controlling algae growth with Consan 20 showing the greatest effectiveness of products labeled for use on turf. The addition of the surfactant Latron CS-7 was necessary to improve effectiveness of Dithane M-45, Agribrom and sodium hypochlorite. The most effective compound tested, Consan 20, proved effective without the addition of the surfactant that caused phytotoxicity in field tests.

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IN VITRO ACTIVITY AND EFFICACY OF CHLOROTHALONIL FOR CONTROL OF *ALTERNARIA* ROT OF TOMATOES. J.O. Kuti, K.S. Creekmore and R.L. Schading. Dept. Agronomy and Resource Sciences, Texas A&M University-Kingsville, TX 78363.

Alternaria alternata (Fr.:Fr.) Keissl. (syn. *A. tenuis* Ness.) causes severe black rot of tomato under field conditions in south Texas. Efficacy and *in vitro* activity of the fungicide Bravo® (chlorothalonil) for control of *A. alternata* on tomato was investigated. Chlorothalonil was effective in reducing *in vitro* conidia germination of *A. alternata* and was fungistatic at concentrations between 10-50 $\mu g\ ml^{-1}$ and fungicidal at concentrations above 500 $\mu g\ ml^{-1}$. Even though there was a significant reduction of *A. alternata* mycelia growth by chlorothalonil, the fungal growth rate was similar between 50 and 500 $\mu g\ ml^{-1}$ treatments. The fungicide was also effective in decreasing the number of infected fruits and disease severity caused by *A. alternata* in postharvest dip treatments using red ripe 'Floradade' tomatoes.

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FUNGICIDAL CONTROL OF BACTERIAL LEAF BLIGHT OF LETTUCE. R. N. Raid, and R. T. Nagata. Everglades Research and Education Center, University of Florida, Belle Glade, FL 33430-8003.

Seven fungicide treatments utilizing the fungicides copper hydroxide and maneb, applied alone and in combination, were evaluated for their control of a natural outbreak of bacterial leaf blight of lettuce, incited by *Xanthomonas campestris* pv. *vitians*. Disease pressure during the test was severe. All treatments resulted in significant reductions in blight severity when compared to the non-treated control. When applied alone, the efficacies of copper hydroxide (1.4 kg ai/ha) and maneb (1.8 kg ai/ha) were not statistically different, however, tank-mixtures of the two fungicides provided for significantly improved control over single-fungicide treatments. Percentages of marketable heads obtained were 12, 30, 40, 42 and 72% for the non-treated check, copper hydroxide, maneb, and copper plus maneb applied weekly, and copper plus maneb applied two times per week, respectively.

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IN VITRO SUPPRESSION OF FUSARIUM SOLANI, THE CAUSAL AGENT OF SUDDEN DEATH SYNDROME OF SOYBEAN. K. S. McLean[1] and G. W. Lawrence[2]. [1]Department of Agriculture, Northeast Louisiana University, Monroe, LA, 71209 and [2]Department of Entomology and Plant Pathology, Mississippi State University, Mississippi State, MS 39762.

Six chemicals, Benomyl, Captan, Carboxin, Metalaxyl, Pentachloronitrobenzene, and SM-9 were examined for their efficacy for in vitro suppression of Fusarium solani, the causal agent of sudden death syndrome (SDS) of soybean. Each chemical was incorporated into Potato Dextrose Agar by filter sterilization at rates of 5, 10, and 15 ppm. A 5 mm diameter agar disk containing F. solani was inverted on the agar surface and placed in a controlled growth chamber at 25 C. At 7 and 14 days after inoculation, all chemicals significantly reduced fungal growth compared to the non treated control. The greatest reduction in F. solani colony diameter was observed in the benomyl and carboxin treatments at all concentrations with an 66% and 45% reduction of fungal growth respectively.

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EFFECT OF RESIDUAL ACTIVITY OF FUNGICIDES ON DICARBOXIMIDE RESISTANCE DYNAMICS IN BOTRYTIS. G. W. Moorman and R. J. LEASE, Department of Plant Pathology, The Pennsylvania State University, University Park, PA, 16802.

Botrytis cinerea populations initiated from spores, 5% of which were resistant to the dicarboximide fungicide vinclozolin, were cycled at 10 day intervals over a 30 day period on excised Pelargonium leaf tissue from plants that had been sprayed once with single fungicides or mixtures of fungicides. At the end of each incubation period, the number of infected leaf disks was recorded as an indication of disease control and the proportion of resistant spores was assayed by plating some on fungicide-free and vinclozolin amended agar (20 µg vinclozolin/ml). Resistance increased quickly in all treatments containing vinclozolin. Vinclozolin maintained the selection pressure in favor of resistance for the duration of the experiments as a consequence of its long residual activity. It was concluded that once resistance is present, dicarboximide fungicide use should cease in greenhouses, particularly when disease pressure is high.

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BICARBONATES AND *BOTRYTIS*: II. EFFECTS WITH SURFACTANTS ON *IN VITRO* COLONY GROWTH OF *BOTRYTIS CINEREA*. Palmer, C. L., A. E. Winston, R. K. Horst, and H. W. Israel. Depts. of Flor. & Orn. Hort. and of Plant Path., Cornell University, Ithaca, NY, USA 14853, and Church & Dwight Co., Inc., Princeton, NJ, USA 08540.

To determine effects of $KHCO_3$ and various surfactants on *Botrytis cinerea* Pers., radial growth of spore-initiated colonies was examined on PDA supplemented with 0.00 and 0.05 M $KHCO_3$ in combination with 29 surfactants used in agricultural and food service industries. Media were adjusted to pH 7.5 after addition of 1% (v:v) surfactant. Colony diameters were measured daily from 0 to 144 h. Changes in colony growth habit were recorded. Surfactants alone had several effects on colony diameter: no, slight, severe, and complete inhibition [Tween 85, Tween 20, Renex 36, and Ethomeen T/25, respectively]. Also, some surfactants altered colony habit and color. Addition of bicarbonate increased inhibition. We conclude that surfactants may have fungicidal properties which will enhance effectiveness of bicarbonate sprays used to control *B. cinerea*.
(Supported by Church and Dwight Co., Inc., Princeton, NJ 08540.)

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COPPER TOLERANCE AND STREPTOMYCIN RESISTANCE IN STRAINS OF *PSEUDOMONAS SYRINGAE* FROM NORTHWEST NURSERIES. H. J. Scheck, J. W. Pscheidt and L. W. Moore. Department of Botany and Plant Pathology, Oregon State University, Corvallis, OR 97331-2902

Grower reports of disease control failures led to the collection and testing of *Pseudomonas syringae* isolates for tolerance to copper and streptomycin (strep). During the springs of 1992 and 1993, 246 isolates were obtained from 14 genera of woody ornamentals with tip diebacks, leaf spots and branch cankers in commercial nurseries and 32 from unsprayed diseased lilacs in landscapes. *P. syringae* isolates collected in 1982 and 1983 and stored at -80C were also tested. Isolates were characterized as *P. syringae* based on fluorescence on King's media B, a negative test result for both cytochrome oxidase and arginine dihydrolase activity, and pathogenicity on lilac tissue culture plantlets. On casitone yeast extract media amended with copper sulfate, 50% and 26% of the 246 isolates from 1992/93 were tolerant of 0.16 and 0.40 mM of copper respectively. 37% were resistant to 1 mg/L strep on amended Kings media B. 30% were tolerant to both 0.16 mM copper and 1mg/L strep while 11% were tolerant to 0.4 mM copper and 1 mg/L strep. Of the 151 isolates from 1982/83, 25% were tolerant to 0.16 mM of copper, 6% were strep resistant and none were tolerant of both. One isolate from an unsprayed landscape was tolerant to 0.16 mM of copper. These data indicate that resistance has increased and growers need to explore new methods for control of bacterial blight.

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FUNGICIDE SENSITIVITY AMONG *RAMULISPORA HERPOTRICHOIDES* (EYESPOT) ISOLATES IN SOUTH AFRICA. B. Robbertse, P.W. Crous and G. Holz, Department of Plant Pathology, University of Stellenbosch, Stellenbosch 7600, South Africa.

Sixty-seven isolates of *Ramulispora herpotrichoides* were obtained from wheat at six sites in the Swartland area. Isolates were tested *in vitro* for sensitivity to benzimidazole and sterol inhibitor fungicides. All isolates were fast growing with an even colony margin (W-type). Their growth was totally inhibited on PDA amended with 1μg/ml carbendazim, indicating that no shift in sensitivity occurred in the population. The mean prochloraz concentration calculated to inhibit fungal growth by 50 % (IG 50 value) was 0.043 $\pm$ 0.029 μg/ml, which is as low as those showing baseline sensitivity in Europe. Of the representative isolates screened against 2 μg/ml triadimenol, 44 % were sensitive, while 56 % were resistant. Isolates which were most sensitive to prochloraz and triadimenol came from a site where no fungicides have been applied against eyespot, and those least sensitive to triadimenol from a site where prochloraz has been used for the past seven years. These results indicated that South African isolates are carbendazim and prochloraz sensitive, but that they differ in sensitivity towards triadimenol.

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Molecular Marking of the Stem Rust Resistance Gene, *rpg4*, in Barley. I. Borovkova, Y. Jin, B.J. Steffenson, and J.B. Rasmussen. Department of Plant Pathology, North Dakota State University, Fargo, ND 58105.

Pathotype Pgt-QCC of *Puccinia graminis* f.sp. *tritici* is virulent on all commercial barley cultivars in the U.S. Barley line Q21861 recently was found to carry a recessive resistance gene, designated *rpg4*, effective against QCC. RAPD markers linked to and flanking *rpg4* have now been identified by bulked segregant analysis from a population of doubled haploid lines derived from a cross of Q21861 and SM89010. The markers were identified by screening 410 10mer oligonucleotide primers. The closest flanking markers mapped 3.5 and 4.3 cM from the gene. Preliminary data from crosses of Q21861 with morphological marker lines and from RFLP analyses suggest that *rpg4* may be located near the telomere of the minus arm of chromosome 7. The molecular markers may facilitate the introgression of *rpg4* into new barley cultivars.

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RESISTANCE TO DWARF BUNT AMONG WINTER WHEAT CULTIVARS. B.J. Goates, USDA, ARS, P.O. Box 307, Aberdeen, Idaho 83210

Control of dwarf bunt caused by *Tilletia controversa* is currently provided by the incorporation of resistance into cultivars. The use of highly resistant cultivars can reduce yield losses to insignificant levels in environments that are conducive to high levels of infection. Numerous cultivars were tested in the field for three or more seasons under extreme disease pressure. Nurseries were inoculated with a composite of pathogenic races that originated from different areas of the Pacific Northwest United States. The cultivars Blizzard, Bonneville, Hansel, Manning, Promontory, Survivor, Weston, and Winridge were very resistant; cultivars Eltan, Luke, and Lewjain were resistant; cultivars Kmor, Madsen, Moro, and Ute were moderately resistant; and the cultivars Andrew, Batum, Hyak, Sprague, and Tres were moderately susceptible. Most cultivars tested were susceptible or highly susceptible.

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CHROMOSOMAL LOCATION OF WHEAT LEAF RUST RESISTANCE GENE *Lr43* DERIVED FROM *TRITICUM TAUSCHII*. T. Hussien, R.L. Bowden, B.S. Gill, Department of Plant Pathology and T.S. Cox, USDA-ARS, Kansas State University, Manhattan, KS 66506-5502.

Triticum tauschii, the diploid D-genome progenitor of bread wheat, is a valuable source of genes for diversifying resistance to the leaf rust fungus *Puccinia recondita* f. sp. *tritici*. Recently, three new leaf rust resistance genes have been transferred from *T. tauschii* to common wheat: *Lr41* (in germplasm KS90WGRC10), *Lr42* (in KS91WGRC11) and *Lr43* (in KS91WGRC16). Standard monosomic analysis was used to determine the chromosomal location of *Lr43*. KSWGRC16 was crossed with each of the seven D-genome monosomic lines. F2 populations from selfed, 41-chromosome F1 plants were inoculated with leaf rust isolate PRTUS6 in a greenhouse. The number of resistant to susceptible seedlings did not deviate significantly from a 3:1 ratio in the noncritical crosses. The ratio was 50:1 in the cross with the 7D monosomic line indicating that *Lr43* is located on this chromosome. The chromosomal arm location of this gene is currently under investigation.

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CHROMOSOMAL LOCATION OF GENES FOR RESISTANCE TO *PUCCINIA STRIIFORMIS* IN WHEAT CULTIVARS DRUCHAMP, STEPHENS, AND YAMHILL. X. M. Chen, R. F. Line, and S. S. Jones. Dept. of Plant Pathol. and USDA-ARS, WSU, Pullman, WA 99164-6430.

Wheat cultivars Druchamp, Stephens, and Yamhill were reported to have stripe rust resistance genes *Yr*3a and *Yr*Dru; *Yr*3a and *Yr*Ste; and *Yr*2, *Yr*4a, and *Yr*Yam; respectively. Each cultivar was crossed with Chinese Spring and 21 monosomic or monotelosomic Chinese Spring lines. F_2 seedlings from monosomic F_1 plants were tested with *P. striiformis* races. The chromosomal locations of the six genes in the three cultivars were determined. *Yr*2 is on chromosome 7B; *Yr*3a on 1B; *Yr*4a on 6B; *Yr*Dru on 6B; *Yr*Ste on 2B; and *Yr*Yam on 4B. The chromosomal locations of two additional genes were also determined, one in Druchamp (*Yr*D) on 6A and one in Stephens (*Yr*S) on 3B. *Yr*2, *Yr*3a *Yr*4a, *Yr*Dru, *Yr*Ste, *Yr*Yam, *Yr*D, and *Yr*S are effective against races CDL-1 and CDL-35; CDL-1, CDL-21, and CDL-29; CDL-1; CDL-21 and CDL-45; CDL-6 and CDL-21; CDL-1 and CDL-35; CDL-1, CDL-35, and CDL-45; and CDL-1 and CDL-45, respectively.

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POWDERY MILDEW RESISTANCE GENES IN SELECTED WINTER WHEAT LINES AND CULTIVARS. R. R. Persaud, P. E. Lipps and *K. G. Campbell, Department of Plant Pathology and *Department of Agronomy, The Ohio State University, Wooster, OH 44691.

Seedlings of seven soft red winter wheat cultivars and four elite breeding lines developed for Ohio were tested for the presence of powdery mildew resistance genes using 14 isolates of *Blumeria graminis* f. sp. *tritici*, previously characterized for their virulence. Differential reactions by isolates could detect *Pm*1, *Pm*3a, *Pm*3b, *Pm*4a, *Pm*17, *Pm*2 and/or *Pm*6, and *Pm*3c and/or *Pm* 5. A major gene (*Pm*3a) for powdery mildew resistance was detected in AGRA GR915, but AGRA GR863, Cardinal, Clark, Dynasty, Freedom and Titan did not carry any of the resistance genes that could be detected by the set of isolates used. However, each of the elite breeding lines tested had major resistance genes. The gene *Pm*3a was present in OH 470 and OH 493-1 and the gene *Pm*17 was detected in OH 464. OH 490 contained *Pm*2 or *Pm*6, or possibley both genes. These genes could not be distinguished because isolates used could not differentiate between the two genes.

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RUSSET BURBANK GENETICALLY IMPROVED FOR RESISTANCE TO POTATO LEAFROLL VIRUS. C. Lawson*, W. Kaniewski*, P. Thomas, T. Mowry, G. Reed, T. Mitsky*, J. Zalewski*, and Y. Muskopf*. *Monsanto Company, St. Louis, MO 63198

Potato leafroll virus (PLRV) is a serious virus disease of potato. Russet Burbank has now been genetically improved to be highly resistant to PLRV infection and the disease symptoms caused by the virus. Various cDNAs of open reading frames from the PLRV genome were introduced as transgenes into potato. At least 20 transgenic lines of each gene construct were assayed by

field screens at 2 locations for PLRV foliar symptoms, virus presence in leaves, net necrosis in tubers, and virus presence and titer from tuber sprouts. The PLRV replicase gene conferred an extremely high level of resistance to PLRV at a frequency of ~25% of the transgenic lines. In some of these lines, no obvious PLRV foliar symptoms were observed at any time during the field season, virus was undetectable in foliar tissue, net necrosis was absent in tubers, and virus was not detected in tuber sprouts. Two years of field data indicate that this resistance is highly effective.

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COAT PROTEIN MEDIATED RESISTANCE TO BARLEY YELLOW DWARF VIRUS IN BARLEY. R. M. Lister, J. R. Vincent, and P. F. McGrath, Dept. Botany and Plant Pathology, Purdue Univ., W. Lafayette, IN 47907; P. G. Lemaux and Y. Wan, UC Berkeley/USDA Plant Gene Expression Center, Albany, CA 94710.

Some barley plants among those (Plant Physiology, 104: 37-48, 1994) derived from cv. Golden Promise calli biolistically co-transformed with the selectable marker *bar* and the Purdue barley yellow dwarf virus (P-PAV isolate) coat protein gene construct (BYDV-cp) showed resistance to BYDV. Over 200 fertile regenerants (R1 plants) have been tested for resistance so far, by infecting them using viruliferous *Rhopalosiphum padi* aphids and subsequently assessing virus contents by ELISA. Resistance (reduced virus content) was apparent in about 10% of the plants, and especially so in about 2%, which yielded extracts with ELISA values less than one-tenth of those for controls. Correlation of resistance with the presence of BYDV-cp is being examined by PCR, and progenies (R2 plants) from resistant R1 plants are being developed for further testing.

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COAT PROTEIN MEDIATED RESISTANCE TO BARLEY YELLOW DWARF VIRUS IN OATS. R. M. Lister, J. R. Vincent, C.-H. Lei and P. F. McGrath, Dept. Botany and Plant Pathology, Purdue Univ., W. Lafayette, IN 47907; B. A. Larkins, Plant Sciences Dept., Univ. Arizona, Tucson, AZ 85721; K. A. Torbert, W. P. Pawlowski, H. R. Rines* and D. A. Somers, Dept. Agronomy and Plant Genetics, Univ. Minnesota, St. Paul, MN 55108. *USDA-ARS Plant Science Research Unit.

Calli of GAF/Park oat (Crop Science 29: 798-803, 1989) were biolistically co-transformed with constructs representing the coat protein genes (CPGs) of the P-PAV, MAV-PS1, or NY-RPV isolates of barley yellow dwarf virus (BYDV), and the *bar* gene for herbicide resistance. Transformed, herbicide-resistant callus tissue was then screened by PCR for the CPGs. Fertile regenerants were recovered from some CPG-transformed lines. About 5% of 286 progeny from these (R1 plants) and of 332 of the subsequent generation (R2 plants), showed a high degree of resistance to the BYDV isolate corresponding to the introduced CPG in tests in which they were infected using viruliferous aphids and their virus contents subsequently assessed by ELISA. Assay values for extracts from these plants did not exceed 10% of those for control extracts.

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COMBINING ABILITY ANALYSIS OF RESISTANCE TO EASTERN FILBERT BLIGHT IN SELECTED HAZELNUT CULTIVARS. N.K. Osterbauer[1], T.L. Sawyer[1], S.A. Mehlenbacher[2], and K.B. Johnson[1]. [1]Dept. of Botany & Plant Pathology and [2]Dept. of Horticulture, Oregon State University, Corvallis 97331.

Inheritance of resistance to eastern filbert blight, caused by *Anisogramma anomala*, was studied in *Corylus avellana* seedlings. Seven parent cultivars were crossed in 12 combinations. Meristematic tissue of 8-wk-old seedlings was inoculated three times over three weeks with *A. anomala* (10^7 ascospores/ml). After a cold dormancy, the proportion of wood diseased was measured. Offspring of the parent VR6-28, which is heterozygous for a single dominant resistance gene, segregated 1:1 for resistance. Offspring of the remaining parents were subjected to general combining ability (GCA) analysis as level of resistance appeared to be quantitative. Based on GCA values, 'Gem', 'Tonda di Giffoni', 'Willamette', and 'Casina' were superior parents for transmission of resistance whereas 'Tonda Gentile delle Langhe' and 'Ennis' were poor parents. General combining ability was not significant in this study, suggesting resistance is not under additive genetic control. To verify these results, a field study using 1-yr-old trees from the 12 crosses is underway.

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SAPROPHYTIC INCREASE OF *PHIALOPHORA GREGATA* IN SOYBEAN RESIDUE. E.A. Adee and C.R. Grau. Dept. of Plant Pathology. Univ. of Wisconsin, Madison, WI 53706.

The stability of the saprophytic population density of *Phialophora gregata* (Pg), causal agent of brown stem rot (BSR) of soybean, as it survives in residue is unknown. The density of Pg was determined from individual stems, or residue confined in mesh bags, which were sampled periodically from field and growth chamber experiments. The average density of Pg in stems buried in the soil, sampled monthly from Dec. - June, with a low initial density (8.59×10^4 colony forming units (cfu)/g in Nov.) had increased to 5.52×10^5 compared to stems with high initial density (1.05×10^6 cfu/g) that had decreased to 3.05×10^5. The population density of Pg in residue positioned on the soil surface or buried was 150 to 350% greater than the initial density when sampled in Feb. and April. However, when sampled in May and June, the density of Pg in residue on the soil surface was 900% greater than the initial density compared to the density in buried residue which was 130 and 16% of the initial density, respectively for the months. Similar amounts of inoculum of Pg (cfu/g x g/m^2) remained in the field in residue on the soil surface after 81 wk from residue with a lower initial density of Pg (6.18×10^3 cfu/g) compared to higher initial density (2.33×10^4 cfu/g) (6.82 and 7.04×10^7 cfu/m^2, respectively) The 81 wk is the time between harvest and planting of a subsequent soybean crop in a corn/soybean rotation. At a cooler growth chamber temperature (1 C) for 41 d, the density of Pg in residue on the soil surface was greater (88.5×10^3 cfu/g) than at 23 C (6.58×10^3 cfu/g). In the same study, the density of Pg in residue of a BSR-resistant cultivar increased from undetectable (<15 cfu/g) to 1.12×10^3 cfu/g after 41 d at 23 C. The increased density of Pg between soybean crops could be an explanation for the sharp increase in severity of BSR from the first to the second consecutive yr of soybean, especially with no-tillage.

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SUDDEN DEATH SYNDROME OF SOYBEAN: PHYTOTOXICITY OF CULTURE FILTRATE OF *FUSARIUM SOLANI*. H. Jin[1], G. L. Hartman[2] and J. M. Widholm[3]. [1]Dept. of Plant Pathology, [2]USDA/ARS, [3]Dept. of Agronomy, University of Illinois at Urbana-Champaign, 1102 S. Goodwin Ave., Urbana, IL 61801

Blue colony-forming *Fusarium solani* cause sudden death syndrome of soybean. Stem injection of soybean seedlings or immersion of seedling shoots with roots excised into crude culture filtrate (isolate 269) caused foliar interveinal chlorosis. The culture filtrate caused soybean calli browning and was quantified by absorbency at 330 nm of 80% acetone extracts of calli. The isolate was grown on 1% sucrose containing extracts of either soybean cotyledons, leaves or stems and on a semi-defined liquid medium containing salts, sucrose and yeast extracts. After two weeks, culture filtrate of each medium was incorporated into callus medium (5%). Calli transferred to callus medium had significantly more browning 3 days after incubation when the medium contained culture filtrate from semi-defined liquid medium. On semi-defined liquid medium inoculated with $1x10^4$ conidia/ml, fresh mycelial weight and phytotoxin production evaluated by callus bioassay peaked at 9 days. Culture filtrate exposed to six temperatures (4, 28, 37, 50, 75 and 100 C) for 30 minutes showed that the phytotoxin(s) was most active at 4 and 37 C; less active at 28, 50 and 75 C; and was inactivate at 100 C based on callus bioassay.

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FREQUENCY AND DISTRIBUTION OF PATHOTYPES OF PHIALOPHORA GREGATA IN IOWA. K.M. Tubajika and X.B. Yang. Department of Plant Pathology, Iowa State University, Ames, Iowa 50011.

Foliar symptoms of brown stem rot caused by Phialophora gregata pathotype I, have been reported in other Midwest states but not Iowa. Frequency and distribution of two pathotypes in Iowa were determined from samples of soybean residue in early spring from fields at 50 randomly selected locations. The pathogens were isolated with a selective medium for P. gregata. Pathotypes I and II were confirmed by foliar and stem symptoms on Kenwood and Jack varieties following root-dip inoculations in greenhouse trials. P. gregata was recovered from 92% of the samples. Fields were found in which either type I or type II predominated. Random isolation of P. gregata from soybean stems and roots in 1993 indicated a frequency of 78% pathotype I. Overall frequency of type I was higher than that of type II in term of colony-forming unit count with a ratio of type I:type II of 4:1. Number of colony-forming units per gram residue varied from $1.0x10^4$ to $4.1x10^5$.

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FREQUENCY OF ISOLATES OF PYTHIUM SPP. CAUSING DAMPING-OFF OF BOTH CORN AND SOYBEAN IN ROTATION FIELDS. B.Q. Zhang and X.B. Yang. Department of Plant Pathology, Iowa State University, Ames, IA 50011.

Experiments were conducted to determine the frequency of Pythium isolates pathogenic to both corn and soybean. Pathogenicity of Pythium isolates from different corn/soybean rotation fields in Iowa was tested. Reduction of seed germination (RSG) at 10 C was used to measure pathogenicity for pre-emergence damping-off. Among 69 pathogenic Pythium isolates tested for pre-emergence damping-off, 30% were highly pathogenic to both corn and soybean (RSG > 80%). Fifty percent of isolates highly pathogenic to soybean had moderate to low pathogenicity to corn. Some isolates were pathogenic to either soybean or corn. In post-emergence damping-off experiments, 20% of isolates were highly pathogenic to both soybean and corn. Isolates which caused pre-emergence damping-off had different pathogenicity in causing post-emergence damping-off. Differences in the frequency of pathogenicity were also found when different varieties were used.

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A NEW VIRUS OF CORN AND WHEAT IN WESTERN KANSAS. D.J. Jardine, R.L. Bowden, Dept. of Plant Pathology, Kansas State Univ., Manhattan KS 66506, S.G. Jensen, USDA-ARS, Dept. of Plant Pathology, Univ. of Nebraska, Lincoln, 68583 and D.L. Seifers, Ft. Hays Expt. Station, Hays KS 67601

Corn plants exhibiting virus-like symptoms were found in a Gray County field in June 1993. Symptoms consisted of severe stunting and a pronounced chlorosis

in the youngest leaves. The chlorosis was a general mosaic with flecking or streaking. As plants matured, new growth was chlorotic while the older tissue reddened along the leaf margins beginning at the tip. The reddening progressed down the leaf followed by a necrosis that also began at the tip. Severely affected plants died. Ear and kernel size were reduced. Fields of blue corn and popcorn were also found to be infected in Wichita County. Wheat from Stanton, Morton, and Ellis Counties was found to be infected with the same virus by ELISA testing in early 1994. Symptoms on wheat were similar to those of wheat streak mosaic (WSM) including mottled, streaked leaves, and stunting. Unlike WSM, however, plants eventually died. Initial characterization of virus-like particles suggests a possible link to the tenuivirus group. Studies on a potential vector are being conducted.

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PATHOGENICITY OF ISOLATES OF SCLEROTINIA TRIFOLIORUM AND S. SCLEROTIORUM TO ALFALFA CULTIVARS UNDER CONTROLLED INOCULATION CONDITIONS. R. G. Pratt and D. E. Rowe, USDA, ARS, Forage Research Unit, P.O. Box 53267, Mississippi State, MS 39762

Plants of eight cultivars and one experimental population of alfalfa were inoculated separately with each of five isolates of S. trifoliorum and S. sclerotiorum in each experiment. Inoculations were performed by dusting particles of infested grain over foliage of 4-wk-old plants. Plants were maintained at ca. 20 C under fluorescent growth lights and with an intermittently saturated atmosphere. Survival was evaluated at 24 days after inoculation. Isolates differed in virulence, but all caused death of some plants. Cultivars differed in susceptibility, and responses to isolates of both Sclerotinia spp. were generally similar. An experimental population developed for resistance to S. trifoliorum also manifested resistance to the isolates of S. sclerotiorum.

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EFFECT OF CUTTING PRACTICE AND INOCULUM LEVEL ON CROWN AND ROOT ROT OF BIRD'S-FOOT TREFOIL. D. P. Whittington and R. B. Carroll. Dept. of Plant and Soil Sciences, Univ. of Delaware, Newark, DE 19717-1303.

Bird's-foot trefoil cultivars Viking (susceptible) and GA-1 (resistant) were grown in the greenhouse for 6 weeks (4 plants/pot) and then subjected to Mycoleptodiscus terrestris (Gerdemann) at three inoculum levels. One month later, plants at each inoculum level were clipped using practices that reflect high, moderate and low cutting stress. Experimental design was a RCB with 5 replications/treatment and the experiment was repeated twice. After 25 weeks of the cutting regime, plants were harvested and rated for crown and root rot. Inoculum level did not result in significant differences in disease severity, root weight or forage yield. Variety had a significant effect on disease development and root weight only. Cutting stress resulted in significant differences in all variables measured.

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DEVELOPMENT OF EXPERIMENTAL POPULATIONS OF WHITE CLOVER WITH AND WITHOUT RESISTANCE TO PEANUT STUNT VIRUS AND OZONE. M. R. McLaughlin, G. A. Pederson, and A. S. Heagle, USDA, ARS, Forage Unit, Mississippi State, MS 39762-5367, and Air Quality Unit, Raleigh, NC 27603.

Clones of white clover, *Trifolium repens* L., tolerant (tol) (NC-R) or sensitive (sen) (NC-S) to ozone (O_3), were crossed with a clone which is heterozygous for monogenic dominant, hypersensitive resistance (res) to peanut stunt virus (PSV). Both NC-R and NC-S are susceptible (sus) to PSV. Seed harvested from NC-R and NC-S were planted in cone-tainers in the greenhouse. Six to 8-wk-old seedlings were randomized, exposed briefly to a high level of O_3, and rated for injury. Plants tolerant and sensitive to O_3 were selected from NC-R and NC-S progeny, respectively, and their reactions to PSV were determined. Populations of O_3-tol/PSV-res, O_3-tol/PSV-sus, O_3-sen/PSV-res, and O_3-sen/PSV-sus were identified. These populations can be used to study white clover persistence in field experiments in uncontrolled environments with variable O_3 and PSV levels.

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EFFECTS OF IRRIGATION METHOD AND INOCULUM LEVEL ON SPREAD OF PHYTOPHTHORA PARASITICA DURING VINCA PRODUCTION. A. Fallon[1], S.L. von Broembsen[1], and J.M. Dole[2], Depts. of Plant Pathology[1] and Horticulture[2], Oklahoma State University, Stillwater, OK 74078

Spread of P. parasitica from a central infestation to surrounding plants during greenhouse production was studied for vinca (Catharanthus roseus) grown in flats or 2.5 inch pots and irrigated by capillary mat (CM), ebb and flow (EF) or handwatering (HW). Spread in flats was 65.3, 94 and 100% for CM, EF and HW, respectively. For pots, spread was less and was dependent on initial concentration of soil inoculum. Spread in EF was 54.8, 4.7 and 2.4% for 10, 1 and 0.1% inoculum, respectively. Spread occurred only at the 10% level for CM (4.7%) and HW (2.4%). P. parasitica was recovered from EF reservoirs, CMs and trays under HW plants during the trials.

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INCREASED INCIDENCE OF BOTRYOSPHAERIA CANKER OF WOODY ORNAMENTALS IN GEORGIA IN 1993. Wakar Uddin, Extension Plant Pathology, University of Georgia, Athens, GA 30602

Samples received in the Plant Disease Clinic at the University of Georgia indicated higher incidence of Botryosphaeria canker in landscape woody ornamentals in Georgia in 1993 than the previous year. Four additional species were diagnosed with Botryosphaeria canker in 1993 with high disease incidence in samples of wax myrtle and leucothe (each with 50% samples positive for *Botryosphaeria* spp.), followed by hydrangea (21%), euonymous (20%) and holly (8%). Leyland cypress, rhododendron and flowering cherry were affected most in both years, with 43%, 15%, and 33% samples diseased in 1992, and 60%, 19%, 33% in 1993, representing a 39%, 21%, and 0% increase over the previous year for the three ornamentals, respectively. *B. dothidia* was the predominant species of *Botryosphaeria* isolated from samples of woody ornamentals in 1993. Bark-splitting, due to prolonged freezing temperatures in Georgia during the blizzard of March 1993, was evident in almost all the samples diagnosed with Botryosphaeria canker and appears to have contributed to the increase in disease.

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CURVULARIA BLOTCH OF LISIANTHUS. J.P. Jones and B.K. Harbaugh. GCREC, Univ. of Florida, Bradenton 34203.

During fall 1992 and spring 1993 light tan to beige blotches developed on the leaves, stems, and flowers of greenhouse-grown lisianthus (Eustoma grandiflorum (Raf.) Shinn.). These blotches enlarge and coalesce into large areas without distinct margins on the leaves and blooms. A Curvularia sp. was isolated consistently from these blotchy areas. In order to establish pathogenicity, the fungus was grown 7 days in pure culture on plates of potato-dextrose agar (PDA). Then a spore suspension (3×10^4 spores/ml) was prepared and misted onto several GCREC breeding lines and cv. Blue Lisa. After 14 days incubation at 23C all noninoculated plants were healthy, whereas disease symptoms, identical to those occurring naturally in the greenhouse, developed on 98% of the inoculated plants. Curvularia sp. sporulated profusely on diseased leaf and stem tissue and was reisolated consistently onto PDA plates. Several of the GCREC lines were highly tolerant to the disease. The optimum temperature for disease development was 24C with considerably less at 32C and none at 16C. Mancozeb, chlorothalonil, and iprodione applied as sprays resulted in excellent disease protection.

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SURVIVAL OF *Discula* STOCK CULTURES AND CONIDIA. S. D. McElreath and F. H. Tainter. Department of Forest Resources, Clemson University, Clemson, SC 29634-1003.

Viability of 157 stock cultures of 44 isolates of *Discula destructiva* and 22 cultures of 3 isolates of *Discula*, Type 2 was determined. Cultures were grown on potato-dextrose agar slants in 1/2 oz square bottles with loosened screw caps. Caps were tightened and bottles were stored at 4° C. Fifty-four/62 (87%) of the cultures of *D. destructiva* stored for 27-28 months, 27/57 (47%) stored for 31-32 months and 10/38 (26%) stored for 34-37 months were viable. Only 1/22 (4.5%) cultures of *Discula*, Type 2 was viable after 32 months. Survival of conidia from 5 isolates of *D. destructiva*, stored in sterile distilled water at -15° C for 36 months, was very low. Conidia from 2 isolates were non-viable and germination of those from the other 3 was less than 0.005%.

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Survey of Pythium and Phytophthora spp. in Irrigation Water Used by Colorado Commercial Greenhouses to Determine Source of Pathogen Introduction. L. Pickett

Pottorff and K. L. Panter, Colorado State University Cooperative Extension, Golden, CO.

Studies were initiated to determine if irrigation water is a source of Pythium and Phytophthora spp. introduction into Colorado commercial greenhouse cropping systems. Water from 9 greenhouses (3 well water, 3 municipal water, and 3 water holding ponds) was surveyed for the presence of Pythium and Phytophthora spp once a month from June to August. All greenhouses had a documented Pythiaceous root disease history. Pythium dissotocum and Pythium rostratum were recovered from two different holding pond sources. No Phytophthora was found in any water source. All municipal, well and one holding pond water sources were free from Pythiaceous fungi. Pathogenicity tests are currently being conducted with P. dissotocum and P. rostratum on cucumbers and lupines. Water holding ponds appear to be the only possible source of Pythium introduction into greenhouses via irrigation. Pythium and Phytophthora spp. are more likely to be introduced into Colorado greenhouse cropping systems via sources other than irrigation water.

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A NEW DISEASE OF HELICONIA CAUSED BY PYRICULARIOPSIS. J. Y. Uchida and C. Y. Kadooka. Department of Plant Pathology. University of Hawaii, Honolulu HI 96822

Several cultivars of exotic Heliconia spp. are exported by the tropical flower industry in Hawaii. A new foliar disease caused by a Pyriculariopsis sp. has been discovered on the island of Hawaii, in a major Heliconia production area. Disease symptoms are small to large, elongate, dark leaf spots, frequently clustered at the midrib. Expanding rots of the midrib cause blight distal to the rots, followed by necrosis of the entire blade. Individual spots are brown to reddish brown. Large (110 X 36 mm) blighted areas, light brown to gray with chlorotic borders, independent of midrib rots also occur. Extensive leaf loss occurs during wet periods. Sporulation of Pyriculariopsis was good on autoclaved heliconia leaves but limited on several commonly used culture media. Pathogenicity was confirmed by inoculations of 'Dwarf Jamaican' and H. muticiana with conidial suspensions followed by disease reproduction and pathogen reisolation.

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INCIDENCE OF MAIZE DWARF MOSAIC IN SWEET CORN HYBRIDS WITH RESISTANCE TO MDMV. M.R. Kerns and J.K. Pataky. Department of Plant Pathology, University of Illinois, Urbana, IL 61801.

Twenty hybrids with resistance to MDMV from at least one inbred parent were evaluated in field trials in 1993. Plants were inoculated three times with MDMV strains A, B, or a mixture of A and B and compared with an uninoculated control in 2 or 3 replicates. Incidence was rated from ca. 50 plants per experimental unit and yield (ear weight) was measured from ca. 20 plants from the middle two rows of each four row plot. Incidence varied among hybrids, ranging from about 3 to 100% for all three inoculation treatments (A, B and A-B), and means were about 20%, 50% and 30%, respectively. All of the resistant hybrids had some plants that were symptomatic. Three hybrids, Bi Guard, Dallas, and HMX 9352 S, had a low incidence (< 10%) of symptomatic plants when inoculated with either strain. Many hybrids had relatively low incidence (<25%) when inoculated with strain A or the combination of strains A and B, but relatively high incidence (>40%) when inoculated with strain B. Some hybrids showed a high incidence of sectoring of symptomatic and asymptomatic tissue. Yield was reduced by as much as 24% by strain A, 38% by strain B, and 25% by strain A and B as compared to the non-inoculated control.

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INHERITANCE OF RESISTANCE TO *Fusarium moniliforme* IN SWEET CORN. C. Nankam and J. K. Pataky, Department of Plant Pathology, University of Illinois, Urbana, IL 61801.

The inheritance of resistance in two sweet corn inbreds to kernel infection by *Fusarium moniliforme* was evaluated using two inbreds IL125b (resistant), Ia2256b (susceptible). Generation mean analysis used the F1, F2, F3 and backcrosses to both parents. When data were analyzed based on generation of the embryo and endosperm, additive, dominance, and additive x dominance genetic effects accounted for 34%, 30%, and 28% of the variation in symptomatic infection of kernels. For asymptomatic infection of kernels, the dominance genetic effect was not significant, but additive and additive x dominance genetic effects accounted for 18% and 44% of the variation. Additive and dominance effects accounted for 74% and 23% of the variation in symptomatic infection and 43% and 35% of the variation in asymptomatic infection when data were analyzed based on the generation of the ear parent (pericarp, silk and maternal tissue of kernels). Frequency distributions were skewed toward the resistant parent in all four analyses. Additional studies of inheritance of resistance to *F. moniliforme* will be evaluated in 1994 from progenies of crosses using additional sweet corn inbreds.

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INOCULATION METHODS TO SCREEN *sh2* SWEET CORN FOR SEED AND SEEDLING REACTIONS TO *Fusarium moniliforme* AND *Penicillium oxalicum*. C. Nankam and J.K. Pataky. Department of Plant Pathology, University of Illinois, Urbana, IL 61801.

Two inoculation techniques were tested in field and greenhouse experiments for screening of *sh2* sweet corn for seed and seedling reactions to either *Fusarium moniliforme* or *Penicillium oxalicum*. A significant genotype by inoculation interaction was observed for stand counts at 20 and 27 days after planting in the field and at 11 and 21 days after planting in the greenhouse. Stands were reduced substantially when seeds, inoculated by imbibing spore suspensions of *F. moniliforme* or *P. oxalicum*, were compared to stands from control plots and plots inoculated with sterilized sorghum seed infected with either fungus. None of the inbreds and hybrids evaluated displayed a high level of resistance to either fungus, although reductions in stand due to *F. moniliforme* were less for two hybrids, SW 445 and SW 610B. Approximately one hundred *su* and *sh2* lines will be evaluated in field, greenhouse, and seed germinator studies in 1994 to further evaluate the ability of the inoculation procedure to differentiate genotypes and to identify resistant and susceptible germ plasm.

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SCREENING OKRA GERMPLASM FOR RESISTANCE TO VERTICILLIUM WILT. R. S. Potti[1], G. J. Weidemann[1] and E. Lamb[2]. [1]Dept. of Plant Pathology and [2]Dept. of Horticulture and Forestry, University of Arkansas, Fayetteville, AR, 72701.

Verticillium wilt can cause significant losses of many crop plants including okra (Abelmoschus esculentus (L.) Moench) in the cooler regions of the United States. Over 150 okra accessions from a worldwide collection were screened for resistance to Verticillium dahliae. Okra seedlings, grown in vermiculite, were root-dip inoculated with a conidial suspension containing 1-2 x 10^6 spores/ml, and transplanted into 7cm pots containing a commercial potting mix. After four weeks in the greenhouse, the plants were rated for plant height suppression and mortality in comparison to uninoculated controls. In dose response experiments conducted in the greenhouse with a standard cultivar, Jade, plant height suppression and mortality were proportional to the inoculum concentration. Several accessions showed less height suppression or mortality than did susceptible cultivars. Results suggest that genetic resistance occurs in okra accessions that could be incorporated into commercial cultivars.

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RESISTANCE IN POTATO TO VERTICILLIUM WILT. T. Suganda and N.A. Anderson, Department of Plant Pathology, University of Minnesota, St. Paul, MN 55108.

Verticillium albo-atrum (VA) and *V. dahliae* (VD), the causal agents of Verticillium wilt of potato are both present in the Red River Valley of Minnesota and North Dakota. Host resistance in five commercial cultivars to both species was evaluated at four locations. Vascular colonization (Log_{10} CFU + 1/0.1 ml) at 100 days after planting and percent yield loss at maturity was determined in 1993. Reddale had the lowest vascular colonization 1.26 (VA) and 1.36 (VD) and lowest percent yield losses, 10.69% (VA) and 8.6% (VD). Vascular colonization for Russet Norkotah was 1.69 (VA) and 2.67 (VD) and had the highest yield losses of 33.59 and 25.93% respectively. Kennebec had the highest vascular colonization, 1.99 (VA), 3.07 (VD) and yield losses of 27.24 and 18.11% respectively. Russet Burbank was susceptible based on CFU counts of 1.92 (VA) and 1.82 (VD) but yield losses were only 12.0 and 9.78% respectively. Norland had intermediate resistance with CFU values of 1.34 (VA) and 1.64 (VD) and yield losses of 12.0 and 9.78%. Vascular colonization was a good indicator of yield loss for Reddale, Norland, Kennebec, but not for Russet Norkotah and Russet Burbank in 1993.

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MONOCLONAL ANTIBODIES TO POTATO VIRUS Y (PVY) HELPER COMPONENT ARE USEFUL FOR PVY STRAIN IDENTIFICATION. T. Canto[1], P. Ellis[2], G. Bowler[2], and D. López-Abella[1], [1]Centro de Investigaciones Biológicas, C/. Velázquez, 144. 28006. Madrid, Spain and [2]Agriculture Canada Research Station, 6660 NW Marine Drive, Vancouver, B.C., Canada, V6T 1X2

A panel of 10 monoclonal antibodies (MAbs) and a rabbit antiserum (PAb-HC) were prepared against purified PVY helper component (HC). The MAbs and the PAb-HC were compared in direct antigen- binding ELISA for reactivity with HC of 22 isolates of PVY, potato virus A (PVA), tobacco etch virus (TEV) and pepper mottle virus (PepMoV). Neither the MAbs nor the PAb-HC reacted with PVA and TEV HC. The immunoreactivity of one MAb was similar to the PAb-HC; it reacted with HC of all 22 PVY isolates tested and PepMoV HC but failed to react with those of PVA and TEV. Another MAb reacted with HC of all the common strains of PVY (PVY^O) but not with any of those of tobacco veinal necrosis strains (PVY^N). The other MAbs could be used to differentiate HC subgroups within the PVY^O and PVY^N strain groups.

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ETIOLOGY OF THE MULTIVIRUS COMPLEX AFFECTING TOMATOES IN ALABAMA. E. J. Sikora and R. T. Gudauskas. Department of Plant Pathology, Alabama Cooperative Extension Service, Auburn University, AL 36849.

In 1992, a multivirus epidemic reduced fruit production by an estimated 25% in the major tomato growing region of Alabama. Cucumber mosaic virus (CMV) alone or in combination with potato virus Y (PVY) and/or tobacco etch virus (TEV), was responsible for the crop failure. To monitor virus incidence in 1993, fields were sampled weekly from the time of transplanting through harvest. A total of 23 fields were surveyed for CMV, PVY, and TEV, as well as tobacco mosaic virus (TMV) and tomato spotted wilt virus (TSWV). Results indicate that the viruses were not introduced through infected transplants. Virus incidence remained low until 3 weeks after transplanting in the earliest settings (April-May). Virus incidence increased rapidly thereafter and often exceeded 90% by harvest. Symptoms in the early settings were mild and often did not appear until late in crop development. Yields were not adversely affected. Viruses were detected earlier and incidence increased rapidly in fields transplanted after the first week in June. In these fields, viruses were detected within two weeks after transplanting and symptoms appeared earlier, and were more severe than in earlier settings. In most cases, yield was significantly reduced. All five viruses were detected at varying levels (CMV 21.4%; PVY 15.8%; TEV 5.9%; TSWV 13.0% and; TMV 7.8%).

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BIOQUANTIFICATION OF AIRBORNE MOVEMENT OF A SULFONYLUREA HERBICIDE. M. A. Bhatti, G. I. Mink, A. S. Felsot, and R. Parker, Washington State University, Richland WA 99352.

Twenty six plant species were evaluated for use in a biological assay to quantify airborne movement of chlorsulfuron, a sulfonylurea herbicide. 'Othello' beans and 'Dark Skin Perfection' peas exposed to sub-lethal concentrations ($\geq$ 1μg/ml and $\leq$ 7.8 μg/ml) of chlorsulfuron produced diagnostic, quantifiable diffuse chlorotic spots within 4-6 days. However, chlorsulfuron concentrations below 1 μg/ml enhanced fresh biomass of beans and peas. In another experiment, diagnostic chlorotic spots developed on plants placed in an enclosed space up to 30 minutes after release of chlorsulfuron aerosols into calm air. In field experiments, movement of chlorsulfuron at 3-10 mph wind speed, was detected 300 meters downwind of the release point; as the distance from the release point increased, the number of discrete chlorotic spots decreased. With wind speeds of 13-20 mph, number of chlorotic spots at a given distance were greater than at the lower wind speed. The data suggested that biological indicator plants can be used to monitor airborne movement of phytotoxic chemicals like sulfonylurea herbicide, especially when analytical methods are not readily available.

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SPRAY NOZZLE WEAR MEASURED BY ELECTRON BEAM ANALYSIS. C.R. Krause, D.L. Reichard, R.D. Brazee and R.D. Fox. USDA, Agricultural Research Service, Application Technology Research Unit, Ohio State University, 1680 Madison Ave., Wooster, OH 44691.

Worn nozzles on spray equipment can severely affect the efficiency of crop management systems while causing unnecessary pesticide contamination of non-target areas. Electron beam analysis (EBA), which has been applied to direct measurement of fungicide deposition, was used to observe both worn and unused brass and stainless steel spray nozzles. Wear patterns and other changes were observed on both types, with X-ray spectra displaying chemical constituents of each nozzle material. EBA could provide nozzle manufacturers with needed information on nozzle mechanics improving performance and enhancing crop protection systems for growers.

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ANALYSIS OF BELL PEPPER BACTERIAL SPOT EPIDEMICS IN NORTH CAROLINA. C. S. Kousik and D. F. Ritchie, Department of Plant Pathology, North Carolina State University, Raleigh, NC 27695.

Bacterial spot of bell peppers caused by *Xanthomonas campestris* pv. *vesicatoria* was related to environmental factors in experiments conducted in 1991 and 1993. Epidemics were initiated by introducing diseased plants as initial inoculum source into plots. In 1991 maximum temperatures correlated significantly with disease severity ratings (r=0.77-0.81). Similarly, in 1993, log transformed disease severity ratings (LDR), correlated (P=0.05) with minimum (MIN, r=0.68-0.82) and maximum (r=0.57-0.73) temperatures, total rainfall (PR10, r=0.51-0.57) and rainy days (PRD10, r=0.57-0.64) in a 10-day period six days prior to the disease ratings. In 1993 epidemics progressed in a bimodal pattern. An initial peak in disease severity during mid Jun developed after a single hail storm on Jun 5, followed by a gradual decline until end of Jul. A second peak developed in early Aug, followed by a gradual decline. Spatial autocorrelation analysis (LCORR2), indicated a significant (P=0.05) effect of wind direction in disease spread. Stepwise regression was used to select environmental variables that influenced disease progress. The model that accounted for the largest amount of variation (R^2=0.81) in 1993 was Y=-5.3 + 0.32MIN + 0.29PR10, where Y=LDR, at any given time during the season. Similarly, models with maximum or minimum temperatures during rainy periods (PRD10), alone and combined with total rainfall (PR10) were developed. Rainfall two wk prior to disease ratings and temperatures during the wk before disease ratings significantly influence disease progress.

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YIELD LOSS OF BELL PEPPERS DUE TO THE BACTERIAL SPOT PATHOGEN, *XANTHOMONAS CAMPESTRIS* PV. *VESICATORIA*. C. S. Kousik and D. F. Ritchie, Department of Plant Pathology, North Carolina State University, Raleigh, NC 27695.

Bacterial spot of bell peppers is an important disease in pepper growing regions in the Southeast. Copper or copper + maneb concentrations and spray intervals were used to generate a range of foliar disease severities recorded on a 0-9 scale. In 1991, two sites, one with copper resistant and sensitive, and other with only copper sensitive strains were used to initiate bacterial spot epidemics on cv. Bell Captain. In 1992 (cv. Jupiter) and 1993 (cv. Jupiter, King Arthur & Rebell) a mixture of three races (1,2,3) of pathogen strains resistant and sensitive to copper were used. Yield loss and area under the disease progress curves (AUDPC) were correlated over years (r=0.71-0.89, P >0.0001). The mean yield loss in 1991 was up to 51% and 75% in untreated checks inoculated with the copper resistant and copper sensitive strain sites respectively. In 1991 losses up to 64 % were observed in similar checks. Total loss for some treatments were observed in 1993, in part due to a hail injury, which drastically defoliated the plants and increased disease severities during the initial flowering stages. Disease during flowering and fruit set stages had significant impact on yield. A critical point yield loss model for cv. Jupiter based on 1992 data was validated in 1993, and predicted and actual losses were significantly correlated (R^2=0.72). Based on 1991 and 1993 data, multiple point (disease severity rating) models selected using Mallow's C_p statistic, improved yield loss estimations and correlations (R^2=0.77-0.98). The use of copper sprays in combination with resistant hybrid pepper resulted in less disease and greater yields in 1993.

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Effect of temperature on infection of soybeans by the southern biotype of the stem canker pathogen, *Diaporthe phaseolorum* var. *caulivora*. J.C. Rupe and E.A. Sutton, University of Arkansas, Fayetteville.

To determine the effect of temperature on infection rate, 2-wk old greenhouse-grown seedlings (cv. Walters), were inoculated with a 10^6 ascospore/ml suspension of the southern biotype of *D. phaseolorum* var. *caulivora*. Inoculated seedlings were placed in a dew chamber at either 15, 20, 22, 25, 27, 30, 32, or 35 C. Four randomly selected seedlings were removed after 8, 24, 36, 48, 72, and 96 hours. Stems and petioles of each plant were cut in 1 cm sections, surface disinfested, and placed on PDA to determine the percent infected. Fastest infection occurred from 22 to 30 C and required 40 hr of leaf wetness to reach 50% infection. The infection rate dropped sharply above and below this temperature range. These data will be used in a model to determine the need for early fungicide application to control stem canker.

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IDENTIFICATION AND CHARACTERIZATION OF PATHOTYPES OF *UROMYCES APPENDICULATUS* FROM NEBRASKA WITH IMPLICATIONS FOR THE DEVELOPMENT OF RESISTANCE. C.M. Sandlin, D. O'Keefe, and J.R. Steadman, Dept. of Plant Pathology, University of Nebraska, Lincoln.

Samples of the bean rust fungus *Uromyces appendiculatus* were collected from 19 sites throughout western Nebraska in 1992. Eighty one isolates were purified in the greenhouse and characterized using differential virulence on a set of 19 standard *Phaseolus vulgaris* differential genotypes. Most of the isolates fell into one of five virulence groups. The most frequently encountered virulence group corresponds to Race 54 described by Stavely (Plant Dis. 68:95-99). The rise in frequency of Race 54 corresponds to the introduction and wide spread use of the bean cultivar Olathe as a rust resistant pinto in Nebraska and Colorado. Race 54 is highly virulent on Olathe, as are the isolates from the other four common virulence groups encountered in this study. RAPD analysis of selected isolates will be presented. The objective of the RAPD work is to determine the genetic similarity of the isolates of Race 54, compared to other isolated from Nebraska and Latin America.

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ISOLATES OF *UROMYCES APPENDICULATUS* WHICH OVERCOME ADULT PLANT RESISTANCE OF ANDEAN *PHASEOLUS VULGARIS*. C.M. Sandlin & J.R. Steadman, Dept. of Plant Pathology, University of Nebraska, Lincoln.

A pathotype of the bean-rust fungus has been found to be virulent on PC-50 and other pubescent bean lines of Andean origin reported to have adult plant resistance. Large uredinia (types 5 and 6) have been observed on field-grown PC-50 in the Dominican Republic since 1991. Greenhouse studies conducted in Nebraska demonstrated that the pathotype is also virulent on primary and adult leaves of several other pubescent Andean bean genotypes considered to have race-nonspecific adult-plant resistance. Inoculation of standard bean rust differential genotypes and Andean and Mesoamerican landrace material has shown that the Dominican Republic isolates and other distinct isolates from the Americas and Africa, are highly virulent on Andean genotypes, but not on genotypes of Measoamerican origin. RAPD analysis of these Andean specific pathotypes will be presented.

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GEOSTATISTICAL ANALYSIS OF EPIDEMIC DEVELOPMENT CAUSED BY *PHYTOPHTHORA CAPSICI* IN COMMERCIAL BELL PEPPER FIELDS. Robert P. Larkin[1], Marcia L. Gumpertz[2], and Jean B. Ristaino[1], [1]Dept. of Plant Pathology and [2]Dept. of Statistics, North Carolina State University, Raleigh NC 27695-7616.

Geostatistics were used to characterize temporal changes in the spatial patterns of disease severity during Phytophthora epidemics in seven bell pepper fields. Semivariograms constructed over four directions (0, 45, 90, and 135° azimuth) demonstrated increasingly strong spatial dependence and anisotropy with time in four of the seven fields, indicating aggregated patterns with distinct directional orientation. Spatial dependence was strongest and correlations among adjacent quadrats were highest in the within-row direction (r=0.60 to 0.76) at later stages of the epidemic compared to other directions (r=0.30 to 0.65). The range of spatial dependence was >16 m in the within-row direction, but was generally lower in the across-row direction (3 to 15 m). Pathogen spread occurred predominantly down rows in these fields. Semivariograms for disease were flat throughout the season in three fields, indicating no spatial dependence and a random distribution of disease. Geostatistical analysis provided a useful tool for quantification of the spatial and temporal dynamics of epidemic development in these fields.

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CLONAL FREQUENCY AND DISTRIBUTION OF *SCLEROTINIA SCLEROTIORUM* IN FIELD POPULATIONS OF CANOLA **Y. Kohli**, Brunner, L. J., Anderson, J. B., Morrall, R. A. A, and Kohn, L. M. Depts. of Botany and Statistics, Univ. of Toronto, Erindale College, Mississauga, Ont., Canada L5L 1C6 and Dept of Biology, Univ. of Saskatchewan, Saskatoon, Canada S7N 0W0.

In a two year study, four canola fields were sampled intensively in grids to determine the spatial distribution and frequency of clones of *Sclerotinia sclerotiorum* during the disease cycle of sclerotinia stem rot. Clone frequencies in samples taken from petals (inoculum stage) were compared with frequencies in samples from stem lesions to screen for evidence of selection. 638 clones were identified by DNA fingerprinting among 2751 isolates. For each clone, the observed average euclidean distance between individual isolates of the same clone was compared to the expected average distance based on 5,000 randomized data permutations. Little deviation from random spatial distribution of clones between petal and lesion samples and within the total sample for each field was observed in either year. Chi-square analysis of clone frequencies showed no significant difference between petal and lesion samples in 1991, but significant differences in 1992. Significant differences in clone frequencies were observed between both fields in 1991 and between both fields in 1992.

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UTILIZING A LARGE DATABASE TO STUDY LEAF RUST RACE AND VIRULENCE GENE DISTRIBUTION. D. L. Long, USDA/ARS. Cereal Rust Laboratory, St. Paul, Mn 55108 and J. J. Roberts, USDA/ARS. Georgia Agricultural Experiment Station, Griffin, GA 30223.

Eight years (1986-93) of U.S. wheat leaf rust survey data are available in computer database format. Methods of sorting and tracking races using data from 4,588 collections (6,520 isolates) enable detailed analysis of 183 races and, hence, the 14 virulence genes which define these races. Race phenotype and individual gene frequency can be easily traced to study patterns of virulence occurrence, relationships to cultivar resistances and competitive fitness of genes for virulence. The database can be used to study the origin and spread of races which become important and/or predominant. For example, monitoring race PNM-10,18, is important since it is the first reported incidence of combined virulence to Lr9 and Lr24. This race is also unusual in its widespread distribution in the first year it was detected. By tracking race location and prevalence each year, we can discern patterns of overwintering, dispersal and distribution of races. This will make a comprehensive evaluation of certain facets of wheat leaf rust population genetics feasible.

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SOURCES, AND SPREAD OF POTATO VIRUS Y (PVY) AND CUCUMBER MOSAIC VIRUS (CMV) INTO PEPPER (*CAPSICUM ANNUUM*) CROPS IN NATAL, SOUTH AFRICA. K.Budnik, J.V. da Graça and M.D. Laing. Dept. of Microbiology and Plant Pathology. University of Natal, P.O.Box 375 Pietermaritzburg 3200, South Africa.

PVY and CMV pose a serious threat to commercial pepper production in the Natal province, South Africa. The spread of the viruses both into and within a field of peppers on the Natal South Coast was monitored bi-weekly, starting at the time of planting. A survey of possible virus weed hosts was also carried out in the same area. All samples collected were tested for PVY and CMV using ELISA. The location of each sample was recorded to illustrate spread into the pepper crop from surrounding source plants. PVY was detected in the first week after planting and 100% infection was observed two months later. CMV spread into the crop was slower and virus incidence was low. *Nicandra physaloides* and *Solanum nigrum* were the most prevalent alternate hosts to PVY while *Bidens pilosa*, *N. physaloides*, *Galinsoga parviflora*, *Chenopodium album*, *C. carinatum*, and *Tagetes minuta* were common hosts to CMV. Although no TSWV was detected in peppers, the weed survey indicated several weed species as hosts to the virus and therefore pose a potential threat.

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TEMPORAL AND SPATIAL CHARACTERIZATION OF WATERMELON MOSAIC VIRUS 2 (WMV-2) INCIDENCE. Mora-Aguilera,G.,[1] Webb,S.E.,[2] Purcifull,D.E.,[1] Zettler,F.W.,[1] Chellemi,D.O.[3] and Kok-Yokomi,M.L.[2] Plant Pathology Department, University of Florida, Gainesville,FL 32611[1]; CFREC, Leesburg, FL 34748[2]; NFREC, Quincy,FL 32351[3].

Average apparent infection rates of WMV-2 epidemics ranging from 0.193 to 0.225 logit units/day (r^2 > 0.93, $p \leq 0.05$) were found in four experimental watermelon plots (sown 24 March 1993) at Leesburg, Florida. Average time to onset of epidemic and epidemic duration at ≥ 95% incidence were 30 and 45 days, respectively. Fruit yield of 253 individual plants, but not fruit sugar content, was directly correlated with the number of days that plants remained healthy (r-spearman of 0.73, p<0.01 and 0.31, p=0.32, respectively). Random and aggregated patterns, determined by Gray's two-dimensional distance class analysis, were observed during development of the epidemics. Edge effects and clusters of at least nine plants were detected on some dates. *Uroleucon pseudambrosiae, Aphis middletonii,* and *A. gossypii* were the most common aphid species caught on green tile traps (137, 137 and 119, respectively, of the 751 aphids caught). Respective population peaks of these aphids occurred on 14 May, 25 April and 5 May. Factor analysis, varimax rotated biplot displays, and one month lag-time regression of factor scores with incidence change (Y) were used as a basis for determining the most important vectors, *U. pseudambrosiae* (Up) and *A. gossypii* (Ag). Their numbers were significantly correlated to change in disease incidence { Y = 0.20 + 0.11(Up) + 0.07(Ag), R^2 =0.77,p<0.01, Cp-Mallows=2.75}.

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EPIDEMIOLOGICAL STUDIES ON APHID-TRANSMITTED VIRUSES AFFECTING MELONS IN CALIFORNIA. K. C. Umesh[1], J. Valencia[2], W. D. Gubler[1], R. E. Plant[3], and B. W. Falk[1]. [1]Dept. of Plant Pathology, [2]UC Cooperative Extension, [3]Dept. of Agronomy, University of California, Davis, CA 95616.

Studies were conducted to identify the spatial and temporal incidence of aphid-transmitted viruses of melons in California. The most common viruses were watermelon mosaic potyvirus-2 and cucumber mosaic cucumovirus, followed by squash mosaic comovirus and cucurbit aphid-borne yellows luteovirus. Virus incidence was mapped in 42 fields located throughout the San Joaquin Valley of California. Migratory aphid populations were continuously monitored at two sites, and local aphid populations were assessed in 20 fields. Virus incidence and severity varied among the fields. Migratory aphid populations peaked in the spring and late summer, whereas local aphid populations were relatively high in some fields throughout the summer. Virus incidence was not correlated with migratory aphid populations, but was often evident ca. 2 weeks after high local aphid activity. JMS stylet oil was tested in 7 field trials for control of virus spread. The efficacy of JMS stylet oil varied with inoculum pressure, but delayed initial infection and virus spread.

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THE GENETICS OF MATING INCOMPATIBILITY IN *DIDYMELLA RABIEI*. A. D. Wilson[1], and W. J. Kaiser[2]. [1]USDA Forest Service, Southern Hardwoods Laboratory, P.O. Box 227, Stoneville, MS, 38776, and [2]USDA Agricultural Research Service, Western Regional Plant Introduction Station, Washington State University, Pullman, WA, 99164.

Didymella rabiei, the teleomorph of *Ascochyta rabiei*, is a serious pathogen that causes a devastating blight of chickpea (*Cicer arietinum*) in Eurasia and the U.S. Pacific Northwest. Fifteen single-ascospore isolates derived from diseased chickpea stems, collected at a single location in Genesee, Idaho, were paired in all possible combinations on sterile chickpea stems pieces incubated on moist sterile filter paper in glass petri dishes for 6 weeks at 10 C or in nylon mesh bags placed on the soil surface outdoors to overwinter for six months. Successful matings were indicated when large numbers of viable ascospores were discharged from mature pseudothecia. Pairings between sympatric isolates indicated that the fungus is

heterothallic with a unifactorial (bipolar) mating incompatibility system. Papazian test pairings between allopatric isolates of each mating type from three locations in Idaho and Washington demonstrated that the fungus does not have multiple alleles at the single mating locus. This was corroborated by similar pairings between mating types from different areas of the world. The nuclear condition of all phases of the life cycle was examined using Giemsa stain. Both ascospores and conidia were commonly multinucleate and appeared to undergo multiple mitotic divisions prior to germination. Somatic hyphae derived from ascospores and conidia were predominately uninucleate.

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ARMILLARIA ROOT DISEASE IN DOUGLAS-FIR PLANTATIONS THINNED, PRUNED, AND FERTILIZED TO INCREASE TREE VIGOR. Pablo H. Rosso and Everett M. Hansen, Dept. of Botany and Plant Pathology, Oregon State University, Corvallis, OR 97331.

The relationship between tree vigor and Armillaria root disease was compared in four Douglas-fir plantations in western Oregon that were subject to different treatments to enhance tree growth and growth efficiency. Plots on each site were thinned to three different density levels, and in each density level four sub-plots, one fertilized, one pruned, one fertilized and pruned, and one left untouched, were set. Growth was measured every two years, and growth efficiency was calculated for each tree. Approximately 6 years after the experiment was started, *Armillaria* infections were evident on all sites. All trees in the four sites were inspected for signs and symptoms of *Armillaria* infection. Vigor status of symptomatic trees infected by *Armillaria* was compared with vigor of healthy trees. In each site, 7 to 11 fungal samples were taken and diploid-diploid and haploid-diploid pairings were carried out to determine the number and size of clones and to identify the species of *Armillaria* present. Disease levels varied at each site. One site had only a few diseased trees, and disease was absent within the sub-plots. In the rest of the sites disease was present in 6 to 9 of 12 sub-plots. Trees in all vigor categories showed signs and symptoms of aggressive *Armillaria* infection. Although other species were found, *Armillaria ostoyae* was responsible for most of the root infection. Reproducibility and ease of the pairing methodology was assessed.

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ASH YELLOWS INCREASE IN YOUNG WHITE ASH POPULATIONS. W. A. Sinclair and H. M. Griffiths; Dept. Plant Pathology, Cornell Univ., Ithaca, NY 14853

Ash yellows (AshY) epidemics were compared in terms of disease incidence and rate of increase in six white ash (*Fraxinus americana*) populations on four sites in central New York State in 1990-1994. Each of 110 to 307 ash per population was tested with DAPI (4',6-diamidino-2-phenylindole·2HCl) for MLO infection and observed for symptoms annually. Based on DAPI results, initial incidence ranged from 10% to 45%, and AshY increased 0% to 8% per year. The overall average rate of increase was 4.9% per year. Highest rates occurred in populations (reproduction, saplings, or trees up to 30 yr old) not mixed with other woody species. No increase occurred in a population of ash reproduction intermixed with other species beneath an ash overstory in which AshY was increasing. AshY incidence and increase based on symptoms were closely correlated with results of DAPI tests.

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Influence of root rot on Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) seedling survival on reforestation sites and assessment of *Fusarium* and *Cylindrocarpon* root colonization. P. E. Axelrood[1], M. Lam[1], R. Peters[1], G. Shrimpton[2] and D. Trotter[2]. [1]B.C. Research Inc., 3650 Wesbrook Mall, Vancouver, B.C and [2]Ministry of Forests, Nursery Extension Services, Green Timbers Reforestation Centre, 14275 - 96th Ave, Surrey, B.C.

Numerous Douglas-fir plantations established in southwestern British Columbia have exhibited poor performance and in some cases high seedling mortality. Concern has been expressed regarding a connection between *Cylindrocarpon* and *Fusarium* root rot and/or symptomless root infections of nursery grown Douglas-fir seedlings and survival and growth of seedings on reforestation sites. Field trials were planted at three locations with Douglas-fir container grown seedlings from four conifer nurseries. Seedlings from the same nursery which appeared healthy or had root rot had similar levels of root colonization by *Fusarium* (14% - 16%) and *Cylindrocarpon* (52% - 66%), as determined by isolating these fungi from bleach treated roots. *Cylindrocarpon destructans* was isolated most frequently from seedling roots. Seedling survival and seedling growth was significantly reduced four months after planting for seedlings with root rot symptoms. The proportion of roots colonized by *Fusarium* and *Cylindrocarpon* could not be used as a predictor of seedling performance on reforestation sites.

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EFFECT OF WATER STRESS ON PATHOGENICITY OF TWO *EUCALYPTUS* CANKER PATHOGENS. I.P. v.d. Westhuizen[1], M.J. Wingfield[1], W.J. Swart[2] & G.H.J. Kemp[1], [1]Department of Microbiology and Biochemistry, University of the Orange Free State, Bloemfontein 9300; [2]Department of Plant Pathology, University of the Orange Free State, Bloemfontein 9300

Eucalyptus species are extensively planted by the South African forestry industry. *Cryphonectria cubensis* and *Endothia gyrosa* are two closely related canker pathogens of *Eucalyptus*. *Cryphonectria cubensis* is restricted to areas of high rainfall and temperature whereas *E. gyrosa* has a wider distribution. The aim of this study, was to determine whether a relationship exists between virulence of these fungi and water stress. Artificial inoculation trials were conducted with both pathogens on trees under stress as well as under conditions of normal water availability. Stress conditions were regulated by using a pressure bomb. Trees were more susceptible to *C. cubensis* under non-stressed conditions while the opposite was true for *E. gyrosa*. This is consistent with field observations where *C. cubensis* is more severe in areas of higher rainfall and *E. gyrosa* under conditions of drought stress.

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PITCH CANKER DISEASE OF WHITE PINE SEEDLINGS IN INDIANA. T.S. McCay-Buis[1,3], T.S. Abney[2], R.B. Cummings[1] and D.M. Huber[3]. Division of Entomology & Plant Pathology[1], Indiana Department of Natural Resources, Indianapolis, IN 46204, and USDA-ARS[2] and Department of Botany & Plant Pathology[3], Purdue University, W. Lafayette, IN 47907.

Two-year-old nursery grown white pine seedlings (*Pinus strobus* L.) were found in Stark County, Indiana with resinous cankers on the main stems. A *Fusarium sp.* was isolated on potato dextrose agar and pathogenicity was established by inoculating 2-yr-old white pine (*Pinus strobus* L.) and Virginia pine (*P. virginiana* Mill.) seedlings between 5 and 10 cm below the growing point of the inoculated branch. Resulting lesions girdled the branch and killed foliage distal to the point of inoculation. A known isolate of the pitch canker pathotype of *Fusarium subglutinans* f. sp. *pini* caused identical symptoms on both white pine and Virginia pine seedlings. Wounded and unwounded controls remained disease free. Species identification of the original isolate of *Fusarium* from the canker on white pine in Indiana is currently in progress.

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HICKORY DYING IN WISCONSIN ASSOCIATED WITH ATTACK BY *SCOLYTUS QUADRISPINOSUS* AND AN UNDESCRIBED *CERATOCYSTIS* SP. E. B. Smalley, Department of Plant Pathology, University of Wisconsin-Madison, 1630 Linden Dr., Madison, WI 53706.

Mass attacks on hickory in Wisconsin by *Scolytus quadrispinosus* were observed on *Carya ovata* in Dane (1986), Rock, Columbia, Dodge, Green Lake and Taylor Counties (1993) and on *C. cordiformis* in La Crosse, Vernon and La Fayette Counties (1986 to 1990), and Chippewa County (1993). In 1986, I isolated a previously undescribed *Ceratocystis* species from bark cankers and discolored wood associated with beetle attacks. Additional strains were isolated again in 1993 from dying hickory in Rock, Green Lake, and Chippewa Counties. Pure cultures were obtained from bark lesions, discolored xylem, larvae and occasionally from adult beetles. Perithecia developed naturally in brood galleries and were produced in abundance on PDA. In field and greenhouse trials, the fungus was highly virulent on members of the Juglandaceae (e.g. hickory, shagbark hickory, pecan, butternut and black walnut), and suggests that it plays a major role in hickory dying following outbreaks of the beetle.

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INITIATION OF SECONDARY GROWTH IN LOBLOLLY PINE ROOTS ALTERS PROTECTION FROM INFECTION. **C.H. Walkinshaw. USDA Forest Service, Box 5500, Pineville, LA 71361-5500.**

Numerous studies describe the microflora of roots but fail to consider relationships between secondary root development and rhizosphere microorganisms. This study on loblolly pine (Pinus taeda L.) roots considers the variables of site and time of the year on incidence of shedding outer cells and on fungal hyphae in root tissues. This shedding precedes diameter growth and causes temporary exposure of cells and intercellular spaces to the soil microflora. In Central Louisiana, 60% of the roots sampled shed their cortex or cork cambium in September. Numerous microorganisms appear within the mass of cells that are being shed and infection of the root by facultative parasites may follow. Microscopical examination of sectioned roots confirms these infections. The process of shedding in loblolly pines roots may regulate rhizosphere microbial growth by nutrient depletion in surface layers of the root.

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OCCURRENCE OF *PYTHIUM* SPECIES ISOLATED DISEASED SOYBEAN SEEDLINGS IN IOWA SOYBEAN FIELDS. S.S.A. Rizvi, X.B. Yang, G.L. Tylka, and D.C. McGee. Department of Plant Pathology, Iowa State University, Ames, IA 50011.

Species of *Pythium* isolated from soybean seedlings with post-emergence damping-off symptoms in 41 Iowa soybean fields in 1993 were determined. A total of 131 isolates of *Pythium* were keyed out based on morphology. *Pythium irregulare*, *P. ultimum*, *P. aphanidermatum*, *P. sylvaticum*, and *P. myriotylum* were identified in 38.9, 27.5, 17.6, 14.5, and 1.5% of the samples, respectively. In most cases, different species were isolated from plants in the same fields. In one field sampled in June, 88.9% of isolates were *P. ultimum*. A field in which all isolates were typed as *P. aphanidermatum* was found in late spring. *Pythium irregulare* was recovered from plants collected throughout period of sampling (mid-June to late July) whereas other species were recovered at fewer sampling times.

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OCCURRENCE AND FREQUENCY OF METALAXYL INSENSITIVITY AND MATING TYPES OF *PHYTOPHTHORA INFESTANS* IN THE COLUMBIA BASIN OF OREGON AND WASHINGTON. Philip B. Hamm, *Barbara A. Fry, and Joy Jaeger. Hermiston Agricultural Research and Extension Center, Oregon State University, P.O. Box 105, Hermiston OR 97838, *Department of Plant Pathology, Cornell University, Ithaca NY 14853.

Late blight is a sporadic disease in the semi-arid potato production area of the Columbia Basin of Oregon and Washington. During the past three years cooler temperatures and high rainfall have caused increasing incidence and severity of the disease. Metalaxyl was widely used in the area during all years to control late blight. In 1992, 30 isolates of *Phytophthora infestans* were collected from wide ranging areas and all were sensitive to metalaxyl (10 µg/ml). In contrast, all but two of 62 isolates collected in 1993 belonged to intermediate or insensitive groups. The mating type of the 30 isolates collected in 1992 was A1, whereas at least 5 of 62 isolates collected in 1993 were A2. This is the first report of the A2 mating type in Oregon or Washington.

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EARLY BLIGHT RESISTANCE FROM WILD *SOLANUM* SPECIES J. P. Helgeson, R. V. James and W. R. Stevenson. USDA/ARS and Dept. of Plant Pathology, University of Wisconsin, Madison, WI 53706.

Solanum brevidens, S. bulbocastanum, and *S. polyadenium* are highly resistant to many plant pests including, *Alternaria solani.* Although these species are sexually incompatible with potato, fertile somatic hybrids were obtained by fusing protoplasts of the wild species and potato. Eleven different somatic hybrids and their sexual progeny have been tested in the field at Hancock, Wisconsin. All somatic hybrids were more resistant than the potato (PI 203900 or Russett Burbank) used in the fusions but less resistant than the wild species. Progeny from crosses of the hybrids with susceptible potato lines segregated for resistance. Progeny as susceptible as the potato parent and lines as resistant as the wild species were recovered. We have tested three backcross generations from one somatic hybrid and have highly resistant selections which have good tuber characteristics and outyield the potato cultivar parents.

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EFFECT OF EARLY SEASON IRRIGATION REGIME ON SEVERITY OF POTATO EARLY DYING AND TUBER YIELD. M. R. Cappaert, M. L. Powelson, N. W. Christensen, W. R. Stevenson, and D. I. Rouse, Oregon State University, Corvallis, and University of Wisconsin-Madison, Madison.

Varying the application of water prior to tuber initiation was assessed as a method to manage potato early dying in cultivar Russet Burbank in field plots in eastern Washington and central Wisconsin. Treatments were pre-tuber initiation irrigation regime (50, 100, or 150% estimated consumptive use by the plant) and inoculum density (noninfested or infested with 5, and 25 or 50 cfu V. dahliae/g soil). Severity of potato early dying was, on average, 31% lower in the deficit compared to the excessive irrigation treatment and 2-fold greater in infested plots compared to noninfested plots. Effect of pre-tuber initiation irrigation on total tuber yield was inconsistent. In contrast, the higher levels of inoculum significantly ($P \leq 0.05$) reduced tuber yield. Early season irrigation management may be a viable option to minimize losses due to potato early dying in some production areas.

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COMPARISON OF VASCULAR COLONIZATION BY *VERTICILLIUM DAHLIAE* IN WILT RESISTANT AND SUSCEPTIBLE POTATOES INFECTED IN THE FIELD OR GREENHOUSE WITH POTATO VIRUS X, POTATO LEAFROLL VIRUS OR BOTH VIRUSES. L. Gyenis and E. Banttari, Department of Plant Pathology, University of Minnesota, St. Paul, MN 55108.

Field experiments from 1990-1993, indicated primary infection of Verticillium wilt resistant, cv. 'Reddale', and susceptible, cv. 'Kennebec' potatoes with potato virus X (PVX) or potato leafroll virus (PLRV) generally increased stem colonization by *Verticillium dahliae* VD.

In 1993 a field experiment evaluated vascular colonization by VD of Reddale and Kennebec infected with PVX, PLRV or both viruses the previous season in the field, or in the greenhouse. Stem colonization was measured by MAb-based I-C ELISA.

Stem colonization by VD was higher in both cultivars grown from seedstocks infected with PVX + PLRV in the field as compared to plants from virus-free seedstocks. Verticillium colonization was also higher, but more variable in plants grown from seedstocks inoculated with these viruses in the greenhouse as compared to those from virus-free plants.

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MORPHOLOGICAL AND RAPD ANALYSIS OF ISOLATES OF *DIAPORTHE PHASEOLORUM* FROM SOYBEAN. F. A. Fernández and R. T. Hanlin, Department of Plant Pathology, University of Georgia, Athens, GA 30602-7274.

Isolates of *Diaporthe phaseolorum* vars. *caulivora* (DPC), *meridionalis* (DPM) and *sojae* (DPS), were grown on various substrates in the laboratory and inoculated into soybean plants in the greenhouse. Isolates varied in their ability to produce pycnidia and/or perithecia on the different substrates. Both alpha and beta conidia were observed in DPC and DPS isolates whereas only alpha conidia were observed in DPM isolates. Morphological characters such as ascus length, ascus width, ascospore length and ascospore width were measured microscopically for each isolate/substrate combination. Statistical cluster analysis of data showed DPM and DPC isolates (highest and lowest values for all characters respectively) forming distinct clusters whereas clustering of DPS isolates overlapped both DPM and DPC clusters. DNA was extracted from DPC, DPM and DPS isolates and amplified via PCR by using 10 base-long oligonucleotide primers of random sequence (RAPD analysis). Preliminary screening of thirty primers yielded eight primers which generated distinct band patterns for these isolates. Random primers can potentially be used for distinguishing DPC, DPM and DPS isolates in soybean.

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IDENTIFICATION AND DISRUPTION OF A GENE INVOLVED IN THE AFLATOXIN BIOSYNTHETIC PATHWAY OF ASPERGILLUS PARASITICUS. N. Mahanti[1], D. Bhatnagar[2] and J.E. Linz[1]. [1]Michigan State University, East Lansing, MI [2]USDA ARS, New Orleans.

Aflatoxins are polyketide-derived secondary metabolites produced by the fungi Aspergillus parasiticus and Aspergillus flavus and are known to be the most potent naturally occurring animal carcinogens. An understanding of the aflatoxin biosynthetic pathway will help eliminate aflatoxin contamination of food and feed crops. Several genes have been cloned that are associated with this complex pathway. Recently, we have been able to clone a gene by complementation of a mutant (UVM8). UVM8 has a mutation prior to norsolorinic acid which is the first known stable intermediate in the aflatoxin biosynthetic pathway. The UVM8 gene has been successfully disrupted and the disruptants are unable to produce any detectable amounts of aflatoxin or any other known intermediates. These strains are suitable for use in biocontrol since most other aflatoxin-blocked mutants accumulate toxic intermediates. The large size of the transcript and its functional location at the beginning of the pathway suggest that UVM8 may be a polyketide synthetase gene. The fragment of DNA used to complement UVM8 is currently being sequenced to help elucidate the structure and the function of the gene.

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USE OF RAPD MARKERS TO DISTINGUISH *DIDYMELLA BRYONIAE* FROM RELATED *PHOMA* SPECIES ISOLATED FROM CUCURBITS. A. P. Keinath[1], M. W. Farnham[2], V. B. DuBose[1] and T. A. Zitter[3], [1]Clemson University, Coastal REC and [2]USDA, ARS, U.S. Vegetable Lab., Charleston, SC 29414 and [3]Cornell University, Ithaca, NY 14853.

Didymella bryoniae (anamorph *Phoma cucurbitacearum*) causes gummy stem blight and black rot of cucurbits throughout the US. *D. bryoniae* is often isolated simultaneously with other *Phoma* spp., such as *P. exigua*, from diseased cucurbit foliage, stems and seed. Although *D. bryoniae* and *Phoma* cannot be distinguished from each other in the field, the species can be identified based on cultural characteristics and pathogenicity (Phytopathology 83:1393). Twenty-two isolates of *D. bryoniae* and eight isolates of *Phoma* spp. were obtained from diseased watermelon, cantaloupe,

cucumber, pumpkin and squash grown in SC, NY, FL and CA. Total DNA was extracted from all isolates and amplified using PCR primed with random oligonucleotide decamers. Five primers were used to evaluate RAPD patterns on agarose gels. Generally, all primers were useful to distinguish *D. bryoniae* from *Phoma*. Four of five primers used revealed at least one band unique to all *D. bryoniae* or all *Phoma* isolates. Seven additional fragments were present in all *D. bryoniae* isolates, except two of the three isolates from NY. PCR primers generated from these unique *D. bryoniae* fragments should be useful to identify this species.

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RAPD ISOLATION OF FINGERPRINT PROBES FOR ANALYSIS OF COLLETOTRICHUM SPECIES. D. Cawthon, W. Dyson, [1]J. C. Correll, and D. D. Rhoads. Dept. of Biological Sciences and [1]Dept. of Plant Pathology, University of Arkansas, Fayetteville, AR 72701.

We have been analyzing genetic diversity of isolates of Colletotrichum gloeosporioides and C. acutatum from diverse plant hosts. The availability of reliable and informative fingerprint probes would greatly facilitate analysis of phylogenetic relationships in these taxa. Hybridization of labelled genomic DNA has been used to screen a battery of RAPD products to identify repetitive elements from these fungi. RAPD products identified were then used to probe Southern blots of DNA from various isolates to compare fingerprint patterns. This approach has identified RAPD products that detect numerous bands in a species specific manner. This simple technique should be generally applicable to other plant pathogenic fungi.

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GENOME COMPLEXITY OF *COLLETROTRICHUM* FROM MAIZE AND SORGHUM. Reena Randhir and Robert M. Hanau. Department of Botany and Plant Pathology, Purdue University, West Lafayette, IN 47907-1155.

We determined the genome complexity of the corn pathogen, *Colletrotrichum graminicola*, and the closely related sorghum pathogen, *Colletrotrichum sublineolum*. The GC content of the nuclear DNA of *C.graminicola* as estimated by melting temperature analysis was 52.2 %, and that of *C. sublineolum* was 53 %. C_0t analysis employing S1 nuclease digestion and a modified second order equation was used to estimate genome size and the amount of repetitive DNA. Least-square analysis of the reassociation data indicated that the haploid genome of *C. graminicola* was 4.9 x 10^7 bp, and that of *C. sublineolum* was 5.3 x 10^7 bp. The single-copy component of *C. graminicola* was 93.5% whereas 6.5% was repetitive DNA. For *C. sublineolum* the single-copy component was 92.4% and 7.6% was repetitive. Genomic reconstruction experiments confirmed the genome sizes as estimated by C_0t analysis. C_0t analysis was also used to determine the relatedness of the two genomes. Nuclear DNA from both species was mixed in equal proportions and allowed to reassociate. Reassociation occured over longer C_0t values than when each was in its pure state. This confirmed that the primary structure of the two genomes was different though closely related.

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GENETIC ANALYSIS AND MOLECULAR CHARACTERIZATION OF CONIDIATION MUTANTS IN *COLLETOTRICHUM GRAMINICOLA*. Juan Wang, Jeff Rollins, and Robert M. Hanau. Department of Botany and Plant Pathology, Purdue University, West Lafayette, IN 47907-1155.

Colletotrichum graminicola (teleomorph: *Glomerella graminicola*) is a fungal pathogen that causes anthracnose of maize. Asexual reproduction by means of conidia serves as a major mechanism of dispersal. Therefore, the process of conidiation can be an important target for disease control. Three distinct conidiation phenotypes have been identified among 10 field isolates under investigation. These phenotypes are: (i) light-dependent conidiation (wild-type), (ii) light independent-conidiation (Con1 mutant), and (ii) aconidiation (Aco1 mutant). Progeny from crosses between wild-type and each of the mutants showed a 1:1 segregation of wild-type vs. mutant phenotype, indicating a single gene mutation in both classes of mutants. Genetic analysis also suggests that the aconidiation phenotype is linked to *PYR1*, a gene involved in uridine biosynthesis and located on the largest chromosome. A dual approach involving map-based cloning and cloning by complementation is being used to recover the genes responsible for the Con1 and Aco1 phenotypes.

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CHARACTERIZATION OF *APHANOMYCES EUTEICHES* USING MOLECULAR MARKERS. Dean Malvick, Craig Grau*, Deborah Samac, and James Percich. Department of Plant Pathology, University of Minnesota, St. Paul, MN 55108 and *Department of Plant Pathology, University of Wisconsin-Madison, Madison, WI 53706.

Isolates of *Aphanomyces euteiches* Drechs., a root-infecting pathogen, can be separated into subpopulations based on their pathogenicity on pea (*Pisum sativum*), bean (*Phaseolus vulgaris*), alfalfa (*Medicago sativa*), and red clover (*Trifolium pratense*). A fifth subspecific group of *A. euteiches* isolates is avirulent on these hosts. We are investigating more definitive methods to distinguish and characterize isolates including randomly amplified polymorphic DNAs (RAPDs) and restriction fragment length polymorphisms (RFLPs). RFLP analysis using a low copy-number genomic probe from *A. euteiches* pathogenic on peas can distinguish between some pea and bean isolates. RAPD analyses were carried out with 46 ten-base oligonucleotide primers and total genomic DNA from five isolates representing each of the five subspecific pathotypes. Most (76%) of the primers amplified DNA from one or more of the isolates, and at least one amplification product profile was distinct for each pathotype. The preliminary data suggest that RAPDs and RFLPs can characterize genetic differences among isolates of the Oomycete *A. euteiches*.

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CHARACTERIZATION OF *CONEJO*, A PUTATIVE RETROELEMENT IN *MAGNAPORTHE GRISEA* M.A. Meyn[1], L. Farrall[2], F.G. Chumley[2], B. Valent[2], and M.J. Orbach[1]. Dept. of Plant Pathology, University of Arizona, Tucson 85721[1] and DuPont Experimental Station, Wilmington 19880[2]

Dispersed repetitive DNA segments known as "MGRs" were identified in *Magnaporthe grisea* rice pathogens (Hamer et al. 1989, PNAS 86: 9981-9985). Here we report the characterization of one of the MGR elements and show that it has features in common with the LINE (long interspersed element) class of retrotransposons. This element is also present at low copy number in *M. grisea* strains pathogenic on grasses other than rice. Restriction sites in element copies are conserved in strains from a wide geographic distribution. The element is 6.3 kbp in length, and sequencing of the 3' half revealed the presence of a 928 amino acid residue ORF that shows strong homology to reverse transcriptase sequences found in other LINEs. Further sequence analysis of several element copies, has identified the 5' and 3' ends of the element, and has revealed 10-12 bp direct repeats outside of the element copies, which supports the classification of this element as a LINE member. Future work will attempt to identify conditions under which this putative element transposes and the role that it may play in strain instability.

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IDENTIFICATION OF *TY1-COPIA* GROUP RETROTRANSPOSON SEQUENCES IN *PHYTOPHTHORA INFESTANS*. P. W. Tooley[1] and D. J. Garfinkel[2], [1]USDA, ARS, Frederick, MD 21702 and [2]Advanced Bioscience Laboratories-Basic Research Program, NCI-Frederick Cancer Research and Development Center, Frederick, MD 21702.

Transposable elements were identified in *P. infestans* by PCR amplification of retroelement-related sequences followed by sequencing of the PCR products. Primers used flanked the active site coding region of reverse transcriptase and were specific to retroelements of the *Ty1-copia* group (Flavell *et al.* 1992. Molec. Gen. Genet. 231:233-242). PCR products of ca. 280 and 360 bp were amplified from five *P. infestans* isolates from Mexico and the U.S. using the above primers. PCR products of both sizes obtained from Mexican isolate 580 were cloned and six of the products sequenced. Although sequence heterogeneity was observed, two sequences showed substantial amino acid sequence similarity with transposable element *Tnt1*, a *Ty1-copia* group member from tobacco. Southern blot hybridization revealed that the elements are present in multiple copies within the *P. infestans* genome, with enough banding pattern diversity observed among isolates for the elements to have potential use in strain detection, genetic mapping, and population studies.

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SEQUENCE COMPARISON OF THE CUTINASE AND PECTIN LYASE GENES OF HOST PREFERENTIAL ISOLATES OF *COLLETOTRICHUM GLOEOSPORIOIDES*. C FULTON[1], P R MILLS[2] AND J E COOPER[1], [1]DEPARTMENT OF APPLIED PLANT SCIENCE, THE QUEEN'S UNIVERSITY OF BELFAST, NEWFORGE LANE, BELFAST BT9 5PX, NORTHERN IRELAND; [2]HORTICULTURE RESEARCH INTERNATIONAL, WELLESBOURNE, WARWICK, UK CV35 9EF.

Successful penetration and colonization of plant host species by pathogenic fungi often requires the production of a variety of degradative enzymes. Cutinase and pectin lyase have been shown in previous studies to be important factors in pathogenicity of the phytopathogenic fungal species, *Colletotrichum* (Dickman M B & Patil S S, (1986) Physiol. Mol. Plant Path. *28*:235-242; Wijesundera R L C *et al*, (1984) J. Gen. Micro. *130*: 285-290.). In this study we have isolated the cutinase gene and a pectin lyase gene (*pelA*) by PCR amplification from genomic DNA using primers designed from published sequences. The genes were isolated from cultures derived from single-spore isolates exhibiting changes in host preference following repeated passage through a "non-host". The 1.6Kb cutinase and 2.5Kb pectin lyase PCR products were subsequently cloned into pBluescript and partially sequenced.

Variation in the gene sequences between isolates of *C. gloeosporioides* could be related to differences in pathogenicity/host specificity.

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RESTRICTION MAPPING AND GENE ORDER IN THE MITOCHONDRIAL GENOME OF SIXTEEN SPECIES OF PYTHIUM. **Joseph Prenger and Frank N. Martin, Plant Pathology Dept., University of Florida, Gainesville, Florida 32611**

Restriction maps have been constructed for the mitochondrial genomes of 16 species of Pythium. The location of genes encoding cytochrome oxidase I and II (CoxI and CoxII),

and the large and small subunits of ribosomal RNA (LRS and SRS) have been placed on these maps. A fifth gene, ATPase 9, has been placed on the map of P. oligandrum and will be examined for the remaining species as well. The order of CoxI, CoxII, LRS, and SRS appear to be conserved in most, but not all of the species examined. The LRS and SRS coding regions are part of the inverted repeat in all species, while CoxI and CoxII are located within the repeated region in some species and in the unique, single copy DNA in others. Data will be presented comparing gene order and restriction maps for the sixteen species examined. Intraspecific polymorphisms in restriction maps and gene order for geographically separated isolates of nine species also have been examined and will be discussed in relation to the types of variation associated with interspecific polymorphisms. The utility of using these criteria for taxonomic purposes and isolate identification will be discussed.

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GENETIC TRANSFORMATION OF AN ECTOMYCORRHIZAL FUNGUS BY PARTICLE BOMBARDMENT. S.N. Bills, D.L. Richter, and G.K. Podila. Michigan Technological University, Houghton, MI USA 49931.

The ectomycorrhizal fungus *Paxillus involutus* (Bat. ex Fr.) Fr. was transformed using particle mediated gene transfer. Transformation was determined using the hygromycin B phosphotransferase gene (*hph*) as the selectable marker and β-glucoronidase gene (GUS) as a reporter gene. Southern blot analysis confirmed that the vector DNA for both *hph* and GUS genes was integrated into the fungal genome. Variations in the number of multiple gene copies and rearrangements were found in the transformants. The hygromycin resistant transformants were mitotically stable maintaining both the *hph* and GUS genes in the fungal genome six months following transformation. Western blot analysis determined that the GUS gene was capable of translating its protein product in the transformed fungus. Enzyme assays of GUS extracts determined that β-glucoronidase was active in the transformed fungi. Pure culture synthesis experiments showed that the ability of *P. involutus* to form ectomycorrhizae with *Pinus resinosa* Ait.was not altered by transformation. We have already used this transformation technique successfully with another ectomycorrhizal fungus *Laccaria bicolor* (Maire) Orton. These results provide the first report of a successful transformation of an ectomycorrhizal fungus using particle bombardment.

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Distribution and occurrence of *Leptosphaeria maculans* virulence types in canola fields. G. S. Mahuku, P.H. Goodwin, and R. Hall. Dept. of Environmental Biology, University of Guelph, Guelph, Ontario. N1G 2W1.

The occurrence of the highly virulent and weakly virulent types of *Leptosphaeria maculans*, causal agent of blackleg of crucifers, was studied in two canola fields in southern Ontario. Using a PCR-based assay with primers specific for these virulence types, leaf, stem and crown lesions were directly examined. The highly virulent type was detected in all plant tissues tested, whereas the weakly virulent type was detected only in leaf lesions. Of 96 leaf lesions examined, 48 tested positive for the highly virulent type, and 12 for the weakly virulent type. Both types occurred in 16 lesions. There was no correlation between leaf lesion size and virulence type. Only the highly virulent type was detected in individual pseudothecia on stubble. Seed from these fields contained both the highly virulent and weakly virulent types. The highly virulent type is thus non-specific in the kind of plant tissue that it infects, whereas the weakly virulent type appears to be limited to leaves and seeds. Our study shows the advantages of using a PCR-based assay to directly detect a fungal pathogen in infected plant tissue.

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ANALYSIS OF THE ACCURACY OF PREDICTED WEATHER DATA ON THE IMPACT OF APPLE DISEASE MANAGEMENT. D. L. Truxall and J. W. Travis. Department of Plant Pathology, The Pennsylvania State University, University Park, PA 16802

Predicted weather data has become available which can allow for delivery of complex models and expert systems. To measure accuracy of the predictions, data were collected during the 1993 growing season at a standard meteorolgoical site, manually and with automated dataloggers. Data was predicted concurrently for the same site. The differences between the temperature measurements and predictions were not significantly different according to Student's t-test at $\alpha = 0.05$. Relative humidity was significantly different only during July 1993. To examine the impact of the accuracy on apple disease management, sensitivity analysis was performed on the Penn State Apple Orchard Consultant, an expert system for apple orchard management. The sensitivity analysis was performed with respect to the apple scab recommendations provided by the program. The percent change in temperature, which caused the recommendation of the expert system to change, indicated that the error associated with predicted temperature data would rarely result in an incorrect decision with regards to apple scab management.

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EFFECT OF TILLAGE, PLANTING DATE, AND CULTIVARS ON THE SOIL POPULATION DENSITY OF *MACROPHOMINA PHASEOLINA*. J. A. Wrather, S. R. Kendig, T. L. Niblack, and G. S. Smith, University of Missouri, Portageville, MO 63873.

Our objective was to determine the effect of tillage, planting date, and cultivars on the soil population density of *Macrophomina* phaseolina microsclerotia. The experimental design was a split-split plot with three tillage treatments (no-till, conventional-till, and ridge-till) as main plots, three planting dates (mid May, early June, and late June) as subplots, and four cultivars randomized within subplots. Soil samples were collected at soybean planting in 1991, 1992, and 1993 and analyzed for colony forming units of *M. phaseolina*. The soil population of *M. phaseolina* was significantly greater in no-till and conventional-till plots than ridge-till plots. The soil population was significantly greater in mid May than late June plantings, and was significantly greater where Forrest, Hartwig, and Essex were planted rather than Rhodes.

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USE OF ENVIRONMENTAL MONITORING EQUIPMENT TO FORECAST BLACK ROT (GUIGNARDIA BIDWELLII) INFECTION PERIODS IN 'SEYVAL BLANC' GRAPEVINES IN MISSOURI. J.D. Hill and J.F. Moore, Southwest Missouri State University, State Fruit Experiment Station, Mountain Grove, MO 65711.

Black rot (Guignardia bidwellii) is the most important fungal disease affecting fruit quality and yield of grapevines in Missouri. The fungus can infect the leaves and fruit of susceptible grape cultivars. Black rot infection periods can be determined by continually monitoring moisture and temperature conditions (Spotts, 1977). Certain fungicides applied within 72 hours of the beginning of an infection period will eradicate the black rot fungus. This study was conducted during three growing seasons to compare the efficacy of protective and curative fungicides applied for the control of black rot based on environmental monitoring equipment. Each experiment was designed as a randomized complete block, using 5, 2-vine replicate plots in 6-, 7- and 8-year old 'Seyval blanc' grapevines. Sprays were applied at 180 psi and 200 gal/A. Environmental monitoring equipment (Envirocaster black rot model 0.05, Neogen Corp.) was used to forecast black rot infection periods. Adequate black rot control was achieved by following Envirocaster model predictions, reducing spray applications by as many as 3 sprays per season. Data from a hydrothermograph and a DeWitt leaf wetness meter closely paralleled environmental data recorded from the Envirocaster. Curative sprays of Nova 40W were more effective at controlling black rot fruit infections than protective sprays of Dithane 75DF and Captan 50WP.

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AN IBM™/WINDOWS™-COMPATIBLE SYSTEM FOR REAL TIME WEATHER MONITORING AND PLANT DISEASE FORECASTING. W.H. Shaffer and M.M. Hulse. Dept. of Plant Pathology and Electronic Instrument Lab, University of Missouri, Columbia, MO 65211.

A system has been developed to monitor weather and forecast plant disease infection periods in real time. The system uses a Campbell 21X Micrologger™ to constantly monitor environmental parameters including: air temperature, relative humidity, leaf wetness, rainfall, wind speed, wind direction, soil temperature and soil moisture. The Micrologger™ was programmed to automatically transmit this information, via radio transmitter, every two minutes. A radio receiver is used to receive this weather data and input it to an IBM™-compatible personal computer via a serial port. A computer program has been developed to run under Microsoft Windows™ that summarizes the weather data from the Micrologger™ and forecasts infection periods for: apple scab, cedar apple rust, apple blossom fire blight, apple sooty blotch, grape black rot, Pythium blight and brown patch of turf grass and wheat scab. The system is being field evaluated during the 1994 season and the results will be discussed.

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Vital staining of fungal nuclei with the bis-benzimidazole fluorochrome Hoechst 33342. B. D. Nelson, Dept. Plant Pathology, North Dakota State University, Fargo, ND, 58105.

Hoechst 33342 (Molecular Probes Inc., Eugene, OR), a cell permeable, minor groove-binding DNA fluorochrome was evaluated for staining of live nuclei in 37 fungi in 21 genera (*Alternaria, Aspergillus, Absidia, Cochliobolus, Heterobasidion, Fusarium, Hirschioporus, Monilinia, Mucor, Pyrenophora, Phellinus, Polyporus, Phytophthora, Pythium, Rhizoctonia, Rhizopus, Saccharomyces, Sclerotinia, Trichoderma, Verticillium,* and *Zygorhynchus*). Pieces of mycelium from liquid cultures were placed in a drop of stain (50 μg/ml in distilled water [pH 7.2] or phosphate buffered saline [pH 7.4]) on glass slides for two minutes, destained in water or buffer for 15 to 30 seconds, then mounted in 50% glycerol. Using a Leitz epifluorescence microscope with a 340-380 nm excitation filter, clearly stained nuclei were observed in the hyphae of all 37 fungi. Averaged over fungi, the mean percentage of observed hyphal cells with stained nuclei was 91%. In hyphae or conidia with dark pigmentation few nuclei were stained. Nuclear staining in conidia or other reproductive structures varied among fungi, but was generally less successful than in hyphae. Because Hoechst 33342 is a non-intercalating stain that can be removed from live cells, it may be a useful tool in the study of nuclei and DNA in fungi.

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DEVELOPMENT OF A LEAF DISK ASSAY TO PRESCREEN BACTERIA FOR BIOLOGICAL CONTROL OF TARGET SPOT ON TOBACCO. R.L. Ashby, Jr. and B.H. Ownley. Entomology and Plant Pathology Dept., The University of Tennessee, Knoxville, TN 37996.

Several hundred bacteria were isolated from long-term tobacco field soils for potential use as biocontrols of Rhizoctonia diseases of tobacco seedlings grown in float beds. The purpose of this study was to develop a leaf disk assay that could be used to prescreen large numbers of bacteria for biocontrol activity against target spot (Rhizoctonia leaf blight) with minimal time and labor inputs. Inoculum of *Rhizoctonia solani* was placed on sterile, moistened horticultural mix in multi-well tissue culture plates. Disks (12 mm) were cut with a cork borer from newly emerged tobacco leaves, surface-sterilized, dipped into bacterial suspensions, and placed in the tissue culture wells. The prescreening assay was optimized for several parameters including form and age of pathogen inoculum, and length of assay incubation time. Using *Bacillus* sp. BA55 as a test organism, growth medium and incubation time for the bacterial test strains were also optimized. Strain BA55 has been shown previously to provide substantial protection against target spot in greenhouse studies.

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PRODUCTION OF *POLYMYXA GRAMINIS* ZOOSPORES IN WINTER WHEAT USING AN AUTOMATED FLOODING SYSTEM. J.E. Carroll[1], G.C. Bergstrom[1], and S.M. Gray[2], Dept. of Plant Pathology[1] and USDA-ARS[2], Cornell University, Ithaca, NY 14853.

Polymyxa graminis, the vector of wheat spindle streak mosaic virus (WSSMV), is an obligate parasite of roots. To produce synchronous zoospore inoculum, a flooding system similar to that used by M.J. Adams (Rothamsted, England) was adapted for winter wheat. Dried roots containing *P. graminis* were obtained from D.J.S. Barr (Ottawa, Canada). Fine root pieces containing resting spores (cystosori) were placed in the root hair zone of 2-4 day old seedlings grown in sterile sand. In two time course experiments, zoospores were harvested and root pieces were scanned for *P. graminis* structures weekly. Cystosori were found at five weeks. Peak zoospore yields of up to 1.5 x 10^5/ml were obtained between seven and ten weeks. A modified Johnson's solution proved the best of three nutrient solutions tested. Growth of winter wheat, which is intolerant of anaerobic conditions, was unaffected by flooding periods of 2 hr imposed twice daily. Of two WSSMV-susceptible cultivars used, 'Frankenmuth' was a better host for zoospore and cystosorus production than 'Augusta'.

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AN AUTOMATED DEVICE FOR CHARACTERIZING RAINFALL. M. A. Boudreau* and L. V. Madden**, *Dept. of Botany, Eastern Illinois University, Charleston 61920, and **Dept. of Plant Pathology, OARDC/The Ohio State University, Wooster 44691.

An automatic raindrop sampler (ARS) was developed for splash-dispersal studies in plant canopies. The ARS consists of an 18 x 9.5 x 7.5 cm rectangular box constructed of galvanized steel and acrylic sheeting, and employs a moveable shutter to briefly expose a 52 x 76 mm card of water sensitive paper (WSP) to incoming water drops. Frequency and size of drops are determined from colored spots produced on the WSP. The WSP is positioned below the shutter in an upper chamber of the ARS, on a removable platform surrounded by dessicant (to minimize WSP coloration due to high humidity). A 24 VDC pull-type solenoid in a lower chamber opens the shutter via a spring-loaded linkage to a pivoting steel rod. The ARS is small, inexpensive, and operated with low voltage, so multiple units can sample rain simultaneously in several confined locations in the field.

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HOST RANGE OF AAL-TOXIN AND ITS FUNGUS ALTERNARIA ALTERNATA. H.K. Abbas, T. Tanaka, S.O. Duke and C.D. Boyette. USDA-ARS, SWSL, Stoneville, MS 38776.

AAL-toxin was tested on 90 crop and weed species and was phytotoxic to susceptible tomatoes, prickly sida, black nightshade, and duckweed at a concentration of 5 μM or less, causing necrotic spots, wilt and death. At 70 to 1000 μM, redroot pigweed, cutleaf wild geranium, some biotypes of sunflower, small morningglory, alfalfa, crimson clover, sicklepod, hemp sesbania, northern jointvetch, venice mallow, soybean, spurred anoda, jimsonweed, Canadian thistle, field bindweed, sharppod morningglory, multicolored morningglory, cotton and velvetleaf were affected. Resistant tomato lines were not susceptible and monocots, including barley, bermudagrass, corn, johnsongrass, rice, sorghum, and wheat, were largely unaffected even at rates as high as 1000 μM of AAL-toxin. Alternaria alternata was pathogenic to susceptible tomatoes only. AAL-toxin has potential as a mycoherbicide in resistant and heterozygous tomato lines and grass crops to control weedy broadleaf species.

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SEASONAL DYNAMICS OF PUCCINIA CARDUORUM ON MUSK THISTLE. A. Baudoin and W.L. Bruckart, Dept. of PPWS, Virginia Tech, Blacksburg, VA 24061-0331, and USDA-ARS, Frederick, MD 21701.

Puccinia carduorum Jacky, a pathogen of musk thistle, has become established in the eastern USA after its introduction in 1987. Natural population development of the rust was monitored in several natural thistle stands in 1991 and 1992. Average numbers of pustules per leaf were 0-0.7 in early May during early stem elongation, 0-15 (1991) and 10-55 (1992) at seed ripening in late June and early July as old plants died, 0.2-2.5 on young rosettes in September and October, and declining to 0 by early December. Germinability of urediniospores from green tissue ranged from 10-88% (mean 51%) between May 26 and Oct 4, 1992, with no clear seasonal trends. Teliospores were present on dead plants in late July and August, but did not become prevalent on young rosettes until October and November. Latent periods (days from inoculation to first open pustule) on potted plants placed in the field were 13-14 days for inoculations in late April and early May, 8 days in June, 17 days in early October, and about 25 days in late October.

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INHIBITORY SUBSTANCES FROM FUSARIUM MONILIFORME AND ALTERNARIA ALTERNATA ON GROWTH OF DUCKWEED LEMNA MINOR L. Ronald F. Vesonder and Robert E. Peterson, National Center for Agricultural Utilization Research, USDA-ARS, 1815 N. University Street, Peoria, Illinois 61604

Aqueous methanol extracts of rice culture material (RCM) obtained from either Fusarium moniliforme (FM) or Alternaria alternata (AA) showed inhibitory activity on growth of duckweed Lemna minor L (LM). The FM RCM extract was fractionated by HPLC with a water:methanol gradient. Bioassay directed-fractionation led to the identification of the active substances fumonisin B_1, B_2, B_3, a mono-acetylated fumonisin and an unidentified metabolite (UNM). UMN at 7 μg/mL inhibited 47% of the duckweed growth. It was 10 times less active than fumonisin B_1 (IC_{50} 0.7 μg/mL). The residue from the AA RCM extract was fractionated into AAL-toxin, tenuazonic acid, and alternariol monomethyl ether. The latter two metabolites inhibited 50% of the growth of LM at ca. 33 μg/mL versus 3 μg/mL for AAL-toxin. The LM is a rapid bioassay for biological activity of the fumonisins and related compounds as AAL-toxin.

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RELATIONSHIP BETWEEN VIRULENCE, CULTURAL CHARACTERISTICS AND dsRNA IN *CALONECTRIA CROTALARIAE*. K. D. Kim, J. S. Russin, R. A. Valverde and J. P. Snow. Department of Plant Pathology and Crop Physiology, Louisiana State University Agricultural Center, Baton Rouge, LA 70803.

Previously, we reported variation in virulence among isolates of *Calonectria crotalariae* isolates on soybean and a direct relationship between virulence and ability to produce perithecia. Virulence was compared with mycelial growth, production of microsclerotia, and double-stranded RNA (dsRNA) on 25 soybean and peanut isolates. Ten isolates from various hosts such as alfalfa, anthurium, howea, koa, leea, and papaya were added for dsRNA analysis. Ranges for production of microsclerotia and mycelial growth were observed in *C. crotalariae* isolates, however none of the 35 isolates tested contained detectable levels of dsRNA. Isolates with greater levels of virulence generally produced more microsclerotia than did less virulent isolates but growth of mycelium among isolates was inconsistent regardless of virulence. Soybean isolates produced significantly more microsclerotia but not mycelial growth than did peanut isolates. These results suggest that production of microsclerotia as well as perithecia are directly related to variations in virulence in *C. crotalariae*. This suggests that *C. crotalariae* may preserve virulence through genetic recombination.

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CORRELATION BETWEEN TRANSMISSION OF HYPOVIRULENCE AND THE NUMBER OF VEGETATIVE INCOMPATIBILITY (*VIC*) LOCI DIFFERENT AMONG ISOLATES IN A NATURAL POPULATION OF *CRYPHONECTRIA PARASITICA*. Y. -C. Liu and M. G. Milgroom, Cornell University, Ithaca, NY 14853.

We tested the hypothesis that transmission rates of hypovirulence viruses among strains of *C. parasitica* are inversely proportional to the number of vegetative incompatibility (*vic*) genes that are different between isolates. In a sample of 58 isolates collected from a natural population in Finzel, Maryland, we found 32 different vegetative compatibility (vc) groups. Eight isolates, each in a different vc group, were randomly selected and infected with the virus from strain EP42. Transmission rates of hypovirulence among the 8 virus-infected isolates and the other isolates were estimated. The number of *vic* genes different among strains was estimated by crossing the donor and recipient strains and determining the proportion of ascospore progeny which were vegetatively compatible with either of the

parents. The rates of transmission among isolates which differed by one and two *vic* genes were 0.45 and 0.26, respectively. When isolates were different by more than two *vic* genes, transmission occurred in only one out of 24 pairs (4%). This study has provided evidence for a significant negative correlation between the transmission of hypovirulence and the number of *vic* genes different among isolates of *C. parasitica* in a natural population.

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HYPOVIRULENCE ASSOCIATED WITH dsRNA DISCOVERED IN *CRYPHONECTRIA CUBENSIS*. I.P. v.d. Westhuizen[1], W.A. Smit[2], M.J. Wingfield[1], & G.H.J. Kemp[1], [1]Department of Microbiology and Biochemistry, University of the Orange Free State, Bloemfontein 9300. [2]Infruitec; Private Bag X5013; Stellenbosch 7600

Cryphonectria cubensis is responsible for serious losses in commercial *Eucalyptus* plantations around the world. Currently, the only means of controlling the disease is by resistance breeding and selection. The virulence of certain fungal pathogens can be reduced biologically through hypovirulence, as is true in the closely related pathogen *C. parasitica*. Isolates of *C. cubensis* were screened for the presence of dsRNA and other hypovirulence associated traits. Pathogenicity tests were also conducted to link the presence of dsRNA with hypovirulence. Various *C. cubensis* isolates from South Africa were found to contain dsRNA. These were hypovirulent in comparative pathogenicity tests and also displayed other hypovirulence associated traits such as a reduction in oxalic acid production and laccase activity. Results further indicate that naturally occurring hypovirulence might play an important role in reducing the impact of Cryphonectria canker in South Africa.

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DETECTION OF DOUBLE-STRANDED RNA (DSRNA) AND VIRUS-LIKE PARTICLES IN A CANADIAN ISOLATE OF CHONDROSTEREUM PURPUREUM. S.F. Shamoun[1] and R.A. Valverde[2], [1]Natural Resources Canada, Canadian Forest Service, Pacific Forestry Centre, 506 West Burnside Road, Victoria, B.C. V8Z 1M5, Canada and [2]Department of Plant Pathology and Crop Physiology, Louisiana State University Agriculture Centre, Baton Rouge, LA 70803, U.S.A.

Chondrostereum purpureum is an important pathogen of orchard crops and is also under evaluation as a mycoherbicide for unwanted brush species in forestry. Isolates of the fungus from Canada, New Zealand and The Netherlands were analysed by polyacrylamide gel electrophoresis for double-stranded RNA (dsRNA) content. DsRNA molecules of 1.0×10^6 and 1.2×10^6 Daltons and isometric virus-like particles (VLPs) of approximately 30 nm were found in only one isolate (PFC 2064) of the 24 isolates tested. No VPLs were found in isolates in which dsRNA was not detected. The growth rate, mycelial dry weight and virulence of representative isolates were measured. The presence or absence of dsRNA and VLPs in the isolates did not correlate with virulence and mycelial growth. The presence of dsRNA content and VLPs in C. purpureum may provide a valuable marker for genetic, epidemiological and environmental fate studies.

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PRODUCTION OF A MATING INHIBITORY FACTOR BY *USTILAGO HORDEI*. Shirley A. Gerhardt and John E. Sherwood. Dept. Plant Pathology, Montana State University, Bozeman, MT 59717.

Mating in the smut fungi results in the conversion of nonpathogenic sporidia to pathogenic mycelia. Thus, effective disease control should be obtained by disrupting mating in these pathogens. *Ustilago hordei* causes covered smut of barley. When the two mating types (*A* and *a*) of *U. hordei* were grown together in broth culture, a mating inhibitory factor (MIF) was found in the culture medium. MIF was also produced by *a* cells transformed with the *A* pheromone gene, but not by either mating type grown alone. Partially purified MIF completely inhibited mating of *U. hordei* but did not prevent teliospore germination or sporidial growth. MIF also prevented mating of other *Ustilago* and *Tilletia spp.*, but had no effect on the growth of several unrelated fungi or bacteria. MIF inhibited barley seed germination at high concentrations (50X the concentration required to inhibit mating), but had no effect on wheat or barley leaves. MIF is soluble in water and methanol:water (2:1) and is stable to boiling, pronase E and proteinase K.

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GENETIC DIVERSITY IN POPULATIONS OF CRYPHONECTRIA PARASITICA IN EASTERN CHINA. Kerong Wang and Michael G. Milgroom, Department of Plant Pathology, Cornell University, Ithaca, NY 14853

We studied genetic diversity in the chestnut blight fungus, *Cryphonectria parasitica*, in populations in eastern China, where it is a native species. Using restriction fragment length polymorphisms (RFLPs), we studied 314 isolates from 18 populations in Guangdong, Hunan, Fujian, Jiangxi, Zhejiang, Jiangsu, Anhui, Shandong, Beijing and Liaoning provinces, representing areas from 24°N to 41°N latitude. DNA from each isolate was digested separately with the restriction endonucleases *Pst*I and *Eco*RI, and then probed with 10 low-copy, nuclear probes. Two probes (pCB29 and pMS29.1) were monomorphic. Probe pCB19 was monomorphic with *Pst*I but polymorphic with *Eco*RI. The other 7 probes were polymorphic with *Pst*I. Populations in the south tended to be more diverse than those in the north. Overall, genetic diversity in China is much greater than that found in the US.

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RELATIVE *IN VITRO* SENSITIVITY OF TEXAS STRAINS OF *CERATOCYSTIS FAGACEARUM* TO SELECTED TRIAZOLE FUNGICIDES. A. D. Wilson, USDA Forest Service, Southern Hardwoods Laboratory, P.O. Box 227, Stoneville, MS, 38776.

The fungicidal activity and effectiveness of the systemic triazole fungicide (propiconazole) to control the oak wilt fungus, *Ceratocystis fagacearum*, has prompted the need to screen additional triazoles for effectiveness against this pathogen. Ten strains of *C. fagacearum*, isolated from live oaks and red oaks in counties throughout the Edwards Plateau Region of Texas, were tested for their relative *in vitro* sensitivity to selected triazole fungicides including: propiconazole, difenoconazole, myclobutanil, tebuconazole, and triadimefon among others. The strains were tested for 6-8 weeks at 21 C on solid potato dextrose agar (PDA) and in Neopeptone broth (NPB) for the effects of these triazoles on radial growth and dry weight accumulation, respectively, at concentrations of 0.1 PPB to 1000 PPB triazole active ingredient. Additional tests at intermediate rates allowed determinations of minimum effective concentrations (MEC) for total growth inhibition or fungicidal activity. None of the triazoles inhibited growth at 0.1 PPB, yet all triazoles provided varying percent growth inhibition up to 100% at concentrations of 1.0 to 1000 PPB in PDA and NPB, with MECs of 100-200 PPB for most strains, although exceptional strains required MECs up to 500 PPB for total growth inhibition.

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PCR DETECTION OF MYCOPLASMA-LIKE ORGANISMS IN HAWAII. W. B. Borth, J. S. Hu, D. Ullman, and V. Jones, Depts. of Plant Pathology and Entomology, Univ. of Hawaii, Honolulu, HI, 96822.

Papaya (*Carica papaya* L.) and macadamia (*Macadamia integrifolia* Maiden & Betche.) are afflicted with severe disorders of unknown etiology in Hawaii. Nucleic acids isolated with an MLO-enrichment protocol from peduncles of macadamia showing decline symptoms and from woody roots of papaya with non-ripening symptoms were used as templates in PCR. Two oligomers which prime the PCR amplification of a conserved 542-bp sequence between the 16S and 23S ribosomal RNA genes of Peach Yellow Leaf Roll (Western-X disease) MLO were used to screen samples from these species with PCR. Fragments of ~ 542-bp were amplified from symptomatic macadamia and papaya but not from respective healthy control plants raised from seed in the greenhouse, or non-symptomatic plants from fields without these disorders. These results suggest that MLOs are involved with papaya non-ripening and macadamia decline in Hawaii. Sequencing of PCR products and comparisons with known MLO sequences to establish the identity of the putative MLOs are in progress.

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MULTIPLEX PCR ASSAYS FOR DETECTION AND RAPID IDENTIFICATION OF UNKNOWN MYCOPLASMALIKE ORGANISMS. D.E. Gundersen[1,2], I.-M. Lee[1], R.E. Davis[1], and D.T. Kingsbury[2]. [1]Mol. Plant Pathology Laboratory, ARS, USDA, Beltsville, MD 20705; [2]George Washington Univ., Dept. Micb. & Imm., Washington, D.C. 20037.

A series of oligonucleotide primers for PCR were designed based on unique 16S rRNA gene sequences (signatures) that were specific to previously designated mycoplasmalike organism (MLO) 16S rRNA groups. Using these group specific primer pairs, different size fragments were amplified, each identifying a single MLO 16S rRNA group. Multiplex PCR using these specific primer pairs in combination(s) allowed simultaneous detection and identification of unknown MLO(s). Nested-PCR using a MLO 16S universal primer pair followed by the multiplex group specific primers was employed to increase sensitivity in the case of low titer MLO infections.

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RFLP ANALYSES OF RIBOSOMAL PROTEIN GENES REVEAL STRAIN DIVERSITY IN MLO 16S rRNA GROUPS I AND III. D.E. Gundersen[1,2], I.-M. Lee[1], and R.E. Davis[1]. [1]Molecular Plant Pathology Laboratory, ARS, USDA, Beltsville, MD 20705; [2] George Washington Univ., Dept. Microbiol. & Immunol., Washington D.C. 20037.

PCR-amplified ribosomal protein gene sequences from a wide range of strains in MLO 16S rRNA group I (aster yellows MLO and related strains) and group III (peach-X MLO and related strains) were compared by restriction fragment length polymorphism (RFLP) analyses. RFLP profiles indicated at least six distinct patterns among strains in MLO group I and at least seven distinct patterns among strains in MLO group III. Strain differentiation by analyses of ribosomal protein gene sequences was consistent with that by analyses of 16S rRNA gene sequences. However, ribosomal protein gene sequences afforded a more refined level of strain differentiation. For example, three distinct ribosomal protein gene sequences profiles were identified for strains in MLO 16S rRNA group III-A.

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MIXED-MLO (MYCOPLASMALIKE ORGANISM) INFECTIONS ARE COMMON IN PERENNIAL FRUIT CROPS IN NORTHERN ITALY. I.-M. Lee[1], A. Bertaccini[2], M. Vibio[2], and D. E. Gundersen[1]. [1]Mol. Plant Pathology Lab, ARS, USDA, Beltsville, MD 20705; [2]Istituto di Patologia Vegetale, Università degli Studi, Bologna, Italy.

Nested-PCR assays using two universal and four MLO 16S rRNA group-specific primer pairs were employed to determine etiologies of diseases associated with pear (decline), plum (leptonecrosis), nectarine (leaf roll), and apricot (chlorotic leaf roll) fruit crops grown in northern Italy. Restriction fragment length polymorphism (RFLP) analyses of MLO 16S rDNA sequences amplified with various combinations of these primer pairs revealed that two to four distinct types of MLOs affiliated with MLO 16S rRNA group I (aster yellows MLO and related strains), group III (peach-X MLO and related strains), group V (elm yellows MLO and related strains), and group X (apple proliferation MLO and related strains) were associated with each disease. Predominant MLO strains associated with pear decline, apricot chlorotic leaf roll, and plum leptonecrosis were identified as members of group X (subgroups A and B). MLO strains associated with nectarine leaf roll were members of groups I and V. Minor MLO strains (one or more distinct types) were also detected in each infected plant.

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GENETIC REARRANGEMENTS IN *SPIROPLASMA CITRI* BR3 LINES DIFFERING IN INSECT TRANSMISSIBILITY. F. Ye, J.E. Rascoe, U. Melcher, and J. Fletcher. Departments of Plant Pathology and Biochemistry & Molecular Biology, Oklahoma State University, Stillwater, 74078.

Spiroplasma citri strain BR3-3X, a wall-less prokaryote isolated from brittle root diseased horseradish, is transmitted by leafhoppers. From this triply cloned isolate, several lines (BR3-T, BR3-P, and BR3-G) which differ in their insect transmissibility and culture history have been developed. Extrachromosomal and genomic DNAs from each line were isolated to search for genes potentially responsible for differences in insect transmissibility. Frequent rearrangements and deletions of extrachromosomal DNA occur among the lines. Almost all fragments of *Eco*RI digested BR3-3X, -T, -P and -G extrachromosomal DNA hybridized with a cloned 8.6 kb extrachromosomal BR3 DNA probe. These fragments accounted for more than 50 kb, suggesting that certain sequences are repeated. *Sal*I digested and pulsed field gel electrophoresis-separated genomic DNA profiles of different lines also show variation, especially in BR3-G, which has lost insect transmissibility. Investigations of relationships between these genetic rearrangements and phenotypic differences such as insect transmissibility are in progress.

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MULTIPLICATION AND SPREAD OF *XYLELLA FASTIDIOSA* WITHIN GRAPEVINE AND OTHER PLANTS. B. L. Hill and A. H. Purcell, ESPM, 201 Wellman, University of California, Berkeley, CA 94720

The xylem-limited bacterium *Xylella fastidiosa* causes Pierce's disease of grapevine and diseases in numerous other plants. After inoculation of plants with *X. fastidiosa* by infective sharpshooter vectors, a grape-virulent isolate multiplied and spread within grapevine and Himalayan blackberry (*Rubus discolor*) and multiplied at the site of inoculation but without evidence of later systemic movement in California mugwort (*Artemisia douglasiana*) and watergrass (*Echinochloa cruz-galli*). The bacterium was first detected (threshold number: 10^4 colony forming units per gm) by culturing from grape, blackberry, mugwort, and watergrass after 4, 32, 30, and 18 days respectively. Maximum bacterial population levels per gram (and percent of infection) detected in these four species were $>10^8$ (100%), 10^7 (58%), $2x10^6$ (20%), and $4x10^5$ (31%) respectively. Evidence of systemic movement of *X. fastidiosa* distal to the inoculation site was found only in grape and blackberry. The bacterium was never cultured or detected by enzyme-linked immunosorbent assay (ELISA) from inoculated Bermuda grass. Host plants may differ widely in their capacities to serve as inoculum for spread of *X. fastidiosa* to vineyards.

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IMPROVED TESTS TO DETECT XYLELLA FASTIDIOSA. Barry L. Hill, Entomology Dept., UC Berkeley, Berkeley, CA 94720, Chung-Jan Chang, Dept. Plant Path, U Georgia, 1109 Experiment Street, Griffin, GA 30223, Walter O. Bliss and Chester L. Sutula, Agdia, Inc., 30380 CR 6, Elkhart, IN 46514.

Previous tests to detect *Xylella fastidiosa* (***Xf***) produced only small differences in the performance of antisera derived from isolates of grape, peach, plum and oak. It appeared that all ***Xf*** isolates may be detected with only one antiserum [as in Sherald, J. L. and Lei, J. D., Plant Disease 75:200-203 (1991)]. We now report that isolates of Pierce's disease from grapevine appear to be distinguishable serologically from other ***Xf***. Antisera produced to these isolates do not react with many of the isolates from tree scorches. Antisera we have produced from isolates from tree scorches are not effective in detecting some grape isolates. The new antisera are used in tests that improve detection of ***Xf***. Our data support the occurence of multiple serogroups in ***Xf***.

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EFFECTS OF ANNUAL WARM-SEASON FORAGE GRASSES ON NEMATODE POPULATIONS AND YIELD OF EGGPLANT. N. Kokalis-Burelle, R. Rodriguez-Kabana, C. F. Weaver, and D. G. Robertson. Department of Plant Pathology, Auburn University, AL 36849-5409.

Microplot experiments were performed to evaluate the effects of several annual warm-season forage grasses on nematode populations and yield of eggplant. Switchgrass (*Panicum virgatum*) cultivars 'Alamo' and 'Cave-in-Rock', 'Red River' crabgrass (*Digitaria sanguinalis*), and 'Eastern' gammagrass (*Tripsacum dactyloides*) were used in rotations with hairy vetch (*Vicia villosa*), annual ryegrass (*Lolium* spp.), and eggplant (*Solanum melongena*) with and without nematicide. Numbers of root-knot juveniles (*Meloidogyne arenaria*), spiral nematode (*Helicotylenchus dihystera*), and nonstylet-bearing nematodes were counted following each crop. Eggplant yield was evaluated throughout the final cropping sequence. All warm-season grasses tested reduced populations of parasitic nematodes but did not reduce numbers of nonparasitic nematodes. Eggplant yield was highest with nematicide following 2 plantings of warm-season

grass and 1 planting of ryegrass in all experiments except gammagrass. Yield of eggplant without nematicide was consistently better following 2 plantings of warm-season forage grass compared to eggplant without nematicide following only 1 planting of grass.

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GLOBODERA TABACUM TABACUM POPULATION DYNAMICS AND DAMAGE TO TOBACCO. J.A. LaMondia, CT Agricultural Experiment Station, P.O. Box 248, Windsor, CT 06095.

Field microplot experiments were conducted from 1987 to 1992 to determine the relationship between the initial density (P_i) of the tobacco cyst nematode (Globodera tabacum tabacum) and fresh weight leaf yield of '0-40' shade tobacco. Leaf yield and total shoot weight were negatively correlated with P_i over a range of densities from 0.1 to 1097 encysted J2 per cm^3 soil ($r=-0.73$, and -0.73, respectively). Nonlinear damage functions (inverse logistic) related G. t. tabacum P_i to yield and shoot weight data, and described losses of less than five percent for P_i under 100 J2 per cm^3 soil. Densities above 100 J2 quickly decreased yields to a maximum loss of greater than 40 percent at P_i of 500 to 1000 J2 per cm^3 soil. Final nematode density (P_f) after each growing season was linearly related to P_i on a log/log plot ($\log(P_f)=1.96 + 0.29\log(P_i)$; $R^2=0.73$).

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EFFECT OF THREE ORGANIC RESIDUES ON NEMATODE POPULATIONS OF GRAPES IN THE GUADALUPE VALLEY, B.C. J. Guevara. MEXICO.INIFAP-CECOEN. APDO 2197. ENSENADA, B.C.MEXICO.

A survey on the occurrence of plant parasitic nematodes in 34 vineyards in Guadalupe Valley showed that of the samples taken Meloidogyne was the major nematode present found in 100%, Criconemella 57% and Xiphinema americanum in 26%. A field trial was conducted to determine the effect of cattle manure, grape and olive residues on grape population nematodes. Whereas in the control group the reproduction rate in the Meloidogyne was found to be 69%, in Criconemella 75% and free-living nematodes 30%; in the olive residues, cattle manure and grape residues groups, the reproduction rate was in Meloidogyne 41,52 and 60%, respectively; in Criconemella -4.5%, 34 and 44% and in free- living nematodes, 75, 39 and 44%. The three organic residues were highest yields in relation to control. It is concluded that olive residues could be used to control plant parasitic nematodes of grapes under Guadalupe Valley conditions.

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NORTH CENTRAL SOYBEAN CYST NEMATODE RESEARCH PROJECT: A MODEL FOR COOPERATIVE REGIONAL RESEARCH AND EDUCATIONAL PROGRAMS. P.R. Thorson[1], G. L. Tylka[1], W. C. Stienstra[2]. [1]Department of Plant Pathology, Iowa State University, Ames, IA 50011, [2]Department of Plant Pathology, University of Minnesota, St. Paul, MN 55108.

In 1993, a regional effort was initiated to determine the effects of susceptible and resistant soybean varieties and nonhost crops on population densities of soybean cyst nematode (SCN), to assess yield loss due to SCN, and to increase awareness of SCN and its management among growers in the North Central Region of the USA. This project is being conducted by cooperating scientists from universities in Illinois, Indiana, Iowa, Kansas, Michigan, Minnesota, Missouri, Nebraska, Ohio, and Wisconsin, and is funded by soybean checkoff funds administered by the North Central Soybean Research Program. A full-time project coordinator was hired, and research objectives and protocols were collectively developed by cooperators to facilitate solidarity among the scientists. Cooperating researchers conducted experiments with as much uniformity as possible, which allowed for combined analysis of data across the region. The coordinator visited all states involved in the project and was instrumental in assuring uniformity of experimental designs and protocols. The success of a project of this magnitude depends upon the collaboration of several scientists and a full-time coordinator.

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THE EFFECT OF THE RENIFORM NEMATODE ON MONOCULTURE SOYBEAN PRODUCTION. I.I. Cornelius and G.W. Lawrence. Department of Entomology and Plant Pathology, Mississippi State University, Mississippi State, MS 39762

In 1993 soybean cv. Coker 156 was planted in field microplots containing a residual overwintered population of Rotylenchulus reniformis to simulate a monoculture production system. Populations at planting averaged 10,571 nematodes/500 cm^3 of soil which was a decrease of 77% from the population at harvest of the previous fall. The highest average population of 16,980 nematodes/500 cm^3 of soil was recovered at 120 days after planting. Soybean yield was reduced in all R. reniformis infested plots compared to the untreated control. Yields ranged from 118 to 215 kg/ha with yield reduction ranging from 6 to 44%. Plant height was reduced in all R. reniformis infested plots compared to the control.

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ULTRASTRUCTURE OF INITIAL DEFENSE RESPONSES OF SOYBEAN RESISTANT TO CYST NEMATODE INFECTION. Y. H. Kim, K. S. Kim and R. D. Riggs. Department of Plant Pathology, University of Arkansas, Fayetteville 72701.

Ultrastructure of early responses to the invasion of soybean cyst nematode, *Heterodera glycines*, race 5, by a recently developed soybean cultivar, Hartwig, was compared with those of the most susceptible line, PI 274420. In both hosts, syncytia were initiated as evidenced by the presence of stylet-penetrated initial syncytial cells and hypertrophy and cell wall dissolution of syncytium component cells. Major differences between the two were; 1) extensive abnormal cell wall thickenings, consisting of electron-dense particulate deposits in a callose-like matrix, which was followed by a rapid necrotic collapse of syncytial cells in Hartwig as early as 2 days after inoculation; 2) continued hyperplasia in PI 274420 evidenced by the presence of cells that were undergoing mitosis and a localized accumulation of several nuclei in a syncytium; and, 3) distended cisternae of rough endoplasmic reticulum in hypertrophied cytoplasm of the initial syncytial cells of Hartwig.

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REPRODUCTION OF THE ROOT-KNOT NEMATODE IN A KENAF-SOYBEAN-COTTON ROTATION SYSTEM. G.W. Lawrence[1], K.S. McLean[2], and J.J. Cornelius[1]. [1]Department of Entomology and Plant Pathology, Mississippi State University, Mississippi State, MS 39762 and [2]Department of Agriculture, Northeast Louisiana University, Monroe, LA 71209.

Plots were established in 1993 to determine the influence of crop rotation on the population development of the root-knot nematode (Meloidogyne incognita race 3) and subsequent effects on kenaf yields. Rotation crops included kenaf cv. Everglades 41 (root-knot susceptible), soybean cv. Forrest (root-knot resistant), and cotton cv. Delta and Pineland 20 (root-knot susceptible). The 1993 growing season represented the first year of a planned three year study. Meloidogyne incognita population densities increased in all plots planted with kenaf. An average population density of 10,180 nematodes/250 cm^3 of soil was recovered from kenaf plants at 182 days after planting. Nematode populations in plots planted with cotton and soybean were 1,854 and 155 nematodes/250 cm^3 of soil, respectively. Kenaf yields were 6,122.5 kg/ha in M. incognita infested plots compared with 21,088.4 kg/ha in the untreated control.

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INFLUENCE OF THE ROOT-KNOT NEMATODE IN MONOCULTURE KENAF PRODUCTION. G.W. Lawrence[1], K.S. McLean[2], J.R. Barillas[3] and J.J. Cornelius[1]. [1]Department of Entomology and Plant Pathology, Mississippi State University, Mississippi State, MS 39762, [2]Department of Agriculture, Northeast Louisiana University, Monroe, LA 71209 and [3]Centro De Tecnologia Agricola, San Andres, La Libertad, El Salvador, C.A.

Kenaf cv. Everglades 41 was planted in microplots which contained a residual overwintered Meloidogyne incognita race 3 population to simulate a monoculture production system. Nematode populations averaged across all infested plots increased from 71 to 21,952 nematodes/500 cm^3 soil at planting and harvest, respectively, with a reproduction factor of $R_f = 407$. The height of kenaf plants were significantly reduced in all infested plots. Average plant height was 0.64m in the infested plots compared with 1.13m in the control. Average dry weight yields were 4,761.9 kg/ha in the presence of M. incognita compared with 19,954.7 kg/ha in the control.

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FTHALIDE, PENTACHLOROBENZYL ALCOHOL, AND PYROQUILON INHIBITION OF AFLATOXIN PRODUCTION BY ASPERGILLUS FLAVUS. M. H. Wheeler and D. Bhatnagar, USDA, ARS, Cotton Pathology Research Unit, Rt. 5, Box 805, College Station, TX 77845 and USDA, ARS, SRRC, P.O. Box 19687, New Orleans, LA 70179.

Two isolates of *A. flavus* were grown in shake cultures with 0 to 8 μg/ml of 2,3,4,5,6-pentachlorobenzyl alcohol (PCBA) or fthalide, and 0 to 30 μg/ml of pyroquilon. The three compounds significantly inhibited the accumulation of aflatoxins B_1 (AFB_1), B_2, and B_{2a} in cultures of both isolates. The effect of PCBA was most pronounced, followed by fthalide and then pyroquilon. In cultures of SRRC-2089, treated with 8 μg/ml PCBA, fthalide, or pyroquilon, AFB_1 levels decreased by 98, 86, and 48%, respectively. At the 8 μg/ml level, fthalide and pyroquilon did not inhibit fungal growth, but PCBA caused a decrease of 12% in mycelial dry weight. Precursor feeding studies with [^{14}C] norsolorinic acid demonstrated that enzymes converting norsolorinic acid and later precursors to aflatoxins were not inhibited by these compounds. This suggested that these compounds inhibited earlier in the aflatoxin pathway.

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A GUS REPORTER ASSAY TO STUDY GENE EXPRESSION AND THE INDUCTION OF AFLATOXIN BIOSYNTHESIS. *J. E. Flaherty*, C. P. Woloshuk, and G. A. Payne. Dept. Plant Pathology, N.C. State University, Raleigh, NC 27695-7616.

Aflatoxins are extremely toxic and carcinogenic secondary metabolites produced by the fungi *Aspergillus flavus* and *A. parasiticus*. Current research is directed at the elimination of these compounds in important food sources. The objective of this research was to develop a method for examining the induction of aflatoxin biosynthesis via expression of two aflatoxin pathway genes, *nor 1* and *ver1*. Toward attaining this objective, the promoter regions of *nor1* and *ver1* were fused to the β-glucuronidase gene (GUS) to form the reporter constructs, GAP12 and GAP13, respectively. These constructs were each co-transformed with a selectable marker into *A. flavus* strain 656-2. Aflatoxin production and GUS activity were determined in the transformants after shifting the cultures from a medium nonconducive for aflatoxin production to a medium conducive for aflatoxin biosynthesis. Transformants harboring either GAP12 or GAP13 displayed GUS expression only when aflatoxin was detected in culture. Thus, both the GAP12 and GAP13 transformants may serve as genetic tools for studying the induction of aflatoxin *in situ* and for identifying substances that affect the expression of genes involved in aflatoxin biosynthesis.

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AFL-1 IN *ASPERGILLUS FLAVUS* AFFECTS THE EXPRESSION OF AFLATOXIN GENES. Charles P. Woloshuk and Galina L. Yousibova, Purdue University, West Lafayette, Indiana 47907.

Aflatoxins are toxic and carcinogenic compounds produced by *Aspergillus flavus* and *Aspergillus parasiticus*. Fourteen years ago, K. E. Papa reported that strain 649 of *A. flavus* contained a dominate mutation at the *afl-1* locus resulting in the loss of aflatoxin production. By the parasexual cycle, we crossed strain 649 with the aflatoxigenic strain 86 (*afl-1*$^+$) to obtain diploids. These diploids did not produce aflatoxin indicating that the *afl-1* mutation is a dominate allele as described by Papa. We also compared the transcript levels of four aflatoxin genes (*nor, ver, omt* and *aflR*) in strain 649 and in the aflatoxigenic strains 86 and NRRL 3357 during the induction period of aflatoxin biosynthesis. Over a 24 hr period, no transcripts were detected in strain 649. In contrast, expression of all four genes occurred in the aflatoxigenic strains. In two diploids (649x86) that were also examined, no expression of the structural genes (*nor, ver, and omt*) was detected; however, there was expression of the regulatory gene *aflR*. These data suggest that the inhibition of aflatoxin biosynthesis associated with the *afl-1* mutation is not totally by suppression of *aflR*, but may also involve direct suppression of *nor,ver*, and *omt*.

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ROLE OF TOXIC METABOLITES PRODUCED BY *CALONECTRIA CROTALARIAE* IN RED CROWN ROT OF SOYBEAN.
K. D. Kim, J. S. Russin, and J. P. Snow. Department of Plant Pathology and Crop Physiology, Louisiana State University Agricultural Center, Baton Rouge, LA 70803.

Calonectria crotalariae, causal agent of red crown rot (RCR) of soybean, induces leaf chlorosis, interveinal necrosis, defoliation, wilting, root rot, and reddish-orange perithecia at stem bases. However, this pathogen could not be isolated from leaf tissues showing symptoms. Therefore, we investigated the possible role of toxic fungal metabolites in development of RCR using nine fungal isolates and 11 soybean cultivars. Cell-free culture filtrates of isolates grown on potato dextrose broth (PDB) were bioassayed for effect on root growth from germinated soybean seeds and symptom development in trifoliate leaves. In a root growth assay, PDB showed phytotoxicity similar to that observed with culture filtrates from virulent isolate (SG915). Therefore, a trifoliate assay with half-strength culture filtrates was adopted for these tests. Dilution end point for culture filtrates from SG915 was determined to be 1:8. The toxic metabolite was unstable when heated (121C, 15min), showing significant reduction in ability to cause wilting. When the culture filtrate of SG915 was tested on trifoliates from 11 cultivars, Cajun (least susceptible in field studies) showed least wilting throughout tests. When culture filtrates from various isolates were tested on Riverside 699 (more susceptible), a wide range of wilting severity was detected. This is the first report suggesting that toxic metabolites of *C. crotalariae* may be involved in RCR of soybean.

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PREFERENTIAL INFECTION OF MAIZE KERNEL EMBRYOS BY ASPERGILLUS FLAVUS. R. L. Brown*, T. E. Cleveland*, G. A. Payne+ and C. P. Woloshuk+. *USDA, ARS, Southern Regional Research Center, P.O. Box 19687, New Orleans, LA 70179 and +Dept. of Plant Pathology, North Carolina State Univ., Raleigh, NC 27695

Maize lines, previously identified in a kernel screening assay as highly resistant, moderately resistant or susceptible to aflatoxin contamination by Aspergillus flavus, were inoculated with an A. flavus transformant containing the E. coli β-D-glucuronidase reporter gene linked to a β-tubulin promoter. Prior to inoculation, kernels to be tested were either wounded in the endosperm, wounded in the embryo region or left unwounded. Inoculated kernels were then incubated for different times up to 7 days under germinating conditions. Results indicate that the embryo region was initially colonized in all infected kernels of those wounded in the endosperm or unwounded, despite the opportunity for entry into the endosperm presented by the wound. Ingress of A. flavus into the endosperm occurred only after longer periods of incubation. The embryo region may provide a more attractive substrate for fungal infection and/or aflatoxin contamination to A. flavus than does endosperm tissue.

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BIOLOGICAL ACTIVITIES OF SYNTHETIC ANALOGS OF FUMONISIN IN PLANTS AND MAMMALIAN CELL CULTURES. H.K. Abbas, T. Tanaka, S.O. Duke, SWSL, Stoneville, MS; G. Kraus, J.M. Applegate, Qiaogong Su, Dep. of Chem., Iowa State Univ., Ames, IA; and W.T. Shier, College of Pharmacy, Univ. of Minnesota, Minneapolis, MN.

Fumonisin B_1 [FB_1], a long chain alkylamine with two tricarboxylic acid moieties, and nine analogs were tested for their biological activity on duckweed (Lemna pausicostata) plants, susceptible tomato (asc/asc) leaf discs, and mammalian cell lines, including dog kidney (MDCK), rat liver hepatoma (H4TG) and mouse fibroblasts (NIH3T3). Analogs 7 and 9 at 10 μM increased cellular leakage and chlorophyll loss from tomato leaf discs. The diester 9 was the most active in the duckweed bioassay but was much less toxic to MDCK and H4TG cells with an IC_{50} of 200 μM compared to 10 μM for FB_1. Analog 9 and FB_1 showed similar low toxicities (IC_{50} = 150 μM) to NIH3T3 cells. Analog 9 appears to have high phytotoxicity and low mammalian toxicity, characteristics required for a safe alternative bioherbicide (supported in part by USDA 93-37201-9561).

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EVALUATION OF CLUSTERING APPROACHES IN THE CLASSIFICATION OF *STREPTOMYCES* SPP. USING FATTY ACID PROFILES. J.H. Bowers, L.L. Kinkel, and R.K. Jones, Dept. of Plant Pathology, University of Minnesota, St. Paul 55108.

Fatty acid profiles contribute significant progress in the classification of microorganisms, however, clustering method (average vs. density linkage), the number of fatty acids in the analysis, and the identity of isolates in the analysis have not been rigorously evaluated in field trials for their influence on the assignment of isolates to clusters. Isolates of *Streptomyces* spp. were obtained from field biocontrol plots and grouped into three independent data sets representing different treatments. Fatty acid profiles were generated by the Microbial Identification System (MIS, Microbial ID, Inc., Newark, DE). For each clustering method, the number of fatty acids and isolates were varied and the data sets analyzed using the CLUSTER procedure in the Statistical Analysis System (SAS). Results were compared to the MIS generated dendrogram at an euclidean distance of 10. The root-mean-square of the total-sample standard deviation and the pseudo F statistic decreased while the R^2 increased as additional fatty acids were included in the analysis. The number of clusters and the Shannon-Wiener diversity index also increased with increasing fatty acids in the analysis. The MIS and SAS analyses did not always produce similar cluster hierarchies. The use of fatty acid profiles in ecological studies will depend on the robustness of the technique.

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EFFECT OF VOLATILE GLUCOSINOLATE DEGRADATION PRODUCTS FROM RAPESEED MEAL ON *APHANOMYCES EUTEICHES* F. SP. *PISI*.
U. Smolinska, M. J. Morra, & G. R. Knudsen. PSES Dept., University of Idaho, Moscow, Idaho 83843.

Volatile compounds from hydrated rapeseed (*Brassica napus*) meal suppressed activity of *Aphanomyces euteiches* f. sp. *pisi* at different stages of the pathogen's life cycle. Meal was used intact, or treated by autoclaving or water extraction to reduce toxicity. Volatiles from intact meal completely suppressed mycelial growth and germination of zoospores on agar, whereas volatiles from detoxified meal had significantly less activity. In growth chamber bioassays, peas (*Pisum sativum*) inoculated with zoospore suspensions and then incubated 24 hr in the presence of volatiles from intact meal, had 50% lower root rot disease severity than in the absence of meal. Volatile glucosinolate degradation products, when passed through soil, decreased infection potential of oospores and resulted in lower disease severity in inoculated peas, compared to controls with oospores that were not exposed to rapeseed volatiles.

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THE EFFECT OF INTERCROPPED OATS ON THE INCIDENCE OF APHANOMYCES ROOT ROT OF PEA
E. R. Kazmar and J. L. Parke, Department of Plant Pathology, University of Wisconsin-Madison, Madison, WI 53706

Root rot caused by *Aphanomyces euteiches* is a major disease of peas (*Pisum sativum* L.) and other legumes. Small grains and legumes are commonly seeded together in establishment of forage crops. To assess the effect of oats (*Avena sativa* L.) on the development of Aphanomyces root rot, peas and oats were sown together in naturally infested soil. In growth chamber

experiments, intercropping of peas and oats significantly reduced the incidence of pea root rot. Oats were effective in suppressing disease at low and moderate, but not at high infestation levels. The mechanism of disease suppression is currently under investigation. A wheat intercrop did not significantly suppress disease. Root rot suppression by oats was not observed in a 1993 field trial under heavy disease pressure.

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ROOT ROT SYMPTOMS AND *APHANOMYCES COCHLIOIDES* POPULATIONS IN A SUGAR BEET FIELD. J.W. Beale[1], C.E. Windels[2], and L.L. Kinkel[1]. [1]Dept. of Plant Pathology, Univ. Minn., St. Paul 55108 and [2]N.W. Expt. Stat., Univ. Minn., Crookston 56716.

To determine the relationship of root rot symptoms and populations of *Aphanomyces cochlioides*, an 8.4 m^2 area in a field of diseased and healthy plants was sampled on 30 July 1993. Soil cores (2 x 20 cm) were collected within rows between each of 15 pairs of roots categorized as having intermediate or severe disease symptoms or as healthy. Sugar beet seedlings were grown in soil samples to bait *A. cochlioides* and to determine a root rot index (0-100 scale). Indices averaged 99 and 96 for soils collected near roots with severe and intermediate disease, respectively; seedlings in soils from the severe category died sooner than those in soils from the intermediate category. The fungus was detected in soil samples collected between 8 of the 15 pairs of healthy plants and indices averaged 76. Thus, higher populations of *A. cochlioides* are typically, but not always, related to disease incidence, which suggests that other factors also influence development of root rot.

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DETECTION OF TWO TOMATO-INFECTING GEMINIVIRUSES IN INDIVIDUAL *Bemisia tabaci* ADULTS. Prem Mehta, J. A Wyman, M. K. Nakhla, and D. P. Maxwell. University of Wisconsin, Madison, WI 53706.

Mixed infections of geminiviruses are known to occur in vegetables; however, the ability of *Bemisia tabaci* to acquire and transmit more than one geminivirus at a time has not been demonstrated. Infectious clones of tomato yellow leaf curl geminivirus (TYLCV) from Egypt and tomato mottle geminivirus (ToMoV) from Florida were agroinoculated individually and together into healthy tomato plants. After 15 days, B-biotype *B. tabaci* adults were fed on these plants for an acquisition-access period of 24 h and then transferred to healthy tomato seedlings for a 72 h inoculation-access period (IAP). Following this IAP, individual whiteflies were examined for viral DNA by a PCR assay with virus-specific primers. Individuals collected from singly infected plants had either TYLCV or ToMoV, while individuals fed on tomatoes with mixed infections contained both viruses. After inoculation with the viruliferous whiteflies, tomato plants were infected with either virus alone or both as expected. Our data show that individual *B. tabaci* adults can acquire two geminiviruses from a source plant with a mixed geminivirus infection and that *B. tabaci* was able to transmit ToMoV and TYLCV from tomatoes infected with clones of these geminiviruses.

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YIELD AND GROWTH CHARACTERISTICS OF SIX HARD RED WINTER WHEAT CULTIVARS IN RESPONSE TO INOCULATION WITH WHEAT STREAK MOSAIC VIRUS PRE AND POST VERNALIZATION. M.A.C. Langham, J. Gellner, and D.G. Gallenberg. South Dakota State University, Plant Science Dept., Brookings, SD 57007.

Six hard red winter wheat cultivars were inoculated with wheat streak mosaic virus (WSMV) before or after vernalization to simulate fall or spring infection periods. Plants were retained in the greenhouse until maturity, and the following growth and yield characteristics measured: number of tillers per plant, height of primary tiller, number of filled spikelets on the primary tiller, number of kernels per plant, number of kernels per tiller, average kernel weight, and total kernel weight per plant. All responses except number of tillers per plant were significantly affected by WSMV infection. The effect on yield was greatest in Rose and least in Triumph 64. Siouxland's yield response was more affected by prevernalization infection while Rose and Triumph 64 were more greatly affected by post vernalization infection. Abilene, Arapahoe, and Brule were affected approximately equally by both pre and post vernalization infection.

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Soybean Mosaic Virus Detected by DAS-ELISA in the Hypocotyl of 24 to 72 Hour Germinating Soybean Seeds. M. A. Bajwa and R. P. Pacumbaba. Department of Plant and Soil Science, Alabama A&M University, Normal 35762.

Soybean mosaic virus (SMV) was detected 50.0% and 48.8% by the double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) technique in the embryos of both mottled and nonmottled seeds of cultivars Stonewall, Hutchingson, Essex, Young, Ransom, and Davis. No SMV from similar cultivars was detected by DAS-ELISA from the stained and nonstained seed coats and endosperm of both mottled and nonmottled seeds. In the interval of 24 to 72 h, SMV was detected by DAS-ELISA at the rates of 31.1% and 31.1% in the hypocotyl of germinating mottled and nonmottled seeds, respectively, of the same cultivars. No SMV was detected by DAS-ELISA in similar cultivars on the stained and nonstained seed coats, epicotyls, and endosperms of both mottled and nonmottled seeds. Greenhouse soybean seedlings 6 to 8 weeks old assayed with DAS-ELISA showed 43.7% and 41.3% SMV on small mottled and nonmottled seeds and 53.9% and 44.5% SMV on big mottled and nonmottled seeds, respectively. Mottled soybean seeds should not be associated with the presence of SMV, since both mottled and nonmottled seeds showed SMV.

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EPIDEMIOLOGY OF SEROTYPES OF MAIZE CHLOROTIC MOTTLE VIRUS. **Stanley G. Jensen, Jennye Matula, and Ben Doupnik, Jr. USDA-ARS, University of Nebr. Lincoln, NE 68583.**

Two serotypes of maize chlorotic mottle virus (MCMV) are distinguishable by Ochterlony agar gel double diffusion (ADD) because the Nebraska wild type (ST^+) carries an epitope not found on serotype ST^-; therefore, ST^+ forms a spur against ST^- when using ST^+ antiserum. All MCMV isolates from Hawaii, Peru, and Mexico were the ST^- serotype. A greenhouse cultured Nebraska isolate proved to be the ST^- serotype and was designated N-ST^-. The objective of this study was to determine the distribution and importance of the ST^- serotype in Nebraska and Kansas. Out of 221 MCMV isolates from 97 locations collected in 1991, 1992, and 1993 only one was of the ST^- serotype. Twenty six additional samples from the same field as this ST^- were all ST^+. Another extensively sampled field contained only ST^+ serotypes. In a long term MCMV screening nursery inoculated annually with N-ST^- natural infection had never been observed. In 1993 several uninoculated control plants were observed with symptoms. ADD analysis showed that while the mechanically inoculated plants were all N-ST^-, most of the infected control plants had the ST^+ serotype. Thus, the Nebraska wild type virus, ST^+, had become established in the field rather than the repeatedly introduced N-ST^-. Cross protection was confirmed. The loss of the epitope may interfere with the overwinter survival of ST^- in this climate.

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EVALUATION OF SOYBEAN GERMPLASM FOR RESISTANCE TO BEAN POD MOTTLE VIRUS USING MECHANICAL AND GROSS-WOUND INOCULATION. **H. A. Diallo, R. C. Gergerich, and S. L. Wickizer. Department of Plant Pathology, University of Arkansas, Fayetteville 72701.**

Bean pod mottle virus (BPMV), a member of the comovirus group, is transmitted by leaf-feeding beetles and causes a widespread disease of soybean [*Glycine max* (L.) Merr.] in the United States. The objective of this study was to screen soybean germplasm for resistance to BPMV using mechanical and gross-wound inoculation. Commercial varieties and plant introduction (PI) lines of soybean were first inoculated with sap from BPMV-infected 'Black Valentine' bean by rub-inoculation of carborundum-dusted leaves and by the gross-wound inoculation technique (Phytopathology 73:936-938), a technique that mimics beetle feeding. All soybean varieties and PIs inoculated with gross-wounding and mechanical inoculation were determined to be susceptible to BPMV when tested by Protein-A ELISA. Symptoms on inoculated plants ranged from symptomless infections, where virus was only detectable by ELISA, through mild to severe mottling of leaves. Virus titer of mechanically inoculated plants was determined and the relationship between BPMV titer and symptom expression was investigated.

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AN ELISA TO DETECT AMERICAN PLUM LINE PATTERN ILARVIRUS. Walter O. Bliss, Chester L. Sutula, Agdia, Inc., 30380 CR 6, Elkhart, IN 46514, Howard E. Waterworth, Natl. Plant Germplasm Quar. Ct. - USDA, 11601 Old Pond Drive, Glen Dale, MD 20769, R. W. Fulton, 5060 Sunrise Ridge, Middleton, WI 53562

Diseases caused by American plum line pattern virus [AmPLPV] have been reported by many since 1941. AmPLPV affects plums, peaches and apricots but has a broader experimental host range among Prunus species. In addition to the U.S., AmPLPV has been reported from Europe, India and New Zealand. A DAS ELISA was developed that detected AmPLPV in leaves and stems from all known infected samples. It identified negatives and differentiated between AmPLPV and other ilar viruses correctly. The optimized ELISA used specific IgG [Fulton, R. W.: Phytopathology **72**:1345-1348 (1982)] coated onto Greiner High Bind polystyrene microwell plates at 5 ug/mL from 0.05M carbonate, pH=9.6. Sample, alkaline phosphatase-IgG conjugate and PNP substrate solution followed a 100 uL protocol with overnight, 2 hour and 1 hour incubations respectively. Healthy peach extract was added to the conjugate diluent to reduce test background. Negative samples produced OD405nm values below 0.10 while positive samples were greater than 0.20. This test appears more reliable than currently used peach GF 305 and Shiro plum indicators and promises to be useful in certification and international quarantine programs.

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MECHANISMS OF GEMINIVIRUS ACQUISITION AND TRANSMISSION BY THE WHITEFLY, *BEMISIA TABACI*. R. C. Rosell and J. K. Brown, Department of Plant Sciences, University of Arizona, Tucson, AZ 85721

Bemisia tabaci is the only whitefly species capable of vectoring whitefly-transmitted (WFT) geminiviruses. Transmission is persistent and vector specific, thus, epitopes exposed on the surface of virus particles must interact directly or indirectly with specialized sites in the insect vector during the feeding process. In order to predict putative viral coat protein epitopes involved in geminivirus/whitefly interactions, GenBank/EMBL nucleotide sequences for WFT geminiviruses were accessed. The GCG (Wisconsin) sequence analysis software was used to search for amino acid sequence similarities between different geminivirus coat proteins. Multiple sequence alignment of coat protein deduced amino acid sequences from WFT monopartite and bipartite geminiviruses indicates 82% similarity (76% identity). Several conserved regions were delineated that have identical amino acid sequences in both mono- and bi-partite WFT geminiviruses. Morphology of the whitefly mouthparts was investigated by electron microscopy and the whitefly stylet arrangement was found to be similar to that of aphids. The following model for the geminivirus/whitefly system, based on luteovirus/aphid system, is proposed. Acquisition of virions by whiteflies occurs during the feeding process via the stylet food canal. Transmission occurs by passage of virions into the accessory salivary glands and through the stylet salivary canal as saliva is injected into the plant during feeding.

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PARTIAL CHARACTERIZATION OF PELARGONIUM LINE PATTERN VIRUS. Ramon Jordan[α] and Suzanne Hurtt[β]. USDA-ARS, US National Arboretum, FNPRU[α] and Plant Sciences Institute, NGRL[β], BARC-W, Beltsville, MD 20705.

The biological, biochemical and serological properties of a virus isolated from *Pelargonium* x *hortorum* exhibiting line and ring pattern symptoms have been determined. The experimental host range of this virus (isolates PLPV CF and D) is similar to that reported for Pelargonium line pattern (PLPV) and ring pattern (PRPV) viruses. Sucrose gradient-purified preparations from the assay host *Chenopodium quinoa* contain spherical particles of about 30-31 nm. Virions consist of a single coat protein of about 36-38 kDa and at least one ssRNA of about 1.5 x 10^6 Mr (and a possible second at about 0.5 x 10^6 Mr). Two dsRNAs (3.1 and 1.1 x 10^6 Mr) are detected in infected tissues. The dsRNA pattern is distinctly different from the dsRNA patterns of carmo-, necro- and tombusviruses and most resembles that associated with dianthoviruses. Antisera to at least 4 other geranium-infecting viruses do not react with PLPV CF, however antisera produced to a Netherlands isolate (PLPV N) did react to isolates PLPV CF and PLPV D. Our antisera produced to PLPV D does react with European and US isolates. Purified preparations of PLPV D produced line pattern symptoms in inoculated cultivars of *Pelargonium* x *hortorum*, could be detected serologically in the infected geraniums, and was recovered by inoculation to *C. quinoa*.

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A SEROLOGICALLY DISTINCT HIGH TEMPERATURE VARIANT DERIVED FROM A GLOXINIA ISOLATE OF IMPATIENS NECROTIC SPOT VIRUS. H. T. Hsu and R. H. Lawson, USDA, ARS, USNA, Floral and Nursery Plants Research Unit, Beltsville, Maryland 20705.

A serologically distinct high temperature variant (HT-1) derived from a gloxinia isolate (Igg) of Impatiens necrotic spot virus (INSV) was compared with tospoviruses from different hosts and geographical regions. HT-1 nucleoprotein was purified from infected Nicotiana benthamiana by extraction with phosphate buffer (0.1 M KH_2PO_4, 0.01 M EDTA and 0.01 M Na_2SO_3, pH 7.2) followed by Nonidet P40 treatment and CsCl centrifugation. The HT-1 nucleoprotein does not react with either INSV or tomato spotted wilt virus (TSWV) antiserum obtained from Agdia, Inc.; nor does it react to antiserum prepared from INSV-B isolated from Schizanthus sp. (California). Polyclonal rabbit antisera prepared to the HT-1 nucleoprotein reacted only with homologous antigens. The HT-1 antiserum did not react with INSV-B, INSV-Igg, or TSWV from lettuce (Hawaii), peanut (Georgia) or tospovirus isolates from tomato, peanut, wax gourd and watermelon from Taiwan.

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THE COAT PROTEIN GENE OF WHITEFLY-TRANSMITTED GEMINIVIRUSES: PRELIMINARY PHYLOGENETIC ANALYSES. J.K. Brown[1], S.D. Wyatt[2], D.R. Frohlich[1], [1]Dept. of Plant Sciences, University of Arizona, Tucson, AZ 85737; [2]Dept. of Plant Pathology, Washington State University, Pullman, WA 99164.

The coat protein (CP) gene of eighty field and/or greenhouse-maintained isolates of whitefly-transmitted (WFT) geminiviruses from a wide range of geographical sites was investigated. Viral DNA was amplified using degenerate primers and polymerase chain reaction (PCR). Primers were designed to flank a region of the coat protein gene having the greatest degree of nucleotide (nt) sequence similarity identified, for a group of well-characterized candidate geminiviruses for which CP gene sequences are available. PCR products of 550 bp were obtained and sequenced. Sequences were aligned using GCG and the multiple sequence alignment program was used to generate a dendogram depicting relationships based on degree of nt sequence similarity. Several non-WFT geminiviruses were used as outgroups. The calculated mean distances of the sequences of interest ranged from 0.05 to 0.57. In preliminary analyses, the virus isolates fell into several major groups that in most cases were grouped by the plant host species from which the virus was originally obtained. Consequently, geographic affiliations, typically apparent from previous distance analyses of the nt sequences of full-length A-components (bipartite) or complete genomes (monopartite) of WFT geminiviruses, were obscured.

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IN SITU DETECTION AND LOCALIZATION OF READTHROUGH PROTEIN OF BARLEY YELLOW DWARF VIRUS. S.-L. Cheng, P. H. Nass, L.L. Domier, and C. J. D'Arcy. Department of Plant Pathology, University of Illinois, USDA-ARS, 1102 S. Goodwin Ave., Urbana, IL 61801.

The virus-encoded structural proteins of BYDV (22kDa coat protein (CP) and 50kDa readthrough protein) were identified by an immunogold localization assay using monoclonal (MAb) and polyclonal antibodies (pcAb) against the CP or the readthrough protein. Seven-day-old Coast Black oat seedlings were inoculated with BYDV-PAV-IL by 30 viruliferous *Rhopalosiphum padi* during a 48 hr inoculation access. Two, 4, or 6 days after the beginning of the inoculation, samples were taken from the midrib of the primary leaf and prepared for electron microscopy. Viral particles were detected with pcAb against PAV and pcAb against 50kDa bacterial fusion protein as well as MAb PAV-IL-22kDa and MAb PAV-IL-50kDa, although the density of labeling with MAbs was lower than that with pcAb. The highest amount of labelling with both sets of antibodies was found in the cytoplasm of infected cells at 4 and 6 days after inoculation. These results suggest that the 50kDa protein of BYDV, produced by readthrough of a stop codon of CP, is expressed concurrently with the 22kDa CP and is accumulated in the cytoplasm of infected cells.

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***IN SITU* LOCALIZATION OF BARLEY YELLOW DWARF RNA AND 17 KDa PROTEIN IN OATS.** **Petra Nass[1)], Leslie L. Domier[2)], Birute P. Jakstys[3)], and Cleora J. D'Arcy[1)]. Department of Plant Pathology[1)], USDA/ARS[2)] and Center for Electron Microscopy[3)], University of Illinois, South Goodwin Ave, Urbana IL 61801.**

Electron micrographs of barley yellow dwarf virus-infected cells have shown fibrillar material in the cytoplasm, nucleus and virus-induced vesicles. Size and appearance of the fibrils suggest they consist of nucleic acid, presumably viral RNA. To test this assumption viral RNA and the BYDV 17 kDa RNA binding protein were localized *in situ*. Seven-day-old oat seedlings were inoculated with BYDV-PAV-IL by approximately 30 viruliferous *Rhopalosiphum padi* during a 48 hour inoculation access. Samples of infected and noninoculated oat seedlings were taken at 1,2,3,4 and 5 days post-inoculation and prepared for electron microscopy. Ultrathin sections were either used for *in situ* hybridization of RNA or immunolocalization of 17 kDa RNA binding protein. Fibrillar material in the cytoplasm and nucleus, but not in virus-induced vesicles, was specifically labelled in both assays. The results indicate that the fibrillar material contains viral RNA.

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MAPPING STRUCTURAL ELEMENTS OF THE TOBACCO MOSAIC TOBAMOVIRUS COAT PROTEIN THAT ELICIT N' GENE HYPERSENSITIVITY. Z. F. Taraporewala and J. N. Culver, Center for Agricultural Biotechnology, University of Maryland Biotechnology Institute, College Park, MD, 20742.

Amino acid substitutions that disrupt the ordered quaternary structure of the tobacco mosaic tobamovirus (TMV) coat protein result in the elicitation of the N' gene hypersensitive response (HR) in *Nicotiana sylvestris*. We have hypothesized that alterations in CP quaternary structure expose a structural motif(s) that is responsible for host recognition and HR elicitation. In this study, additional amino acid substitutions have been created to map the tertiary structural features of the CP required to elicit the HR. Substitutions disrupting the long and short inner loops, RNA binding sites, beta-sheet, amino- and carboxy-termini had no effect on HR elicitation. However, substitutions disrupting the core alpha-helical bundle prevented HR elicitation. Specifically, these changes occurred primarily within the right-radial and right-slewed alpha-helices. This indicates that local conformation of this structural region must be maintained for host recognition and HR elicitation to occur.

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VIRUS RESISTANCE IN TRANSGENIC TOMATO AND TOBACCO PLANTS.

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The new world bipartite geminivirus, chino del tomate (CdTV) causes leaf curl in tomato and tobacco and is transmitted by *Bemisia tabaci*, the sweet potato whitefly. Our goal is to engineer plants to express either recombinant single-chain antibodies (ScFv) and/or anti-sense RNA directed against essential geminivirus encoded products. Focusing on the B encoded components of CdTV, we have sequenced and compared this component to well characterized geminiviruses. An interesting feature of the CdTV B component is the close proximity of BL-1 and BR-1, separated by less than 25 nucleotides. Anti-sense RNA targeted against the BL-1 and BR-1 in transgenic *Nicotiana tabacum, N. benthamiana* , and *Lycopersicon esculentum* have been produced.

Following challenge inoculation with viral DNA using either the particle gun or whitefly mediated transmission, the efficacy of this strategy to protect plants from geminivirus infection will be evaluated by PCR, symptomatology and Northern analysis.

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INFECTION OF TRANSGENIC TOBACCO CONTAINING A MUTANT 183-kDa TMV-REPLICASE GENE BY TMV. X.S. Ding and R.S. Nelson, The Noble Foundation, P.O. Box 2180, Ardmore, OK 73402

Transgenic tobacco expressing a portion of the 183-kDa replicase gene (designated REP21; Donson *et al.*, 1993, MPMI **6**: 635) is known to be highly resistant to systemic infection by TMV. Analysis of 18 plants from a single line expressing this construct showed that chlorotic lesions appeared on leaves inoculated with U1-TMV at 18 days post inoculation (DPI). Immunocytochemical analysis indicated that U1-TMV accumulated in diminishing quantities from mesophyll cells to bundle sheath cells to vein cells of minor veins in these chlorotic lesions. Using grafted plants (REP21 tobacco as scion; Xanthi nn tobacco as rootstock), we found that plants, whose rootstocks were inoculated with U1-TMV, developed systemic chlorotic spots in REP21 leaves (22 DPI). The importance of quantity and timing of virus accumulation in inoculated leaves for effective systemic spread will be discussed.

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GENE VI OF CAULIFLOWER MOSAIC VIRUS AND A SINGLE RECESSIVE HOST GENE DETERMINE SYSTEMIC NECROSIS IN *NICOTIANA CLEVELANDII*. **Lorant Kiraly and James E. Schoelz, Dept. of Plant Pathology, University of Missouri, Columbia MO**

Previous research has shown that gene VI of cauliflower mosaic virus (CaMV) determines systemic movement in solanaceous hosts. CaMV strain D4 induces a systemic mosaic, whereas strain W260 causes a systemic necrosis in the solanaceous plant *Nicotiana clevelandii*. To determine which W260 sequences are responsible for eliciting systemic necrosis in *N. clevelandii*, we constructed chimeric viruses between the D4 and W260 strains. Here we show that gene VI of CaMV not only determines systemic movement but also the development of systemic necrotis vs. mosaic symptoms in *N. clevelandii*. Systemic necrosis occurred only if the gene VI sequence of the virus was from W260. We also have evidence that the systemic necrosis trait in *N. clevelandii* is governed by a single, recessive gene. We made crosses between *N. clevelandii* and *N. bigelovii*, a host which reacts with a systemic mosaic to W260 infection. All F1 plants showed systemic mosaic when inoculated with W260. These plants were selfed and in the F2 generation, the infected plants revealed a 3:1 Mendelian segregation of systemic mosaic vs. necrosis, indicating that the systemic necrosis trait was conditioned by a recessive plant gene.

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CHARACTERIZATION OF THE SOLANACEOUS HOST RANGE OF FIVE STRAINS OF CAULIFLOWER MOSAIC VIRUS. Monir Shababi and James E. Schoelz, Dept. of Plant Pathology, University of Missouri, Columbia MO, 65211.

Previous studies has demonstrated that gene VI of CaMV strain D4 determines systemic infection of several solanaceous species, including *Nicotiana bigelovii*. In contrast, three regions of CaMV strain W260 are involved in systemic infection of *N. bigelovii*. To further characterize the host range of CaMV strains, we have now cloned five additional CaMV strains and determined their ability to infect solanaceous species. The strains NY8153, Harris, and CabbB (ATCC #PV-147) systemically infected *N. bigelovii*, among other solanaceous species, while the other two strains, California and CabbB (Davis), were localized to the inoculated leaves of *N. bigelovii*. To determine whether gene VI of NY8153, Harris, and CabbB(ATCC) is the only gene responsible for systemic infection in *N. bigelovii*, chimeric viruses were constructed between these strains and CM1841, a strain that is unable to systemically infect any solanaceous host. The chimeric viruses, consisting of the gene VI coding region of these strains and genes 1 - V of CM1841, failed to systemically infect *N. bigelovii* , suggesting that the solanaceous host range determinants of NY8153, Harris and CabbB(ATCC) were similar to W260 rather than D4.

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IDENTIFICATION OF A GLOBAL REGULATOR OF SECONDARY METABOLITE PRODUCTION IN *PSEUDOMONAS FLUORESCENS* STRAIN PF-5. N.A. Corbell, J. Kraus, and J.E. Loper. USDA ARS HCRL, 3420 N.W. Orchard Ave., Corvallis, Oregon 97330.

Mutations in the *apd* (antibiotic production) gene of the plant root-colonizing bacterium *Pseudomonas fluorescens* strain Pf-5 pleiotropically abolish the production of an array of extracellular secondary metabolites including pyoluteorin, pyrrolnitrin, 2,4-diacetylphloroglucinol, tryptophan side chain oxidase, HCN, and protease. The *apd* gene product contained consensus sequences of both the histidine protein kinase and the response regulator of two-component regulatory systems. Striking similarity was found between the nucleotide and predicted amino acid sequences of *apd* and *lemA*, a gene required for the pathogenicity of *Pseudomonas syringae* pv. *syringae*. Introduction of a cloned 6.7-kb *Kpn*I fragment containing the *apd*$^+$ gene restored the wild type phenotype to both LemA$^-$ mutants of *Pseudomonas syringae* and Apd$^-$ mutants of Pf-5.

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ANALYSIS OF THE SYRD PROTEIN OF *PSEUDOMONAS SYRINGAE* PV. *SYRINGAE*. Neil B. Quigley. Dept. of Microbiology, University of Tennessee, Knoxville, TN 37996-0845.

DNA sequence analysis predicted previously that the *syrD* gene of *Pseudomonas syringae* pv. *syringae* encodes an ATP-binding cassette (ABC) transporter protein that is likely involved in syringomycin secretion. The *syrD* gene has been subcloned as a promoterless *Bam*HI fragment and expressed in *E. coli* from an inducible plasmid-borne promoter. SDS-PAGE analysis has confirmed that SyrD is ~63 kDa in size. SyrD is not released readily from cells subjected to cycles of rapid freezing and thawing. It is predicted that SyrD is a transmembrane protein associated with the cytoplasmic membrane of *P. s. syringae*. High level expression of SyrD provides the opportunity to test this prediction, and to look for SyrD homologs among diverse toxigenic strains of this pathogen.

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NEW COPPER RESISTANCE GENES FROM *PSEUDOMONAS FLUORESCENS* WITH HOMOLOGY TO GENES INVOLVED IN CYTOCHROME *C* BIOGENESIS. C.-H. Yang and D. A. Cooksey. Dept. of Plant Pathology, Univ. of Calif, Riverside, CA92521.

A cosmid library of DNA from copper-resistant *Pseudomonas fluorescens* 09906 was constructed, and a clone was identified that complemented two of three chromosomal Tn*5* insertions in copper-sensitive mutants. Subclones were mutagenized with Tn*3-gus* and marker exchanged into wild-type 09906 to determine length and orientation of transcriptional units required for copper resistance. A 3.5-kb fragment that contained functional copper-resistance genes was sequenced and the predicted protein products showed strong similarity with two proteins from *Rhodobacter capsulatus*: CcI1, a periplasmic protein that may be a chaperone for ligation of heme with *c*-type cytochromes, and HelX, a periplasmic disulfide oxidoreductase that may retain cysteine residues of apocytochromes in a reduced state prior to ligation of heme.

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ISOLATION AND CHARACTERIZATION OF GENES CODING FOR SYRINGOMYCIN SYNTHETASES IN *PSEUDOMONAS SYRINGAE* PV. *SYRINGAE*. B. K. Scholz, M. L. Hutchison, and D. C. Gross. Dept. of Plant Pathology, Washington State University, Pullman, WA. 99164.

Syringomycin is a necrosis-inducing lipodepsinonapeptide phytotoxin produced by *Pseudomonas syringae* pv. *syringae*. Previous analysis of the *syrB* and *syrC* genes indicated that syringomycin was synthesized by a thiotemplate mechanism. Such mechanisms involve large multienzymatic synthetases that activate and condense amino acids onto a growing peptide chain. In earlier studies, the deduced amino acid sequence of SyrB was shown to contain six signature core sequences characteristic of non-ribosomal peptide synthetases. We hypothesized that for each amino acid in the syringomycin peptide there is a dedicated enzymatic domain containing all six core sequences that are associated with the activation and condensation of each unique amino acid. The *syr* cluster was isolated using pulse field gel electrophoresis and the amino acid-activating domains were mapped using oligonucleotide probes homologous to the core sequences for adenylation (core 2) and thioester formation (core 6) of *syrB*. Based on sequence analysis of the *syr* cluster we propose a model for the biosynthesis of syringomycin.

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CHARACTERIZATION OF THE 2,4-DIACETYLPHLOROGLUCINOL BIOSYNTHETIC LOCUS FROM *PSEUDOMONAS FLUORESCENS* Q2-87. M. Bangera*, H. Hara, D.M. Weller and L. S. Thomashow. USDA-ARS and *Department of Microbiology, Washington State University, Pullman, WA

The antibiotic 2,4-diacetylphloroglucinol (Phl) is produced by certain strains of

fluorescent pseudomonads that have been implicated in the control of soilborne plant pathogens worldwide. Phl production is required for the suppression of take-all disease of wheat by *Pseudomonas fluorescens* Q2-87, and a locus involved in synthesis of the antibiotic was described previously (Vincent *et al.*, (1991), Appl. Environ. Microbiol. 57:2928-2934). A 7-kb subclone from this locus was sufficient to transfer Phl biosynthetic capability to each of 15 heterologous Phl-nonproducing strains of *Pseudomonas* from the rhizosphere of wheat. Mutagenesis of the 7-kb clone with Tn*3*HoHo1 identified at least one promoter and up to three transcriptional/translational units within the putative biosynthetic locus. One unit contained within a 1-kb fragment was sufficient to complement a Phl-deficient Tn*5* mutant of Q2-87. DNA sequence analysis of this fragment revealed open reading frames that encoded predicted proteins with similarity to the chalcone synthase/stilbene synthase family of plant enzymes. Additional sequence analysis is underway to further elucidate the Phl biosynthetic pathway and the evolutionary relationship between the bacterial and plant genes.

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USE OF THE ICE NUCLEATION GENE (INAZ) AS A MARKER TO MEASURE PHENAZINE GENE EXPRESSION IN *PSEUDOMONAS AUREOFACIENS* STRAIN 30-84. W.G. Dilantha Fernando and L. S. Pierson III, Department of Plant Pathology, University of Arizona, Tucson, AZ 85721.

Pseudomonas aureofaciens strain 30-84 produces three phenazine antibiotics. When produced on wheat roots, these phenazines effectively inhibit the take-all pathogen *Gaeumannomyces graminis* var. *tritici*. In order to understand the regulation of phenazine gene expression in the rhizosphere, a reporter strain was constructed within the phenazine biosynthetic gene phzB. A promoterless ice nucleation gene (inaZ) was inserted into the phzB reading frame and the resulting phzB::inaZ fusion was marker exchanged into the 30-84 chromosome. The reporter strain (30-84Ice) is being used to examine the expression of microbial biocontrol genes *in situ*. Ice nucleation activity as a function of bacterial population size will be determined by the droplet freezing assay and serial dilution plating.

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A *RPOS*-LIKE SIGMA FACTOR GENE IS INVOLVED IN PYRROLNITRIN PRODUCTION BY *PSEUDOMONAS FLUORESCENS* PF-5. [1] A. Sarniguet and [2] J. E. Loper. [1]INRA Station de Pathologie Végétale, Centre de Recherches de Rennes, BP 29, 35650 Le Rheu, France and [2]USDA-ARS HCRL, 3420 NW Orchard Ave., Corvallis, OR 97330, USA.

Pseudomonas fluorescens Pf-5, a rhizosphere inhabitant that suppresses several soilborne diseases, produces at least three antibiotics. One of these, pyrrolnitrin, is involved in the biological control of *Pyrenophora tritici-repentis* and *Rhizoctonia solani*. Genomic DNA flanking a Tn*5* insertion that inactivates pyrrolnitrin production by Pf-5 has a high nucleotide homology with the *rpoS* gene of *Escherichia coli*, which has a central role in gene regulation during stationary phase. Several pleiotropic effects caused by an *rpoS* mutation in *E. coli* are also observed with the Tn*5* mutation of Pf-5. These include sensitivities of stationary phase cells to stress imposed by hydrogen peroxide, osmotic shock or aging. A plasmid containing the cloned wild type sequence restores pyrrolnitrin production, tryptophan-side chain oxidase activity and stress resistance to the Tn*5* mutant of Pf-5. We propose that a sigma factor encoded by an *rpoS*-like gene influences pyrrolnitrin production and certain phenotypes expressed by Pf-5 during the stationary phase.

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Cell to Cell Interactions Among Rhizosphere Bacteria Influence the Expression of Phenazine Antibiotics in *Pseudomonas aureofaciens* 30-84. **D.W. Wood and L.S. Pierson III. Department of Plant Pathology, University of Arizona, Tucson, AZ 85721.**

Phenazine antibiotic production in the soilborne biological control bacterium *Pseudomonas aureofaciens* 30-84 is regulated by a diffusible signal molecule. Two genes, *phz*R and *phz*I, have been identified previously and are members of the LuxR / LuxI family of cell density-dependent transcriptional regulators. Members of the LuxR / LuxI family are believed to regulate the expression of their genes in a cell density-dependent manner by sensing the accumulation of a diffusible homoserine lactone (HSL) autoinducer. Using a *phz*B::*lac*Z genomic reporter strain (30-84Z), we have screened bacterial isolates from the rhizosphere of wheat for their influence on phenazine gene expression. Cell-free supernatants from several of these isolates are able to influence the expression of the phenazine reporter strain 30-84Z. In addition, extraction protocols designed to isolate HSL derivatives have been effective in isolating biologically active inducers from several of these strains. These results indicate that other wheat rhizosphere bacteria are capable of producing diffusible signal molecules which can influence the expression of genes essential for biological control in *Pseudomonas aureofaciens* 30-84.

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ISOLATION AND CHARACTERIZATION OF BACTERIAL ENDOPHYTES FROM PRAIRIE PLANTS. P.M. Higley & Anne Vidaver, University of Nebraska, Lincoln, NE 68583-0722.

Endophytic bacteria were collected in 1992 from leaves of 25 species of vegetatively growing prairie grasses and forbs and in 1993 from the stems of 7 species of prairie grasses in the reproductive stage. Seventy-seven and 65%, respectively, of the isolates collected on NBY agar in 1992 and 1993 were Gram negative. Ten of the 36 isolates collected in 1992 could be recovered from stem-inoculated, greenhouse-grown plants at the reproductive stage. The incidence of recovery varied from 10% to 60%, with a mean recovery of 22%. The bacterial titer detected in colonized plants ranged from 3.5×10^3 to 4.0×10^5 CFU/g fresh wt. with a mean titer of 1.8×10^4 CFU/g fresh wt. The 35 isolates obtained in 1993 from naturally-colonized prairie grasses were found at levels ranging from 1.5×10^4 to 5.2×10^9 CFU/g fresh wt. The ability of these bacteria to colonize artificially-inoculated plants is currently being evaluated in the greenhouse.

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PSEUDOMONAS SOLANACEARUM PRODUCES A HOMOSERINE LACTONE-LIKE SIGNAL MOLECULE. L. M. Ganova-Raeva, A. B. Flavier, and T. P. Denny. Department of Plant Pathology, University of Georgia, Athens, GA 30602.

A number of Gram negative bacteria, including some phytopathogens, produce homoserine lactones (HSLs) that act as intercellular signal molecules to regulate various cellular processes. Although structurally similar, these HSLs differentially activate heterologous HSL-recognition systems. We found that wild-type *Pseudomonas solanacearum* releases a presumptive HSL that activates both *Vibrio fischeri* and *Agrobacterium tumefaciens* HSL-recognition systems. Production of the presumptive HSL by *P. solanacearum* was greater in minimal medium than in rich, complex medium. Maximal induction of the *A. tumefaciens* HSL-recognition system by an ethyl acetate extract of a wild-type *P. solanacearum* culture was five-fold lower than by an *A. tumefaciens* HSL preparation. The *P. solanacearum* extract contained little of the volatile, extracellular factor (VEF) also made by this pathogen. Mutation of either *phcA* (a global regulator of virulence-associated genes) or *phcB* (which influences *phcA* expression) in *P. solanacearum* reduced production of the presumptive HSL five fold. Thus, *P. solanacearum* appears to make both the VEF and an HSL that is different from that of *A. tumefaciens*. We are continuing to investigate the involvement of an HSL in regulating density-responsive traits in *P. solanacearum*.

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PURIFICATION AND CHARACTERIZATION OF A VOLATILE, ENDOGENOUS FACTOR THAT RESTORES EXPRESSION OF VIRULENCE-ASSOCIATED GENES IN A *PSEUDOMONAS SOLANACEARUM* MUTANT. A.B. Flavier and T.P. Denny. Department of Plant Pathology, University of Georgia, Athens, GA 30602.

Inactivation of *phcA*, which encodes a LysR-type transcriptional regulator (PhcA) in *Pseudomonas solanacearum*, reduces expression of several virulence-associated genes. Strains carrying a *phcB83*::Tn*5* mutation are reduced in expression of PhcA-regulated genes as well as *phcA*. Unlike *phcA* mutants, the *phcB83* mutant is restored to a wild type (WT) phenotype by a volatile, endogenous, extracellular factor (VEF) produced by WT strains but not by the *phcB83* mutant. We have attempted to isolate and identify the VEF to enable us to investigate its possible role in PhcA regulation of virulence genes. Culture supernatants of WT *P. solanacearum* were fractionated by distillation and the distillates extracted with $MeCl_2$. The organic fractions were washed with HCl and $NaHCO_3$, and then evaporated to dryness. The residue was dissolved in hexane and passed thru a silica gel column, which was then eluted in a stepwise-fashion with solvents of increasing polarity. Presence of the VEF after each purification step was followed using a *phcB83,eps::lacZ* strain whose *lacZ* expression is increased to WT levels in the presence of the VEF. Preliminary GC-MS analysis indicated that the major component of the active fraction is probably 8-hydroxy hexadecanoic acid methyl ester. The compound is absent from similarly processed culture supernatants of the *phcB83* mutant.

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CHARACTERIZATION OF A FACTOR PRODUCED BY PSEUDOMONAS AUREOFACIENS BG33 THAT INHIBITS EGG HATCH OF CRICONEMELLA XENOPLAX D.C.M. Glandorf, B. Leverentz, W.C. Derrick and D.A. Kluepfel, Dept. of Plant Pathology and Plant Physiology, Clemson University, 120 Long Hall, Clemson, SC 29634-0377.

Pseudomonas aureofaciens BG33 is able to kill eggs of the ring nematode *Criconemella xenoplax* which is a major factor in the premature death of peach trees. This study focused on the biochemical and genetic characterization of the egg kill factor produced by *P. aureofaciens* BG33. BG33 produced the egg kill factor in both rich and minimal medium.

Egg kill activity was also detected in cell free culture filtrate of BG33. A Tn5 transposon mutant bank of BG33 was screened for egg kill activity. Eight out of 2100 mutants tested lacked the egg kill phenotype and exhibited different chromosomal insertions of the Tn5. Experiments are in progress to complement these mutants to the wild type phenotype.

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A SEMISELECTIVE MEDIUM FOR DETECTING EPIPHYTIC AND SYSTEMIC POPULATIONS OF *PSEUDOMONAS SAVASTANOI* FROM OLEANDER. H. R. Azad and D. A. Cooksey, Dept. of Plant Pathology, University of California, Riverside, 92521.

A new medium, oleander knot agar (OKA), was developed for isolation of *Pseudomonas savastanoi* (Ps) from oleander plants. OKA contained (in grams per liter): L-serine (2.0), yeast extract (1.0), $NH_4H_2PO_4$ (0.5), K_2HPO_4 (0.5), NaCl (5.0), $MgSO_4 \cdot 7H_2O$ (0.2), SDS (0.4), boric acid (1.0), vancomycin (0.15), cephalexin (0.05), and agar (14.0). Of 34 strains of Ps from several different geographic areas, all grew on OKA, and the mean quantitative recovery for all Ps strains was 41%. OKA was more selective than other media tested, such as BCBRVB, M-71, KBBC, MSP, and PVF-1, and inhibited 89% of saprophytic bacterial strains recovered from oleander tissues. In addition to isolation from galls, OKA was used to detect epiphytic populations of Ps from leaves, stems, and flowers, and to detect systemic movement of Ps in oleander stems.

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PHYSIOLOGICAL AND ULTRASTRUCTURAL COMPARISONS OF STREPTOMYCES SCABIES WITH DEEP PITTING AND NONPATHOGENIC ISOLATES OF STREPTOMYCES SPP. F. Spooner, B. Stein, and R. Hammerschmidt, Dept. Botany and Plant Pathology, Michigan State University, East Lansing, MI 48824

Streptomyces scabies and *S. acidiscabies* cause common scab of potato and differ morphologically from a Michigan isolate which causes deep-pitted scab. The Michigan isolate (DP) has been shown to be morphologically and pathologically different from R. Loria's pathogenic *S. scabies* RL 232 and nonpathogen RL 95 isolates. Both RL 232 and RL 95 produce spiral spore chains and pigments in media, especially peptone yeast iron agar (PYI), with RL 95 showing a weak PYI reaction. The DP isolate produces flexuous spore chains and is negative for pigment production on several standard media. On the basis of physiology, biochemistry, and ultrastructure, the isolates DP and RL 232 appear to be more similar to one another than to RL 95. DP, while pathogenic on potatoes, lacks some of the characteristics given for *S. scabies* and *S. acidiscabies*, so it does not belong to either species.

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SPATIAL PATTERNS IN ICE NUCLEATION TEMPERATURES AND POPULATION SIZES OF *PSEUDOMONAS SYRINGAE PV. SYRINGAE* ON GERMINATING BEAN SEEDS. S.S. Hirano, M.K. Clayton[1], A.J. Dik and C.D. Upper[2], Departments of Plant Pathology, Statistics[1], and Plant Disease Resistance Research Unit, USDA Agricultural Research Service[2], University of Wisconsin, Madison, WI 53706.

Bean seeds were inoculated at the time of planting with *Pseudomonas syringae* pv. *syringae* (Pss) strain B728a for the purpose of examining the relationship between spatial patterns of ice nucleation temperatures (INTs) and populations of Pss. Every seed in 5-m row segments from replicated field plots was dug up and assayed with a tube ice nucleation test. Spatial patterns in INTs and Pss populations as estimated by dilution plating were random for seeds sampled immediately after planting. One day later, the patterns in INTs and pathogen populations were non-random. These patterns were associated with non-random patterns in seed germination. Four days after planting, the patterns in INTs and pathogen populations were once again random. Patterns in INTs and pathogen populations were similar on developing seeds. Non-random patterns in pathogen populations, which appear to be similar to those described previously for bacterial brown spot disease, may appear very early in the life of the host plant. The factors affecting the transient nature of spatial patterns of Pss on germinating seeds and whether there is any relationship between patterns of Pss a few days after planting to those in disease weeks later remain to be elucidated.

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EFFECT OF SOIL SOLARIZATION ON *AGROBACTERIUM TUMEFACIENS* POPULATIONS. A. Raio°, A. Zoina°, M.L. Canfield* and L.W. Moore*.
°Institute of Plant Pathology, University of Naples "Federico II", Via Università 100, 80055 Portici, Italy. *Dept. of Botany and Plant Pathology, Oregon State University, Corvallis, OR 97331-2902, USA.

Methods to control crown gall disease are sparse and not always effective. New approaches are needed. Soil solarization was chosen as an alternative method to test for control of *Agrobacterium tumefaciens* in soil. In 1992 *Agrobacterium* populations in a naturally infested soil were monitored before and after solarization. The population of *Agrobacterium* was 10^4 cfu/gr of soil, but only one of 700 isolates tested was pathogenic. After solarization, the number of *Agrobacterium* had decreased to less than 10^2. Soil temperature at 15 cm depth had reached 45-48 °C. A second experiment was conducted during the summer of 1993. Solarized and untreated soil was infested with a rifampicin resistent mutant of *A.t.* strain B49c/83 to a final concentration of 10^6 cfu/gr of soil. Natural and rif+ *Agrobacterium* populations were weekly monitored on selective media. The maximum temperature reached 52 °C at 5 cm and 38 °C at 20 cm in solarized plots. After four weeks of treatment, B49c/83 was no longer detectable in solarized soil whereas the mean population of *Agrobacterium* in control plots was $3x10^3$ cfu/gr of soil. These results indicate that solarization is an effective method for reducing the population of A.t. in nursery soils.

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USE OF *Syringa* PLANTLETS IN TISSUE CULTURE AND SYRINGROMYCIN DNA PROBES TO RAPIDLY EVALUATE PATHOGENICITY OF *Pseudomonas syringae* ISOLATES. M. L. Canfield, L. M. Bateham, S. L. Lu, H. J. Scheck, and L. W. Moore, Department of Botany and Plant Pathology, Oregon State University, Corvallis, OR 97331-2902.

Pseudomonas syringae is commonly isolated from nursery stock, however the determination of pathogenicity is difficult and labor-intensive. Lilac plantlets in tissue culture and DNA probes were used to detect pathogenic isolates of *P. syringae* from among those collected in 1982, 1983, 1992 and 1993 from 42 naturally infected plant hosts with tip dieback, necrotic lesions or cankers. Bacterial isolates were suspended in water to a concentration of 10^8 cfu/ml and lilac plantlets were inoculated by immersing them in the bacterial suspension. Plantlets were then exposed to -5° C for 10 min followed by incubation in a growth room at 25° C for ten days. Pathogenicity was evaluated by time of onset and extent of tissue necrosis during that time interval. Colony hybridizations were conducted using DNA of syringomycin genes *syrB* and *syrD* of *P. syringae* labled with digoxygenin-11-dUTP as probes to detect pathogens. Of 605 isolates tested, 304 have been identified as pathogenic using these methods. There was considerable variability in the virulence of the isolates tested, with some producing widespread necrosis within 24 hours while others developed necrosis later that spread more slowly.

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PHASEOLUS AUREUS ROXB. INFECTED WITH *PSEUDOMONAS SOLANACEARUM*. H. Abdullah and N.Y.Fuk. Dept. of plant Protection, Faculty of Agriculture, Universiti Pertanian Malaysia, UPM 43400, Serdang Selangor, Malaysia.

Pseudomonas solanacearum was isolated from *Phaseolus aureus* Roxb. (green gram) showing bacterial wilt symptoms in the field. Biovar determination indicated that all isolates were biovar 3. Eventhough the pathogen has been reported on a number of host, this is the first report of the pathogen on this host in Malaysia and possibly elsewhere. Greenhouse inoculation studies showed that the pathogen was also highly virulent to tomato, tobacco, eggplant, chilli and moderately susceptible to tobacco and groundnut. Isolates of the pathogen from *Sesbania rostrata, Stachytarpheta indica, Hyptis suaveolens and Erechtites hieracifolia* were found to be pathogenic to green gram. This indicated that the strain infecting green gram is not a distinct strain of the pathogen but could be similar to those infecting the above mentioned hosts. All varieties tested were found to be suseptible.

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THE PRODUCTION OF CALCIUM OXALATE CRYSTALS BY THE WHITE ROT FUNGUS RESINICIUM BICOLOR: IMPLICATIONS FOR THE TRANSLOCATION OF CALCIUM. Jon H. Connolly and Jody Jellison. Dept. of Plant Biology and Pathology, University of Maine, Orono, ME 04469-5722.

Oxalic acid production is extremely common in fungal systems. Under supersaturating conditions this oxalic acid production can lead to the precipitation of calcium oxalate. The exudation of oxalic acid and the coincident precipitation of the calcium salt has been observed in several plant pathogens and wood decomposers. *Resinicium bicolor* is a white rot organism which produces styloid druses of twinned calcium oxalate crystals in the wood environment. In modified ASTM soil block assays *R. bicolor* produces calcium oxalate crystals in wood and crystals four times larger in mycelial cords growing up the sides of the glass jar. Such accumulations of calcium oxalate at centimeter distances from calcium sources in the wood and soil indicate that this fungus is capable of transporting calcium in its mycelium significant distances. The ability of wood degrading fungi to translocate calcium within their thallus from areas enriched in calcium to sites impoverished in this nutrient could have important implications for nutrient redistribution on the forest floor as downed trees decay.

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STIMULATION OF GERMINATION OF TELIOSPORES OF UROMYCES APPENDICULATUS BY SOME LOW MOLECULAR WEIGHT ALDEHYDES AND DERIVATIVES. R. C. French[1], S. E. Nester[1], and J. R. Stavely[2], USDA, ARS, [1]Frederick, MD 21702; [2]Beltsville, MD 20705.

Previously we reported stimulation of the germination of teliospores of the bean rust pathogen, Uromyces appendiculatus by isobutyraldehyde and methyl isobutyrate and related ester derivatives (J. Agric. Food Chem. 41:1743-7, 1993). Stimulatory compounds were volatile, with at least one carbonyl oxygen and four to seven carbon atoms. Upon extending the study to smaller compounds, activity was found in some two and three carbon compounds. Acetaldehyde stimulated germination to 70% (control 11%) at 21 days, at 10 µl/L. Propionaldehyde stimulated germination to 78% (control 22%) at 21 days, at 25 µl/L. Two water soluble, non-volatile derivatives, calcium propionate and acetaldehyde ammonia, stimulated germination to 76% (control 14%) at 500 µl/L, and to 83% (control 9%) at 50 µl/L, respectively, at 21 days. Ethanol and a trimer of acetaldehyde (paraldehyde), were inactive. Teliospores exposed to vapor from 25% aqueous acetaldehyde for 5 min germinated 43% (control 8%) at 21 days. Ubiquitous compounds such as these may play a role in activating teliospores to initiate the infection process.

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CHARACTERIZATION OF A cDNA CONTAINING CALCIUM-BINDING DOMAINS INDUCED DURING A HYPERSENSITIVE REACTION. J. L. Jakobek and P. B. Lindgren, Plant Pathology, North Carolina State University, Raleigh, N.C., 27695

A cDNA clone was identified corresponding to a transcript which accumulates to high levels in bean during the hypersensitive reaction to *Pseudomonas syringae* pv. *tabaci*. Sequence analysis indicated that this clone, designated pHrs32, represents a novel gene distinct from other plant defense genes currently listed in the gene/protein data bases. This analysis also revealed the pHrs32 insert to be approximately 650 base-pairs (bp) in length having an open reading frame (ORF) with a predicted product of 161 amino acids. The polypeptide defined by this ORF contains 3-putative calcium-binding domains known as EF-hands. This polypeptide shares no amino acid homology with other calcium-binding proteins except within the EF-hands. Northern analysis indicated that the pHrs32 transcript is approximately 900 bp in length. Southern analysis suggests that the pHrs32 insert corresponds to a single gene or a member of a small gene family in bean. When bean is infiltrated with *P. s.* pv. *tabaci* treated with inhibitors of bacterial protein synthesis, the pHrs32 transcript does not accumulate nor does the hypersensitive reaction occur. Preliminary experiments indicate that the pattern of pHrs32 transcript accumulation observed in bean in response to a *P. s.* pv. *tabaci* Hrp⁻ mutant and compatible *P. s.* pv. *phaseolicola* are significantly different from that observed in response to wildtype *P. s.* pv. *tabaci*.

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THE SENSITIVITY OF NORMAL AND TEXAS MALE STERILE CYTOPLASM MAIZE LEAVES TO BIPOLARIS MAYDIS RACE T TOXIN IS REDUCED BY LIGHT. D. S. Park and M. O. Garraway, Department of Plant Pathology, The Ohio State University, Columbus, OH 43210.

Detached leaves of Normal (N) and Texas male sterile (T) cytoplasm maize isolines (cv. W64A) from Ohio and Iowa were infiltrated with various concentrations of BMT-toxin in the light (10,000 lux) for 24 hr at 28 C. They were immersed in DDW then incubated for another 24 or 48 hr either in the dark, or in alternating light (12 hr) and dark (12 hr) cycles, or in continuous light (CL). The sensitivity of leaves to BMT-toxin was evaluated by comparing their rates of loss of electrolytes. The T, but not the N, cytoplasm isolines leaked 3-4 times more electrolytes than the N cytoplasm isolines. Also for T cytoplasm isolines, electrolyte leakage was greatest (3-4 times CL) when toxin-treated leaves were incubated in continuous dark, intermediate (1.5-2 times CL) when leaves were incubated under alternating light and dark cycles, and least when incubated in continuous light (1.0 times CL). Moreover, exposure of BMT-toxin to light for 24 hr prior to its infiltration into detached leaves had no effect on its potency. Therefore, there is a decrease in the sensitivity to BMT-toxin when T cytoplasm maize leaves are exposed to light after toxin-treatment. A light-mediated alteration in the physiology of maize leaves, rather than a light-inactivation of toxin, appears to be involved.

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Extracellular enzymes involved in the potential biocontrol of Rhizoctonia solani by Bacillus megaterium and Trichoderma harzianum. B. L. Bertagnolli, F. K. Dal Soglio, J. B. Sinclair and D. M. Eastburn, Dept. of Plant Pathology, University of Illinois at Urbana-Champaign, Urbana, IL 61801-4709.

A biocontrol strategy using mixtures of the soilborne biocontrol agents, B. megaterium B153-2-2 and T. harzianum Th008, is in development for control of soybean seedling blight and root rot caused by R. solani 2B12 (AG 2-IIIB). Enzyme levels in cell-free culture filtrates of B153-2-2, Th008 and 2B12 were tested by spectrophotometry. Fungal filtrates showed high levels of pectinase, while B153-2-2 filtrates showed high levels of Ca^{++}-dependent endoproteinase and chitosanase A. Comparable levels of pectin lyase were found in B153-2-2 and 2B12 filtrates. Both pectinase and pectin lyase from 2B12 were partially inactivated by Ca^{++}-endoproteinase from B153-2-2. Th008 produced elevated levels of chitinolytic enzymes in the presence of a sterile cell suspension of R. solani. No phospholipase A_1 (Megacin A) activity was detected in any filtrate.

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IN VITRO MYCELIAL GROWTH INHIBITION OF FUNGAL PATHOGENS BY *PSEUDOMONAS AUREOFACIENS* STRAIN 30-84. W.G. Dilantha Fernando and L.S. Pierson III, Department of Plant Pathology, University of Arizona, Tucson, AZ 85721.

Pseudomonas aureofaciens strain 30-84, carries the biosynthetic genes phzB and phzC involved in the production of phenazine antibiotics. Strain 30-84Z contains a promoterless lacZ structural gene fused in frame within the phzB gene, making the strain Phz⁻. Strain 30-84R which carries functional copies of phzB and phzC, also contains additional copies of the transcriptional activator phzR in trans. The ability of strains 30-84 (Phz^+), 30-84Z (Phz^-), and 30-84R (Phz^{++}) to inhibit mycelial growth of several fungal plant pathogens was tested in vitro. The mycelial growth of *Gaeumannomyces graminis* var. *tritici* was decreased 5 fold in three experiments, by strain 30-84R, over the wild type strain 30-84. There was no significant inhibition of mycelial growth by strain 30-84Z. Similar results have been observed with other fungi tested in our initial experiments. This indicates the potential of improving biological disease control through understanding microbial gene regulation.

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EVALUATION OF STRAINS OF ACTINOPLANES SPP. FOR THEIR EFFICACY TO REDUCE SOILBORNE DISEASES CAUSED BY OOMYCETOUS FUNGI. N.I. Khan, A.B. Filonow and L.L. Singleton, Department of Plant Pathology, Oklahoma State University, Stillwater OK 74078.

Clay granules bearing sporangia of Actinoplanes spp. were applied at 0.1% to 10% w/w to nonsterile soil infested with 500-2000 oospores of Pythium ultimum/g. Populations of Actinoplanes spp. at these concentrations were 10^4-10^8 CFU/g of soil. In bench top experiments with table beet or bush bean as hosts, emergence after 4-6 wk was increased and root rot reduced in soil treated with strains ATCC 25844 (A. brasiliensis), W257 and W57 applied at 5% or 10% w/w compared to nontreated controls ($P \leq 0.05$). These strains also were efficacious in field microplots at 5% w/w ($P \leq 0.05$), but not at 0.1% w/w. On the bench, with soil infested with 500 oospores of Aphanomyces cochlioides/g and sugar beet as the host, emergence increased when strains W57 and W257 were applied on granules at 5% w/w, but only W57 reduced root rot over controls ($P \leq 0.05$). Treatments of microplots infested with A. cochlioides with strains W57 and W257 at 5% w/w increased emergence ($P \leq 0.05$) and reduced root rot ($P = 0.1$) compared to controls. The 0.1% w/w level was not effective. Strains also were applied on seeds (10^7 CFU/seed). Seeds were planted in soil infested with 1000 oospores of P. aphanidermatum or P. ultimum/g. On the bench, emergence of pea, cotton or bush bean was increased and root rot severity reduced ($P \leq 0.05$), using seed treated with strains 25844 or K30.

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CLAY GRANULES BEARING ACTINOPLANES SPP. FOR THE BIOCONTROL OF OOMYCETOUS FUNGI IN SOIL. N.I. Khan, A.B. Filonow and L.L. Singleton, Department of Plant Pathology, Oklahoma State University, Stillwater OK 74078.

Strains of Actinoplanes spp. grew and sporulated well on 1/3 strength Czapek-Dox agar at pH 7 and 28-30 C. Continuous exposure to light (150 uE/m²/sec) was inhibitory to growth and sporulation of strains on agar, but lower intensity light (1-4 uE/m²/sec) was not inhibitory. Strains were induced to sporulate on clay granules for application to soil. Granules (Ultra Premium Cat Litter, WalMart) were ground to pass a 30 mesh sieve, collected on a 45 mesh sieve, washed with tap water, autoclaved, cooled, and treated with 1/3 strength Czapek-Dox broth amended with 0.1% peptone. Granules were dried at 70 C for 48 h, inoculated with suspensions of either zoospores or washed, macerated hyphae of Actinoplanes sp., and incubated at 28 C in the dark for 2-3 wk. Some strains, e.g. ATCC 25844, sporulated in 3 days while others, e.g. K30, required 10-14 days. Sporangia of Actinoplanes spp. on granules in buffer released zoospores that parasitized Pythium ultimum oospores. Damping-off and root rot caused by Pythium or Aphanomyces spp. on several hosts were controlled in natural soil using granules treated with Actinoplanes spp.

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CONTROL OF CARDUUS ACANTHOIDES WITH MYROTHECIUM VERRUCARIA IN THE GREENHOUSE IN THE ABSENCE OF DEW. Shaw-Ming Yang, USDA, ARS, Frederick, MD 21702.

Two isolates of Myrothecium verrucaria (Albertini & Schweinitz) Ditmar:Fr., ATCC 90310 and ATCC 18398, were investigated for their ability to control Carduus acanthoides L. (plumeless thistle) in the greenhouse in the absence of dew. ATCC 90310 was isolated from Euphorbia esula L. (leafy spurge collected in China by S. M. Yang) and ATCC 18398 was isolated from submerged balsa wood in Maryland by J. L. Crane. Both isolates killed 2- to 4-week-old, greenhouse-grown seedlings within 2 weeks at 10-40% RH and 20-35 C when the plants were inoculated with conidia plus invert emulsion (IE). The concentration of conidia in the IE was 10^8/ml. Efficacy of the two isolates to kill C. acanthoides was affected by plant age and inoculum dosage. Seedlings were killed by one application of inoculum (100 ml on 40 plants/m²) while 12-week-old plants in the same arrangement required one to three applications of 400 ml inoculum to kill

the plants. The controls receiving IE only remained healthy. Field tests of M. verrucaria in IE are needed to determine the potential of this product for biological control of C. acanthoides.

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ISOLATION AND SELECTION OF BACTERIA FOR MANAGEMENT OF *PYTHIUM APHANIDERMATUM* ON CUCUMBER GROWN IN ROCKWOOL. M. L. Matheny, M. E. Taylor, and M. S. Melzer. EcoScience Corporation, 377 Plantation Street, Worcester, MA 01605.

Rockwool is an inert growth medium used in the production of greenhouse crops. *Pythium aphanidermatum* regularly causes a 7-10% loss of cucumber plants grown in rockwool. A total of 1311 bacteria were isolated from the rhizosphere of rockwool-grown cucumber plants collected in Florida, Ohio, and Ontario, Canada. Bacteria were isolated by four methods: specifically for Gram negative bacteria or Bacilli and nonspecifically on tryptic soy broth agar (TSBA) or nutrient solution agar (NSA). The isolates were screened in a tissue-culture-plate assay for ability to protect cucumber seeds from infection by *P. aphanidermatum*. An isolate of *Pseudomonas*, which consistently provided excellent control, was used as the standard for selection of effective isolates. Approximately 20% (264) of the isolates had activity equal to or better than the standard. Only 2% of the *Bacillus* isolates were effective whereas 26% of the Gram negative bacteria were effective. Of the isolates recovered on TSBA and NSA, 25% and 20%, respectively, were effective. The most promising isolates are being tested in an assay that more closely resembles the cucumber-rockwool production system.

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Mycostop Biofungicide for Greenhouse Ornamentals and Vegetables. J. C. Meneley, AgBio Development Inc., 9915 Raleigh St., Westminster, CO 80030.

Mycostop is a wettable powder formulation of *Streptomyces griseoviridis* Strain K61. It was recently registered for use on container grown ornamentals and vegetables to control *Fusarium, Alternaria,* and *Phomopsis* root rots and wilts. The product is applied as a drench or soil spray or can be used as a transplant dip or seed dressing. Mycostop has been successfully used by commercial growers in Europe since 1990 and in the U.S. late 1993. Suppression or control of other pathogens has also been documented by University and grower trials. A review of Mycostop biocontrol activity will be presented.

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FIELD SCREENING OF A POTENTIAL BROAD-SPECTRUM BIOHERBICIDE FOR *AMARANTHUS* SPP. E.N. Rosskopf, R. Charudattan, and J.T. DeValerio, Plant Pathology Dept., Univ. Florida, Gainesville.

Phomopsis amaranthicola (proposed n. sp.) is a potential broad-spectrum bioherbicide for *Amaranthus* spp. as determined under greenhouse conditions. The pathogen caused symptoms without a dew period when inoculum suspensions were amended with psyllium mucilloid. A field trial was performed with *A. hybridus, A. lividus, A. retroflexus, A. spinosus, A. viridus* and a triazine-resistant *A. hybridus*. Treatments consisted of mycelial and conidial inocula, amended with mucilloid and sprayed once or twice. Disease progress rates and areas under the disease progress curves were significantly higher for inoculated versus noninoculated plots, the latter having been infected after the dispersal of conidia from inoculated plots. Final mortality of all species, except *A. hybridus*, reached 100% in inoculated plots 25 days earlier than in noninoculated plots. A single application of a high dose of conidia (6×10^7 conidia/ml) resulted in the most effective control of the weeds when compared to a low dose (1×10^6 conidia/ml) or a mycelial suspension.

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EFFECTS OF SUBLETHAL HEATING ON MICROSCLEROTIA OF VERTICILLIUM DAHLIAE AND IMPLICATIONS FOR BIOCONTROL. E. C. Tjamos and D. R. Fravel, Agricultural University of Athens, Athens 118-55, Greece and USDA, ARS, BPDL Beltsville, MD 20705.

Microsclerotia (ms) were not heated or heated as follows to simulate soil solarization: H-1 was 31C for 10h then 35C for 14h; H-2 was 33C for 10h then 36C for 14h; and H-3 was 35C for 10h then 38C for 14h. Five days after plating, 96-93% of ms exposed to 1-5 days, respectively, of H-1 had germinated; 90-50% of ms receiving 1-5 days, respectively, of H-2 had germinated; and 92-0% of ms receiving 1-5 days, respectively, of H-3 had germinated. Three days after plating, 40% of colonies from the nonheated controls formed melanized ms and 75% had formed ms by 7 days after plating. In contrast, ms heated 1 day with H-3 produced colonies forming few melanized ms. Only 5% and 10% of these colonies formed melanized ms 6 and 9 days after plating, respectively. There was a synergistic interaction between sublethal heating and the biocontrol fungus Talaromyces flavus in for melanin deposition and an additive effect for wilt control.

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EVALUATION OF THE ANTAGONISTIC POTENTIAL OF CULTIVATED AND UNCULTIVATED NURSERY SOILS AGAINST THE FUSARIUM ROOT ROT PATHOGEN OF CONIFER SEEDLINGS. M. H. Hoefnagels and R. G. Linderman, Oregon State University Department of Botany and Plant Pathology and USDA-ARS Horticultural Crops Research Laboratory, Corvallis, OR 97330

Soil from a cultivated (tilled, fertilized, fumigated) conifer seedling nursery was compared with adjacent, recently cleared but never-cropped forest soil for antagonistic potential *in vitro* against the root rot pathogen, *Fusarium oxysporum*. Soil suspensions (untreated or heated to 60°C for 20 min) were dilution-plated and bacterial colonies were counted after 24, 48 and 72 hr of incubation. After 72 hr, plates were sprayed with *F. oxysporum* conidia, incubated 48 hr, and bacterial colonies with zones of inhibition of *F. oxysporum* were counted. Total bacteria for forest and nursery soils were approximately equal, but a greater proportion of bacteria from forest soil was antagonistic to *F. oxysporum*. Few bacteria visible after 24 hr were antagonistic; most antagonists appeared after 48 and 72 hr. In both soils, a high proportion of bacteria survived heating, indicating they had high populations of spore-forming bacteria. A greater percentage of colonies isolated from heated than untreated suspensions was antagonistic to *F. oxysporum*. These results indicate that forest soil is a good source of antagonists against *F. oxysporum*, and that cultivation may reduce the antagonistic potential of nursery soils.

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EVALUATION OF BACTERIAL ANTAGONISTS AGAINST PHYTOPHTHORA CINNAMOMI IN THE CLOSED-INSULATED PALLET SYSTEM (CIPS). R. G. Linderman, J. L. Marlow, B. Blackburn, and H. W. McDaniel. USDA-ARS Horticultural Crops Research Laboratory, Corvallis, OR 97330

A closed-insulated pallet system (CIPS), which provides constant temperature, moisture, and nutritional conditions, was used to grow snapdragon plants as a host for the evaluation of candidate bacteria as biocontrol agents against the root rot pathogen, *Phytophthora cinnamomi*. Test plants were inoculated with candidate bacterial antagonists 2 wks before transplant into potting mix containing *P. cinnamomi* inoculum. Plants in containers in the CIPS were watered by capillarity from the pallet reservoir, and fertilized by diffusion from a slow-release source on the medium surface. Root rot and wilt were apparent on snapdragon within 2-3 weeks after transplant. Effective antagonists, providing protection against *P. cinnamomi* for the 6 week duration of the experiment, were clearly separated from those that did not provide protection on the basis of shoot size and degree of wiltt as well as percentage of roots from which the pathogen could be recovered. The data indicate that snapdragon is highly susceptible to *P. cinnamomi*, and the CIPS is a valuable, consistent, and reliable system in which to conduct *in vivo* assays to evaluate biocontrol agents isolated from soil or roots.

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SUPPRESSION OF *FUSARIUM SAMBUCINUM* BY VOLATILES FROM *BRASSICA* SPP. H. S. Mayton, R. Loria and S. F. Vaughn. Dept. of Plant Pathology, Cornell Univ., Ithaca, NY 14853 and USDA-ARS, MWA, National Center for Agricultural Utilization Research, Peoria, IL 61604.

Brassica spp. were tested for production of volatile compounds suppressive to *Fusarium sambucinum* (FS) for use in biological control. A 3-mm plug of FS was transferred to water agar with streptomycin and penicillin (100 µg/ml) and inverted over a jar (500 ml) containing 40-day-old chopped leaf tissue alone (10, 20 and 40 g) or with soil (20 g tissue, 100 g soil). Radial growth (RG) of FS was measured after 1, 3, 5 and 7 days. *B. juncea* and *B. nigra* tissue inhibited RG ($\leq$ 50% of the control), while *B. carinata, B. napus* and *B. campestris* did not. Inhibition of RG was greatest with 40 g of tissue. Tissue incorporated into soil was less suppressive than tissue alone. *Brassica* accessions are being screened for suppression of FS using 40 g of leaf tissue; variability within *B. juncea* and *B. carinata* has been observed.

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MICROBES ISOLATED FROM WHITE PINE NURSERY SOIL TO SUPPRESS PATHOGENIC *FUSARIUM* SPECIES. Cynthia M. Ocamb, USDA Forest Service, North Central Forest Experiment Station, 1992 Folwell Ave., St. Paul, MN 55108.

Rhizosphere and non-rhizosphere soils were collected from eastern white pine (*Pinus strobus*) nurseries. Soil was mixed into media containing cellulose, chitin, or pectin, poured into petri dishes, and incubated for 48 hr. *Fusarium solani*, *F. oxysporum*, and *F. proliferatum* were grown on media with pectin, cellulose, or dilute potato broth. *Fusarium* spp. were minced,

mixed with Czapek's agar, and pipetted over the soil dilution plates. Potential biocontrol agents were isolated from inhibition zone centers; the number of strains collected by each method was contrasted with that obtained using Herr's original method (1959). Rhizosphere inhabitants that both inhibit and competitively utilize the same carbon sources as root rot-causing *Fusarium* spp. were selected as potential biological control agents.

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SUPPRESSION OF *PHYTOPHTHORA CACTORUM* IN VITRO BY VOLATILES FROM CANOLA RESIDUES. V. L. Smith, Dept. Plant Pathology & Ecology, CT. Agr. Exp. Stn, New Haven, CT 06504

Decaying crucifer residues produce volatile breakdown products including isothiocyanates which are effective soil fumigants. Canola (*Brassica napus*) cv. 'Humus' has been shown to produce abundant glucosinolates that decay to produce isothiocyanates. Oven-dried, nonsterile, finely ground foliage and petioles (residues) of canola 'Humus', rutabaga, kale, and collards were placed in one quadrant of divided petri dishes at 0.5 g and 1 g rates; the remaining three quadrants were filled with Difco corn meal agar (CMA). Residues were moistened with sterile distilled water (SDW, 1:1, w/w); at no time were the residues in direct contact with the CMA. Plates with 1 quadrant of SDW were used as the control. Radial growth of 4 isolates of *P. cactorum* was measured at 2-day intervals; the experiment was repeated twice with 1 g residues and three times with 0.5 g residues. Only canola 'Humus' significantly reduced growth of *P. cactorum* at both levels of residues; suppression was equal at both rates. In planta suppression of *P. cactorum* on tomato is being studied.

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COMPOST-INDUCED SYSTEMIC RESISTANCE IN CUCUMBER TO PYTHIUM ROOT ROT AND ANTHRACNOSE. [1]W. Zhang, [1]W.A. Dick and [2]H.A.J. Hoitink. Department of [1]Agronomy and [2]Plant Pathology, OARDC, The Ohio State University, Wooster, OH 44691.

Cucumber (*Cucumis sativus L. cv* 'Straight Eight') seedlings germinated in compost-amended or peat mixes naturally suppressive and conducive to Pythium root rot, respectively, were transplanted using a split root technique into peat and compost mixes infested with a mixture of *Pythium ultimum* and *P. aphanidermatum*. Root rot of plants in peat was significantly less on roots paired with compost than peat. In separate tests, cucumber seedlings were inoculated on the first true leaf with *Colletotrichum lagenarium*. They were challenged 7 days later on the second true leaf with the same organism. Mean anthracnose severity ratings in the second true leaf, on a scale of 1 (symptomless) to 4 (leaf death), were 2.9 for inoculated and 3.6 for uninoculated plants. The rating difference associated with plants in compost was even greater with a value of 2.8 for plants in compost and 3.9 for plants in peat. These results suggest that compost induced systemic resistance in cucumber to these pathogens.

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DEVELOPING CITRUS FRUIT COATINGS THAT SUPPORT GROWTH OF THE BIOCONTROL YEAST *CANDIDA OLEOPHILA*. R. G. McGuire and R. D. Hagenmaier, USDA - ARS, 13601 Old Cutler Rd., Miami, FL 33157

Yeasts of the genus *Candida* are epiphytic on fruits of many citrus species, and some have proven useful for biocontrol of *Penicillium* decays. In commercial operations, these yeasts might be applied to fruits during the waxing procedure, but coatings need to be compatible to prolong the survival of the species. Wood resin is immediately toxic, unlike coatings based upon shellac and various waxes. Several components that enhance solubility, emulsification, and applicability of these primary constituents have been examined. *Candida oleophila* will survive for several hours in a shellac latex coating that contains as much as 1.5% KOH (pH 11), but 0.5% NH_3 (pH 9) is the limit. The tolerances for morpholine and ethanol lie between 1 and 2% and between 5 and 6%, respectively. Based upon these results, one dissolved shellac coating and two microemulsions of candelilla and polyethylene wax have been formulated that support survival of this yeast.

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SENSITIVITY OF *HELMINTHOSPORIUM SOLANI* STRAINS TO FUNGICIDES. **C. A. Strausbaugh, R. L. Forster, K. K. Shetty, P. Nolte, and G. E. Kleinkopf. Univ. of Idaho, R.& E. Center, Kimberly, ID 83341.**

Fourteen *Helminthosporium solani* strains, the causal agent of silver scurf on potato, were tested for their sensitivity to thiabendazole (TBZ), thiophanate-methyl plus mancozeb (TopsMZ), imazalil sulfate (IMZ), and CGA-173506 (Maxim). Mycelial plugs from single-spored cultures were placed on malt extract agar amended with 5, 50, 200, and 500 mg ai/L for TBZ, TopsMZ, and Maxim. IMZ was evaluated at 0.5, 5, 20, and 50 mg ai/L. Colony diameters were recorded at 2, 4, and 6 wks and ED_{50} values were calculated through linear regression. Seven strains were sensitive (ED_{50} <5mg ai/L) to TBZ, while four strains were moderately resistant (ED_{50} 23 to 424 mg ai/L), and three strains were resistant (ED_{50} >500 mg ai/L). With TopsMZ, the ED_{50} values for 12 strains ranged quantitatively from <5 to 66 mg ai/L at 6 wks. Two strains were moderately resistant to TopsMZ at 6 wks, with ED_{50} values of 143 and 153 mg ai/L, respectively. All strains were sensitive to IMZ with ED_{50} values of <1 to 5 mg ai/L. The percentage of growth reduction with Maxim ranged from 20 to 54% at 6 wks. The efficacy of TBZ, TopsMZ, Maxim, and IMZ decreased over time with less sensitive strains.

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USE OF COPPER SULFATE FOR LEAF SPOT CONTROL ON SOUTHERN RUNNER PEANUT. A. K. Culbreath and T. B. Brenneman, The University of Georgia, Coastal Plain Experiment Station, Tifton, GA 31793-0748.

Field experiments were conducted during 1991-1993 to evaluate copper sulfate (3.4 kg/ha) and chlorothalonil (Bravo 720, 1.26 kg ai/ha) for control of early (*Cercospora arachidicola*) and late (*Cercosporidium personatum*) leaf spot of peanut (*Arachis hypogaea*). Treatments were applied every 14 days to the cultivar Southern Runner. Leaf spot severity ratings (Florida 1-10 scale) were higher on plants treated with copper sulfate than with chlorothalonil in all three years. Leaf spot ratings for nontreated, copper sulfate and chlorothalonil treatments, respectively, were 9.0, 7.5, and 1.8 (LSD = 1.0) in 1991, 8.1, 5.7, and 4.6 (LSD = 0.9) in 1992, and 7.1, 5.0, and 3.3 (LSD = 0.7) in 1993. Yields for all three treatments were similar in 1991. Yields for the nontreated, copper sulfate and chlorothalonil treatments, respectively, were 3026, 3848, 4783 kg/ha (LSD = 786) in 1992, and 3831, 4954, and 5256 kg/a (LSD = 646) in 1993. The combination of resistance and tolerance in Southern Runner with the suppressive effect of copper sulfate on leaf spot epidemics represents a potential disease control regiment that could reduce dependence on chlorothalonil for peanut production and would provide an option for disease control that does not include synthetic fungicides.

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FUNGICIDE RESISTANCE IN *VERTICILLIUM FUNGICOLA*, A MYCOPATHOGEN OF *AGARICUS BISPORUS*. A. M. Bonnen, Department of Plant Pathology, The Pennsylvania State University, University Park, Pennsylvania, 16802, USA.

To follow the development of fungicide resistance in the *Verticillium fungicola* population over time, 44 isolates, collected from different locations over 43 years, were screened for their resistance to four fungicides, benomyl, N-phenylcarbamate, chlorothalonil and thiabendazole. Resistance or sensitivity was determined through measurement of radial growth on PDA plates amended separately with each fungicide (50 mg active ingredient/l). Of the fungicides studied, only benomyl was found to have resulted in resistance which correlated directly with its introduction for control of disease on mushrooms. N-phenylcarbamate was included in this study in order to determine whether sensitivity to this fungicide was negatively correlated with resistance to benomyl as has been noted in a number of other fungi. Although most of the isolates exhibited negative cross-resistance, many did not. Resistance or sensitivity to thiabendazole or chlorothalonil revealed no discernable pattern relative to usage. However, *V. fungicola* was found to exhibit a high level of resistance to chlorothalonil (55-75%) as early as 1950, well before its development as a fungicide. This level of resistance is surprising since chlorothalonil continues to be relatively effective in controlling *V. fungicola*. It is possible *in vivo* responses to this fungicide are different from responses observed in the *in vitro* plate assays. We are now testing a number of the isolates for their *in vivo* responses to chlorothalonil.

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VARIATION IN SENSITIVITY TO PROPICONAZOLE IN *BIPOLARIS ORYZAE* AND *BIPOLARIS SOROKINIANA*, CAUSAL ORGANISMS OF FUNGAL BROWN SPOT OF WILD RICE. Jason Brantner, Dean Malvick, James Percich, and Robert Nyvall. Department of Plant Pathology, University of Minnesota, St. Paul, MN 55108.

Propiconazole (Tilt, Ciba-Geigy) is the only chemical registered in Minnesota to control fungal brown spot (FBS) of cultivated wild rice (*Zizania palustris*), caused by *Bipolaris oryzae* and *B. sorokiniana*. There have been no studies reported concerning variation in sensitivity to propiconazole in *Bipolaris* spp. from Minnesota wild rice paddies. Variability in propiconazole sensitivity was assessed *in vitro* among isolates of *B. oryzae* and *B. sorokiniana*. The biomass (dry weight) of each isolate was determined after five days of growth at 24 C in potato dextrose broth amended with 0, 0.1, 1.0, and 10.0 µg/ml a.i. propiconazole. The percent growth inhibition at 0.1 µg/ml was 15-44% and 31-71% among isolates of *B. oryzae* and *B. sorokiniana*, respectively. At 1.0 µg/ml, percent growth inhibition was 79-89% among isolates of *B. oryzae* and 99% for isolates of *B. sorokiniana*. None of the isolates grew measurably at 10.0 µg/ml. The results indicate variation in sensitivity to propiconazole exists among isolates within and between the two species.

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FUNGICIDE RESISTANCE IN CALIFORNIA ISOLATES OF *SCLEROTINIA MINOR*. J.C. Hubbard and K.V. Subbarao. Dept. of Plant Pathology, University of California, Davis, c/o U.S. Agricultural Research Station, 1636 East Alisal St., Salinas 93905.

The dicarboximide fungicides iprodione and vinclozolin are used for control of lettuce drop, incited mainly by *Sclerotinia minor* in coastal California. To investigate if the apparent loss of effectiveness of these chemicals is due to fungicide resistance in *S. minor* populations, 20 isolates were tested for resistance to iprodione. Both young and mature vegetative mycelium of each isolate was plated on potato dextrose agar (PDA) amended with 0, 1, 5, 10, 25, and 100 µg/ml of the fungicide. Radial growth was measured, and all wild-type isolates tested were sensitive. However, within 2 wk, all isolates produced measurable growth at ≤5 µg/ml of iprodione. After 5 wk, nine isolates produced growth on plates amended with 100 µg/ml. Germination of sclerotia was tested on similarly amended media, and after 2 wk, 18 isolates had at least 1% germination at 5 µg/ml. After 5 wk, 19 isolates had at least 1 % germination at 100 µg/ml. Seventy-three iprodione-resistant cultures derived *in vitro* were transferred to PDA, and then plated on media amended with 5 µg/ml of either iprodione or vinclozolin. Seventy-one retained resistance to iprodione and, of these, 70 were also resistant to vinclozolin. Greenhouse and field experiments are in progress to evaluate fungicide sprays on lettuce inoculated with fungicide-resistant cultures and wild-type sensitive isolates. Results may provide information about causes for the perceived loss of effectiveness of these chemicals in some lettuce fields in coastal CA.

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IR-4 FUNGICIDE PROGRAM UPDATE - REGISTRATION OF MINOR USES. D. C. Thompson, and W. L. Biehn. IR-4 Project, Cook College, Rutgers University, New Brunswick, NJ USA 08903

During the last five years there have been approximately 500 pesticide tolerance registrations approved on food crops based on work by IR-4. Noteworthy new tolerances and clearances approved by EPA in 1993 include: captan on blackberry and raspberry, metalaxyl on cranberry, metalaxyl on ginseng, and triadimefon on raspberry, and one biological: *Pseudomonas fluorescens* on mushroom. In 1993, IR-4 conducted residue or efficacy trials involving 52 food-use projects with the following fungicides: benomyl, captan, chlorothalonil, fosetyl-Al, imazalil, iprodione, mancozeb, metalaxyl, myclobutanil, PCNB, propiconazole, triadimefon, and vinclozolin, and two nematicides: ethoprop and fenamiphos. The IR-4 program has recently been expanded to meet the increasing pest control needs of producers of minor crops, and minor-use needs in major crops. This expansion will accelerate the registration of present pesticide-use projects and accomodate additional projects. During this expansion, IR-4 has made a commitment to become more involved in the development and registration of biological and biochemical pesticides.

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EFFICACY OF DIMETHOMORPH FOR CONTROLLING PHYTOPHTHORA ROOT ROT OF SOYBEAN. A. F. Schmitthenner and R. G. Bhat, Dept. of Plant Pathology, OARDC, Ohio State University, Wooster, OH 44691.

Dimethomorph, a cinnamic acid derivative manufactured by Shell, was evaluated for its inhibitory action against *Phytophthora sojae*, the cause of soybean root rot. This chemical was compared with known fungicides and chemicals in laboratory, greenhouse and field experiments. Dimethomorph was incorporated into dilute lima bean agar medium at various concentrations, and percent inhibition of radial growth of mycelium of *P. sojae* races sensitive and resistant to metalaxyl or fluorophenylalanine was measured. The growth of all *P. sojae* isolates was completely inhibited by dimethomorph at a concentration as low as 1 mg/l. In a layer test performed under greenhouse conditions, dimethomorph (@ 4.7 g/kg seed) was as effective as Apron in controlling Phytophthora root rot on soybean cultivars Sloan and Conrad. Moreover, under field conditions, dimethomorph (@ 3.1 g/kg seed) reduced root rot damage as much as Apron (@ 1.0 g/kg seed) in two out of three tests.

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EFFECTS OF NATURALLY-OCCURRING AROMATIC COMPOUNDS ON PYTHIUM ROOT ROT OF COTTON. E. M. Bauske, R. Rodríguez-Kábana, and J. W. Kloepper. Department of Plant Pathology, Biological Control Institute, Alabama Agricultural Experiment Station, Auburn University, AL 36849-5409.

Citral, benzaldehyde, and furfural control plant-parasitic nematodes of cotton. To determine if these compounds control disease caused by *Pythium* spp., two greenhouse experiments were done using naturally-infested field soil. Soil was treated with 0.5 ml/kg soil of each compound nine days prior to planting. Pasteurized field soil and nontreated field soil were included as controls. Sixteen seed of the cultivar Deltapine 50 were planted in each pot (replication) with six replications of each treatment arranged in a randomized complete block design. In both experiments, citral delayed germination when compared with the other treatments. After two weeks, no differences in plant stands were detected though mean dry weight was lowest in the citral treatment. Soil application of all three compounds reduced disease when compared with the nontreated soil. *Pythium* spp. were isolated from plants in benzaldehyde-treated, furfural-treated, and nontreated soils, but not from plants in citral-treated or pasteurized soil. Results indicate that aromatic compounds may provide effective control of root rot caused by *Pythium* spp.

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EVALUATION OF WHEATS AND AEGILOPS SQUARROSA FOR NEW SOURCES OF BACTERIAL STREAK RESISTANCE. E.A. Milus[1], S.A. Harrison[2], B.L. Tillman[2], and D.B. Chalkley[1]. [1]Department of Plant Pathology, University of Arkansas, Fayetteville 72701 and [2]Department of Agronomy, Louisiana State University, Baton Rouge 70803.

In 1991 the 5,000 most recent common wheat entries were obtained from the USDA National Small Grains Germplasm Research Facility at Aberdeen, ID, and evaluated in paired single-row plots (one row protected with a fungicide) at Baton Rouge in 1992. Entries highly susceptible to leaf rust, leaf blotch, or bacterial streak were discarded, and 386 resistant lines plus 64 susceptible or resistant checks were re-evaluated in 1993 in replicated hill plots that were protected with a fungicide and inoculated with Xanthomonas campestris pv. translucens. In 1994, the selected lines and checks were re-evaluated as in 1993 and in the seedling stage under controlled conditions where disease reactions on primary leaves were recorded. Based on seedling disease reactions, 60% of the lines were resistant to bacterial streak. The Kyoto Collection of Aegilops squarrosa (254 lines) was obtained from the Wheat Germplasm Resource Center at Kansas State University and evaluated in 1994 in replicated hill plots and in the seedling stage as described above. Based on seedling disease reactions, 23% of these lines were resistant to bacterial streak. The best sources of resistance and the relationship between disease reaction in the seedling stage and bacterial streak severity in the field will be discussed.

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STAND DENSITY EFFECT ON THE DEVELOPMENT OF *PUCCINIA GRAMINIS* F. SP. *TRITICI*-QCCJ IN BARLEY (*HORDEUM VULGARE*). R. Dill-Macky and A.P. Roelfs, University of Minnesota, St Paul MN 55108 and USDA-ARS-MWA, Cereal Rust Laboratory, St Paul MN 55108.

The popular Minnesota malting barley Robust and the stem rust susceptible feed barley Steptoe were examined in plots (3.6 m^2) at each of four stand density treatments in a randomized block with four replicates. The stand densities approximated double, normal, half and quarter of the planting rate (95 kg ha^{-1}) recommended for commercial growers. Rust epidemics were generated using *Pgt*-QCCJ by inoculating surrounding spreader plants at flag leaf emergence. Plots were assessed for rust incidence and severity seven times at 3-4 day intervals from the initial appearance of symptoms. Epidemics spread more rapidly in Steptoe than in Robust irrespective of the stand density. The effect of stand density on rust severity was greater than the effect of genotype. Rust development was up to 40% greater in sparse stands than in dense stands of both Robust and Steptoe, and, significant differences in the severity of the rust epidemics were observed between the stand densities by the second assessment. Reductions in grain size were most severe in sparse stands where rust development was greatest.

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DETERMINING A VIRULENCE THRESHOLD FREQUENCY OF *BLUMERIA GRAMINIS* f.sp. *TRITICI* ON SOFT RED WINTER WHEAT. Amy L. Sowell and Steven Leath, Department of Plant Pathology and USDA-ARS, North Carolina State University, Raleigh, North Carolina 27695.

Knowledge of pathogen virulence is important in predicting powdery mildew epidemics on wheat. The objective of this study was to determine the virulence threshold frequency of *Blumeria graminis* f.sp. *tritici* necessary for a powdery mildew epidemic to occur on soft red winter wheat in the field. Field plots were established in three environments and inoculum was a mixture of two to four isolates combined to produce one of four virulence frequency levels. The isolates were applied to spreader rows adjacent to experimental plots

in the field. Disease development was assessed throughout the season to determine the relationship between initial virulence frequency and subsequent disease development on the host. In the 1993-94 season, there were no significant differences in disease among the treatment levels.

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DIVERSITY OF *GIBBERELLA ZEAE* AT SMALL SPATIAL SCALES. R.L. Bowden and J.F. Leslie, Department of Plant Pathology, Kansas State University, Manhattan, KS 66506-5502.

We previously reported high genotypic diversity in the wheat scab fungus, *Gibberella zeae* (anamorph *Fusarium graminearum*), in a sample of 24 isolates from across Kansas. In order to study diversity at smaller spatial scales, 50 wheat heads were randomly sampled from each of two 0.25-m^2 quadrats in a field of mature wheat with severe scab symptoms in 1993. Isolations of *G. zeae* were attempted from sample positions at the top, middle, and bottom of each head and one isolate was saved per position. Vegetative compatibility groups (VCGs) were used to identify different genotypes. Preliminary results were obtained for the first ten heads in the first quadrat. A total of 19 VCGs were found among the 26 isolates in the sample. Four heads were colonized by three different VCGs, five heads were colonized by two VCGs, and one head had one VCG. One VCG was found colonizing four different heads and another was found on two heads. The frequent occurrence of several genotypes per head suggests that opportunities for sexual recombination exist in the field.

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EFFECT OF TIME OF INOCULATION WITH WHEAT STREAK MOSAIC VIRUS ON YIELD OF SELECTED WHEAT CULTIVARS. S. Muratoti[1] and A. D. Hewings[1,2], Department of Plant Pathology[1], and USDA ARS Crop Protection Research Unit[2], University of Illinois, Urbana IL 61801

The effect of an Illinois isolate of wheat streak mosaic virus (WSMV) on yield in selected hard red spring wheat and soft red winter wheat cultivars is being evaluated. In spring 1993 three hard red spring wheat cultivars, Butte 86, Marshall and Wheaton were planted in yield plots with 3 replications in a RCB design. Virus inoculum was prepared by grinding WSMV-infected leaves in 0.01 M, K_2HPO_4 pH 7.0 (1:2, w/v). Seedlings at Feeke's growth stages of 2, 4, 6 and 8 were mechanically inoculated using an air brush; controls were noninoculated plots. Beginning one week after inoculation, symptoms were rated every 7-8 days using a 0-5 scale, where 0 was no symptoms and 5 was severe stunting and chlorosis. The response of the wheat cultivars to inoculation with WSMV at different growth stages was significant ($Pr > F = 0.0001$) using repeated measures analysis. Analysis using ANOVA and FLSD mean separation showed significant differences in grain yield, 1000 kernel weight and height ($P \leq 0.05$) within the cultivars. Butte 86, a tolerant variety, showed less reduction in yield at all growth stages when compared with Marshall and Wheaton.

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FIRST REPORT OF BOTRYOSPHAERIA CANKER ON PISTACHIO TREES. W.J. Swart and W-M. Botes, Department of Plant Pathology, University of the Orange Free State, Bloemfontein 9300, South Africa.

The cultivation of pistachio nuts (*Pistacia vera*) in South Africa is a relatively recent development with considerable commercial potential. Diseases that could limit production of this new crop must therefore be anticipated and diagnosed early. Dieback of 2-year-old *P. vera* trees, grafted onto *P. atlantica* rootstocks, was observed in trial plantings during the spring of 1992. Close examination of diseased trees revealed the presence of cankers on the lower part of the stem in the vicinity of the graft. Cankers were characterized by the presence of black, uniloculate pycnidia on the bark surface and black discoloration of the cambium below the bark. Isolations from the margins of diseased tissue onto malt-extract agar consistently yielded black fungal colonies and pycnidia bearing mature conidia identified as belonging to the *Sphaeropsis* anamorph of *Botryosphaeria obtusa*. The fungus was shown to be highly virulent to three *Pistacia* species in pathogenicity tests in the field. Small diameter branches of *P. atlantica*, *P. vera* and *P. integerrima* developed cambial lesions measuring 53.50, 51.30 and 15.78 mm respectively, 14 days after artificial inoculation with fungal mycelium. Wounding of branches prior to inoculation resulted in significantly larger cambial lesions. The fungus was successfully reisolated from all cambial lesions. As far as we have been able to determine, there are no reports in the world of *B. obtusa* being associated with a disease of cultivated pistachio trees.

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FUNGICIDAL CONTROL OF PHOMOPSIS FRUIT ROT OF PEACH. H. Barczynska and P. Fenn, Department of Plant Pathology, University of Arkansas, Fayetteville, AR 72701.

Fungicides were evaluated under laboratory conditions to determine their efficacy to control Phomopsis fruit rot. This disease has caused important losses of processing clingstone peach varieties grown in Arkansas. Fruit (var. Babygold 7) in the firm mature stage were washed in water with 0.02% Tween 20, air dried, and treated by dipping in fungicides prepared at recommended rates used to control brown rot and other peach diseases. After 24 hr each fruit was inoculated with one 20 μl drop containing $5X10^4$ pycnidiospores of the Phomopsis sp. cultured on PDA. Incubation was at 21°C in covered flats to maintain high humidity. Incubation period (time to lesion appearance) and lesion diameter were recorded. Incubation periods averaged about 10 days with propiconazole, fenbuconazole, myclobutanil, triforine and ferbam, significantly longer than 6 days for untreated fruit. Chlorothalonil, iprodione, benomyl, vinclozolin and dicloran did not extend the incubation period. Only iprodione and dicloran slowed the rate of lesion expansion.

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SELECTION BY AK- AND AM-TOXINS OF MUTANTS RESISTANT TO BLACK SPOT AND ALTERNARIA BLOTCH FROM GAMMA RAY-IRRADIATED IN VITRO PLANTS OF JAPANESE PEAR AND APPLE. H. Tabira1, H. Otani2, K. Kohmoto2 and M. Shimonaka1, 1Lab. Plant Biotech., Tottori Hort. Expt. Stan., Kurayoshi 682 and 2Fac. Agric., Tottori Univ., Tottori 680, Japan

Apical meristem cultures of Japanese pear cultivars 'Kisui', 'Osa-Nijisseiki' and 'Shinsui', and apple cultivars 'Indo' and 'Ohrin' that are highly susceptible to black spot and Alternaria blotch, were irradiated with gamma-ray and their viability was investigated 60 days after irradiation. At a dose of 80 Gy or more, initial growth of the shoots was inhibited remarkably, but the shoot viability recovered during two or three subcultures. In order to isolate mutated resistant cells from chimeric tissues, axillary buds were repeatedly subcultured on shoot multiplying media. Shoot tips or detached leaves *in vitro* were dipped in AK- or AM-toxin solution at concentrations of 0.05 and 1 µM for 48 hr. Necrosis occurred on AK-toxin-treated pear leaves. A mutant shoot that had 100-fold resistance to AK-toxin compared with the original cv. 'Osa-Nijisseiki' was selected out of 672 shoots irradiated at 80 Gy.Two mutants that had 50-fold resistance were selected out of 1600 shoots of cv. 'Kisui' irradiated at 100 Gy. On the other hand, apple shoots were tested for AM-toxin sensitivity by using leaves of rooted shoots in greenhouse because no necrosis occurred on leaves of cultured apple. Out of 453 shoots of cv. 'Indo' irradiated at 120 Gy, a mutant that had more than 100-fold resistance to AM-toxin was selected. In cv.'Ohrin', a mutant with 50-fold resistance to AM-toxin was obtained out of 24 shoots at 80 Gy. The results show high mutation frequency in gamma ray-irradiation combined with tissue culture techniques, and offer criteria for mutation breeding to improve highly susceptible but commercially valuable cultivars among vegetatively propagating apple and Japanese pear.

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A NEW CANKER DISEASE OF APPLE, PEAR, AND PLUM ROOTSTOCKS CAUSED BY *DIAPORTHE AMBIGUA* IN SOUTH AFRICA. W.A. Smit[1], B.D. Wingfield[2], & M.J. Wingfield[2]. [1]Infruitec, Private Bag X5013, Stellenbosch 7599, South Africa and [2]Department of Microbiology and Biochemistry, University of the Orange Free State, Bloemfontein 9300, South Africa.

Diaporthe ambigua was found to be the cause of a newly recognized disease of apple, pear, and plum rootstocks in South Africa. The fungus was isolated from margins of cankers on rootstocks and branches of diseased trees, and from spores taken from perithecia and pycnidia imbedded in cankers. Characteristic symptoms included sunken, pointed lesions with marginal longitudinal cracks. Key identifying characters were perithecia, separate or in groups, with elongated necks protruding from the bark under moist conditions, and stromata delimited at the outer margins by broad, blackened zones. Pathogenicity tests were conducted on 3-year-old apple, pear, and plum rootstock cultivars. Vegetative compatibility (VC) groups were demonstrated by pairing isolates on oatmeal agar, and the sexual system was studied by inoculating single ascospores onto sterile apple twigs on water agar medium. *D. ambigua* was consistently associated with cankers on apple, pear, and plum rootstocks and testing of Koch's postulates demonstrated its pathogenicity conclusively. The fungus was found to be homothallic. In addition, isolates from one rootstock tended to be of the same VC group, whereas those from adjacent rootstocks usually represented different genetic entities.

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THE RELATIONSHIP OF THE DATE FOR HULL SPLIT TO CONTAMINATION OF PISTACHIO NUTS BY *ASPERGILLUS* SPECIES. M. A. Doster and T. J. Michailides, Dept. of Plant Pathology, Univ. of California, Davis/Kearney Ag. Center, Parlier 93648.

Abnormal pistachio nuts with split hulls, called early splits (ES), frequently have kernels contaminated with *Aspergillus* molds. In 1993, most ES (55%) had their hulls split between 12 and 26 August, although only 18 and 27% split their hulls before and after this period, respectively (commercial harvests occurred in mid September). ES

that split before 26 August showed more than four times greater incidence of *Aspergillus* species and ten times greater infestation by navel orangeworm (*Amyelois transitella*) than ES that split closer to harvest. In addition, most of the ES that split before 26 August had shriveled hulls at harvest time, while only 4% of the ES formed after 26 August did. Since both navel orangeworm infestation and shriveled hulls have been associated with high aflatoxin contamination of pistachio nuts, the ES with earlier hull split probably have more aflatoxin (produced by *Aspergillus flavus* and *A. parasiticus*). The physical characteristics were measured for ES formed during different periods. The ES that split earlier had smaller nut size, more external shell discoloration (including more staining along the suture), smaller fruit weights, and lower kernel moisture. Because these ES that split their hulls so early are very different from normal pistachio nuts, they can be removed during processing.

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INFLUENCE OF TEMPERATURE AND LEAF WETNESS PERIOD ON DEVELOPMENT OF SEPTORIA LEAF SPOT OF TOMATO. S. K. Parker, M. L. Gleason, and F. W. Nutter. Department of Plant Pathology, Iowa State University, Ames, IA 50011.

The influence of temperature and leaf wetness on the development of Septoria leaf spot of tomato was studied in controlled environment experiments. Four-wk-old tomato seedlings (cv. New Yorker) were spray-inoculated with *Septoria lycopersici* spores applied at a density of 25 spores per cm^2 leaf area. Plants were then placed in growth chambers at 10, 15, 20, 25, and 30 C. Plants were exposed to repeated daily dew periods of 0, 4, 8, 12, or 16 h until new lesions ceased to appear. Check plants received an initial 48-h continuous dew period. Incubation period, defined as the number of days past inoculation before 50% of the total lesions were visible, was calculated using linear regression. For leaf-wetness durations of 4-48 h, incubation periods decreased as temperatures increased from 10 to 25 C. No lesions developed at 30 C. At temperatures near the optimum for fungal growth (20 and 25 C), the highest infection frequency was observed on plants receiving a continuous 48-h dew period. At 10 and 15 C, the highest infection frequencies were observed on plants receiving intermittent dew periods of 12 and 16 h. This suggests that at 20-25 C and 48 h continuous leaf wetness, many spores were able to complete the infection process, but at 10 C, a single 48-h continuous moisture period was limiting. The experiment will be repeated in 1994.

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INCREASE OF SEPTORIA LEAF SPOT IN LINEAR TOMATO PLANTINGS. S. K. Parker, F. W. Nutter, and M. L. Gleason. Department of Plant Pathology, Iowa State University, Ames, IA 50011.

Temporal and spatial increase of defoliation damage caused by Septoria leaf spot in linear tomato plantings was monitored in 1992 and 1993. Three plots, each containing a single row of 25 tomato plants (cv. New Yorker) spaced 60 cm apart, were established in new sites each year. Rows were oriented east-west. Inoculum was introduced on 2 July in both years by spray-inoculating the center plant in each row. Defoliation within rows, expressed as a proportion, was assessed over the period from 2 July to 18 August. Defoliation increased at the apparent rate of 0.282 (R^2=0.96) in 1992 and 0.450 (R^2=0.98) in 1993. The data shows that Septoria leaf spot epidemics can increase rapidly given favorable weather conditions. Disease increase was linearly related to rainfall activity (R^2=0.97), which was above normal in both years. Defoliation values decreased as distance from initial foci increased. Disease gradients varied with direction and assessment date. Lack of fit to regression models used to describe disease gradients was significant, indicating that unexamined factors, in addition to distance from foci, influenced disease gradients. Increases in isopath velocities were positively correlated to increases in distance from foci and to increases in isopath level. All analyses indicated that defoliation progressed more rapidly to the east of foci (downwind).

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PHYTOPHTHORA ROOT ROT OF PEPPER: DISEASE SPREAD IN THE FIELD AND EFFECT OF CULTIVATION AND ROGUEING ON YIELD. I. R. Sollars, C. M. Liddell and C.L. Biles. New Mexico State University, Department of Entomology, Plant Pathology, and Weed Science, Box 3BE, Las Cruces, NM 88003, U. S. A.

Two field experiments on Phytophthora Root Rot of pepper (PRR), caused by *Phytophthora capsici*, were conducted in 1992/93 in the Mesilla valley of New Mexico. Plants were grown in a naturally infested field, and alternate rows were irrigated, a common local practice used to reduce PRR levels. In one experiment, rogueing dead plants did not further reduce PRR levels or increase yield of red peppers relative to non-rogued control plots. The initial plants killed by *P. capsici* were randomly distributed throughout the field and plants killed later in the season were only weakly clumped. This pattern is consistent with the hypothesis that initial and subsequent infections by *P. capsici* during the season were predominantly caused by primary soil inoculum (oospores?). Secondary infections by zoospores appear to be less important, probably due to the use of alternate row irrigation. A second experiment, determining the effect of root wounding through cultivation on PRR level and crop yield had four treatments: hoe only; hoe+light cultivation; hoe+heavy cultivation; and hoe+herbicide (Command®). There were no differences between treatments in the yield of green or red peppers, or on PRR levels indicating that heavy cultivation does not contribute to increased levels of disease.

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YIELD LOSSES ASSOCIATED WITH FOLIAR DISEASES OF FRESH-MARKET TOMATOES IN FLORIDA AND THE BENEFITS OF PROTECTANT FUNGICIDES. Ken Pernezny, Lawrence Datnoff, Janice Collins, University of Florida, Belle Glade, FL 33430 and Tom Mueller, Collier Growers, Immokalee.

Three large-scale field tests were conducted on a commercial farm during the 1992-93 and 1993-94 winter vegetable seasons in order to quantify yield losses associated with foliar diseases of fresh-market tomatoes. Data were also obtained on the benefits of protectant fungicides. The benefits of fungicides to Florida tomato farmers were amply demonstrated in the Fall 1992 and Fall 1993 experiments. Total marketable yields were reduced about 30% when no fungicides were used compared to the best treatments with effective disease control. Net returns were over $3000/acre in chlorothalonil treated plots. Much of the loss of marketable yield was due to direct fruit damage from target spot (*Corynespora cassiicola*). Tank-mix sprays of copper/mancozeb provided good early season control of bacterial spot. However, late season target spot damage was much higher in copper/mancozeb plots than in chlorothalonil plots. In Fall 1993, weekly chlorothalonil sprays reduced target spot damage to marketable fruit by 25%. Attempts to measure yield losses in the Spring of 1993 were hampered by extreme weather conditions. An associated bacterial speck epidemic caused severe and uniform damage in all treatments.

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PERFORMANCE OF SOIL SOLARIZATION IN A HUMID, SUBTROPICAL CLIMATE AND ITS EFFECT ON SURVIVAL OF FOUR SOILBORNE PATHOGENS. D.O. Chellemi, S.M. Olson, and D.J. Mitchell. University of Florida, Route 3 Box 4370, Quincy, FL 32351.

Survival of *Fusarium oxysporum f.sp. radicis-lycopersici*, *F.o. lycopersici*, *Phytophthora nicotianae* and *Pseudomonas solanacearum* were examined in 1992 and 1993 in field plots located in northern Florida. Maximum temperatures recorded at depths of 5, 15, and 25 cm were 43.8, 38.9, and 36.5 C in bare soil and 49.5, 46.0 and 41.5 C in solarized soil. Soil solarization significantly ($P \leq 0.05$) reduced survival of *P. nicotianae* and *P. solanacearum* to depths of 25 and 15 cm, respectively. Significant reductions in either *Fusarium* species did not occur below 5 cm. An inverse linear relationship was observed between degree hr accumulation above 40 C and survival of *P. nicotianae* and *P. solanacearum*.

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SURVIVAL OF PUCCINIA RECONDITA INOCULUM ON WHEAT LEAVES AT -10 to 15 C. Eversmeyer, M. G. and Kramer, C. L. USDA/ARS, KSU, Manhattan, KS 66506.

Initiation of rust epidemics in the Central Great Plains wheat growing region depends mainly on the ability of inoculum to survive and infect the host crop. P. recondita still contained within uredinia on living host tissues are able to withstand -10 to 15 C temps for as long as 48 hrs with only a slight loss in germination. However, when host leaves bearing uredinia are excised and subjected to subfreezing temps, germination of the urediniospores is significantly reduced after 24 hrs. Greatest reduction in germination potential occurred when spores separated from the uredinia and host tissues were exposed to the subfreezing temps. Knowledge of probable survival habitats for primary inoculum is critical in defining survival of primary inoculum and in model development of initial phases of epidemics.

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MODELING COMPETITION BETWEEN PATHOGEN STRAINS. M. Newton, L.L. Kinkel, and K.J. Leonard. Department of Plant Pathology, and USDA, ARS Cereal Rust Lab, University of Minnesota, St. Paul, MN 55108.

We developed a mathematical model to quantify competitive interactions between strains of *Puccinia graminis f. sp. tritici* on leaves of wheat seedlings. The model consists of two equations: the first is based on a multiple infection transformation relating numbers of uredinia of a given strain to the inoculum densities of that strain and of a competitor. The second equation, based on a monomolecular model, relates sporulation of a given strain to its own infection density and that of a competitor. Data from growth chamber experiments involving single- and double-strain inoculations were used to estimate the model parameters: infection efficiency, maximum number of infections per leaf, sporulation efficiency, maximum sporulation per leaf, and competition coefficients. The model allows us to distinguish effects of competition from those of population density, and provides insight into the role of each parameter in determining a strain's competitive ability.

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DISEASE AND INFECTION GRADIENTS OF FUSARIUM HEAD BLIGHT (*GIBBERELLA ZEAE*) IN WHEAT. T. Paulitz[1], W. L. Seaman[2], and P. Dutilleul[1], [1]McGill University, Ste. Anne de Bellevue, Quebec, H9X 3V9, and [2]Agriculture Canada, Ottawa, Ontario, CANADA

The ascospore dispersal of *G. zeae* from an area source was studied in 10 X 10-m plots of spring wheat (cv. Max) artificially infested with perithecial inoculum. Corn kernels colonized by *G. zeae* were spread in the center 4 X 4-m area of the plots in the last week of May, 1992 and 1993. Perithecia formed in mid-June and ascospores were released in the first week of July. Two weeks after anthesis, disease (% spiklet infection) was assessed in 0.25-m^2 quadrats sampled in a regular grid pattern. Seeds were collected from the same quadrats in late Aug, and incidence of seed infection was determined by plating on a selective medium. Disease and seed infection were highly correlated ($r=0.83$). Data were mapped with 3-D contour plotting and showed a non-isotropic topology that correlated with night wind direction. Data were also modelled using trend surface analysis. The rate of decline of disease and infection over distance from the inoculum source was lower on the leeward side of the plot. Highest disease and seed infection were measured on the windward edge of inoculated areas. Foci with seed infection >25% were detected in an adjacent non-inoculated plot 10 m away from the inoculum source.

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CITRUS SCAB: TEMPORAL AND SPATIAL RATE-OF-SPREAD ANALYSES OF VARIOUS CONTROL STRATEGIES IN CITRUS NURSERY PLOTS. T. R. Gottwald, USDA, ARS, Horticultural Research Laboratory, Orlando, Florida 32803

The nonlinear Gompertz model was superior to other nonlinear models tested for describing progress of disease incidence of citrus scab in 12 sour orange nursery blocks over time and was used as the basis for spatial and temporal analyses. Aggregation of disease was demonstrated by Lloyd's index of patchiness, variance-to-mean ratio, and ordinary runs analysis, especially early in the epidemic and before 50% disease incidence was achieved. In three nurseries with a central point focus of disease, isopath boundaries moved predominately northward and away from the focus. This was presumably in response to splash dispersal of inoculum by rain showers and sprinkler irrigation. The spatial rate-of-spread (v) was generally greater early in the epidemics and when measured at distances farther from the focus of infection. For scab disease control plots consisting of four treatments, Gompit rates of disease increase (k) were significantly reduced by applications of captafol or copper at 30-day intervals versus a water spray. Benomyl did not effectively reduce the rate of disease progress at one site, presumably due to the presence of fungicide-tolerant strains of Elsinoe fawcettii. Areas under the disease progress curves and areas circumscribed by individual isopath disease levels were reduced most by captafol, and to a lesser extent by copper compared to the water control.

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SPATIAL AND TEMPORAL DYNAMICS OF EPIDEMICS IN APPLE ORCHARDS CAUSED BY *PHYMATOTRICHUM OMNIVORUM*. C. Kenerley[1], K. Ivors[1], D. Appel[1] and S. Nelson[2]. [1]Dept. Plant Pathology and Microbiology, Texas A&M University, College Station, TX 77843 and [2]Dept. Plant Pathology, University of Hawaii, Honolulu, HI 96822.

Epidemics caused by *Phymatotrichum omnivorum* were monitored in five commercial apple orchards during one growing season in Kerrville, TX. Orchards were in the fourth growing season and were naturally infested with *P. omnivorum*. Trees (M7a root stock) were on an A-frame trellis at a density of 435 trees/acre. The orchards ranged in size from 2451 to 3451 trees. Plant mortality at the beginning of the growing season ranged from 8 to 16%. Regression diagnostics for data over 143 days for all orchards indicated that temporal changes in disease incidence were described adequately by simple linear models ($r = 0.97-0.99$). At leaf fall, mortality among the orchards ranged from 20 to 30%. Two-dimensional distance class analysis (2DCLASS) detected significantly nonrandom patterns of disease incidence in all orchards with the orientation of disease clusters occurring both within and across trellis rows. Significant edge effects were detected in 4 of 5 orchards.

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EPIDEMIOLOGICAL STUDIES OF PECAN SCAB CAUSED BY CLADOSPORIUM CARYIGENUM IN OKLAHOMA. A. Marenco, S.L. von Broembsen and J.A. Duthie, Dept. of Plant Pathology, Oklahoma St. Univ., Stillwater, OK 74078

Foliar and nut scab ratings, and defoliation were recorded weekly during the 1993 season for 60 compound leaves and 20 nut clusters on both native pecans and a highly susceptible cultivar, San Saba, at Sparks, OK. Foliar disease ratings increased gradually on San Saba to a maximum of 6.4 on a 1-8 scale at season's end (17 September) with rapid partial defoliation increasing 25-98% in the period 1 July to 15 July. Nut disease ratings increased from 1.2 on 24 June to 5.3 on 29 July. For natives, disease ratings were low throughout the season with a 2.2 foliar rating and a 3.4 nut rating at season's end. Disease development corresponded to monitored weather conditions (ambient temperature, relative humidity, leaf wetness and rainfall) known to be favorable to pecan scab.

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FIELD TEST OF THE PRECISION AND ACCURACY OF ELECTRONIC WETNESS SENSORS. M.L. Gleason, K.J. Potratz, M.L. Hockmuth, S.K. Parker, and G.A. Pearston, Departments of Plant Pathology and Horticulture, Iowa State University, Ames, IA 50011

Measurements of dew-period duration by painted, electronic wetness sensors at the top of the plant canopy in a tomato field and on adjacent turfgrass were compared to visual observations which were made every 20 min. Among individual sensors at each site, the range of response to the onset of dew on tomato leaves was >5 hr on some nights and <1 hr on other nights. Sensors in the tomato field indicated dew onset as much as 2 hr earlier or later than dew became visible on the uppermost leaflets in the crop canopy. A calibration threshold that was derived from a drying curve resulted in underestimation of dew-period duration by up to 2.4 hr. The differential between electronic and visual dew-duration measurements at the top of the tomato canopy was within 1 hr of the differential on turfgrass. These findings emphasize the need to use properly calibrated sensors for dew-period measurements and to calibrate dew-period data in a nearby crop canopy.

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WATER INPUTS AS MEANS TO FORECAST AND CONTROL WHITE MOLD OF BEAN. K. M. Duellman, R. A. Meronuck, and L. L. Kinkel. Dept Plant Pathology, Univ. of Minn., St. Paul 55108.

White Mold (*Sclerotinia sclerotiorum*) greatly limits yield of dry bean in Minnesota. The effect of different amounts of water on white mold progress and the effect of disease on yield were investigated over 4 years. In each year, four treatments were established in an area of intensive bean production on sandy, irrigated soil. Treatments one, two, and three were irrigated when soil water potentials reached 30 centibars (cb), 65 cb, and 100 cb, respectively; a fourth treatment received no supplementary water. Treatments were split into plots receiving fungicide and plots with no fungicide. Total moisture input (irrigation + rain) during a period spanning 10 days before to 10 days after bloom was positively correlated to percent diseased canopy at maturity for all years ($r = 0.83$). Disease and yield were negatively correlated ($r = -0.85$). We conclude that disease progress can be manipulated through irrigation practices during bloom. Furthermore, based on predicted disease-induced yield loss, the economic benefits of spraying can be determined. A disease forecaster and a spray decision aid incorporating environmental and economic factors are being developed from these data.

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SIMULATION STUDIES ON RISK OF RICE BLAST EPIDEMICS ASSOCIATED WITH EFFECTS OF ENHANCED UV-B AND TEMPERATURE CHANGES IN SEVERAL ASIAN COUNTRIES. Y. Luo, D. O. TeBeest, Department of Plant Pathology, University of Arkansas, P.S.Teng, N.G.Fabellar, Division of Plant Pathology, IRRI, Philippines.

RICE-BLAST (Combination of CERES-RICE and BLASTSIM models) was modified to considerate the effect of temperature changes and enhanced UV-B (Ultraviolet-B) on rice growth and blast epidemics. Parameters of UV-B effect on plant net assimilation rate and on blast infection components were incorporated into the model. Validation of the modified model showed that simulated rice growth fit well with observations under enhanced UV-B. Thirty years estimated weather data for each of 59 locations in 5 Asian countries (Japan, China, Korea, Thailand and Philippines) were used to study the effects of temperature changes and enhanced UV-B on blast epidemics. A lower temperature resulted in a higher risk of epidemics in tropical countries, which is opposite to the situations in the cool subtropics where the elevation of temperature was estimated to cause more severe disease. Enhanced UV-B will

cause 9-10% rice yield loss under normal temperature conditions in most locations. Blast with enhanced UV-B will cause 12-20% and 10-14% rice yield losses in the tropics and cool subtropics respectively. Decreasing temperature by 3 degrees under enhanced UV-B will bring about more severe blast epidemics compared with other temperature change conditions in tropical countries. GIS maps were produced to show the risk area of blast in those countries.

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INDIVIDUAL-BASED MODELS TO PREDICT MICROBIAL ACTIVITY IN SPATIALLY STRUCTURED HABITATS. Guy R. Knudsen, Dept. of Plant, Soil & Entomological Sciences, University of Idaho, Moscow, ID 83843.

Population-state models of microbial growth and activity that assume homogeneous mixing (e.g., exponential, logistic, Monod) may become inaccurate or intractable in the presence of spatially structured habitat variability. Individual-based models follow large numbers of individuals simultaneously to describe populations. Microcomputer simulations were developed for i) aerial dispersal and epiphytic growth/survival of aerosolized bacteria, and ii) vegetative growth of a soilborne fungus. Individuals were both a) single bacterial cells or hyphal segments, and b) spatially oriented microhabitats. Model predictions often were similar to "traditional" growth curves, but variability in habitat structure frequently caused large deviations that might be mistaken, using an analytical modeling approach, for chaotic behavior. Difficulties with the individual-based approach include a never-satiated need for computational resources, and a shortage of validation methodologies. The potential of geographic information systems and geostatistics as tools for parameterization and validation of spatial simulation models will be discussed.

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SURVEY OF WOOD COLONIZING FUNGI FOR PITCH REDUCTION

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A new area of biological processing involves pretreating wood with fungi to degrade problematic pitch prior to pulping. Sapwood colonizing fungi have been screened for pitch reduction on pine and aspen. Different species have been tested for their relative effectiveness to decrease pitch as measured by dichloromethane extractables for pine, or ethanol/toluene extractables for aspen.

In addition, eleven isolates of *O. piliferum* and forty-six isolates of *O. piceae* were also tested on pine or aspen. For the *O. piliferum* isolates, on pine 18% did not reduce pitch but 64% reduced pitch by 25% - 35% over the untreated control. For *O. piceae*, the isolates can be put into four groups: twenty four percent of the isolates tested did not reduce pitch, 46% reduced pitch by 1% - 15% when compared to the control, 28% reduced pitch by 16% - 35%, and 2% (one isolate) reduced pitch by 60%. Isolates that were not effective at reducing pitch were weaker isolates.

The screening of the best pitch degrading isolates will be continued in terms of growth temperature range and duration. The paper will also present details of the growth of the fungi in wood cells as analyzed by scanning electron microscopy.

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ESTABLISHMENT OF A LONG-TERM MONITORING SYSTEM FOR RHODODENDRON DIEBACK IN THE GREAT SMOKY MOUNTAINS NATIONAL PARK. T.J. Michaels, B.H. Ownley, K.D. Johnson[1], and R.B. Reeder. Entomology and Plant Pathology Dept., The University of Tennessee, Knoxville, TN 37996 and [1]U.S. Dept. of Interior, National Park Service, GSMNP, Gatlinburg, TN 37738.

A dieback of rhododendron (*Rhododendron maximum* L.) of unknown etiology is occurring in several areas of the Great Smoky Mountains National Park. The purpose of this study was to establish a long-term monitoring system to determine and follow the distribution and incidence of dieback in the Park. A study site was established that consisted of twelve 20m x 20m plots. The plots represented a range of rhododendron stand densities and trunk diameters. Rhododendrons were tagged and rated, based on predetermined ranges for canopy closure (CA), leaf chlorosis (CH), leaf whorls/branch (WH), twig and branch death (TW), and extent of damage caused by leaf colonizers and pests (OT). A dieback rating value, R, was calculated by weighting the variables in the following model: $R=0.05*CA + 0.283*(WH + CH + TW) + 0.10*OT$. Further modeling established that only three of the variables (WH, CH, TW), equally weighted, were necessary to calculate R. Dieback severity was positively correlated with trunk diameter (tree age). Attempts to isolate a specific causative agent were inconsistent. The inability to identify a specific pathogen, coupled with the correlation of disease severity and tree age, suggests that the dieback syndrome occurs gradually and is caused by a combination of environmental stress and opportunistic plant pathogen(s).

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SITKA SPRUCE RESPONSE TO LEPTOGRAPHIUM ABIETINUM. B.L. Illman, Forest Products Laboratory, Madison, WI, 53705, and R.A. Werner USDA/FS Institute of Northern Forestry, Fairbanks, AK, 99775.

Defense mechanisms of Sitka spruce (Picea sitchensis) are elicited in induced reaction zones (IRZ) in inner bark by the blue-stain fungus Leptographium abietinum, a pathogenic fungus associated with spruce beetle, Dendroctonus rufipennis (Werner and Illman, 1994, Environ Entomol). Host response of 6 Sitka spruce trees in Alaska was analyzed by inoculating fungus in 4 circular (2.5 cm) plugs equal distances around trees at about breast height. Samples within and outside IRZ were collected 4 wks after treatment. Methanol extracts of all samples gave a continuous absorption spectrum of 200-360 nm with an unchanging peak at 215. Broad peaks about 280 and 325 were different in healthy vs IRZ. Extracts, separated on a C18 reverse phase column by high pressure liquid chromatography (HPLC), 5-100% acetonitrile gradient, 325 and 280 detection, indicate that some host constitutive phenolics disappeared in IRZ and others were produced. These will be identified by NMR, IR or MS.

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SCANNING ELECTRON MICROSCOPY OF SYCAMORE PATHOGENS ON INOCULATED LEAVES. Vernon Ammon, and Stephen R. Vann, Mississippi Agricultural and Forestry Experiment Station, Mississippi State, Mississippi 39762.

Sycamore seedlings inoculated with the sycamore twig canker fungus, the anthracnose fungus, the canker-stain fungus, and the sycamore dieback fungus were examined with a scanning electron microscope after 1, 2, 4, and 8 days incubation. Conidial germination, appressorial development, and hyphal growth patterns were observed. Conidial germination of the twig canker fungus did not exceed 25% for all incubation periods tested, hyphal growth was sparse and appressorial formation was most often associated with short germ tubes. Conidial germination of the sycamore anthracnose fungus exceeded 75% after 24 hours incubation. Germ tube length varied and most terminated with the production of appressoria. Conidial germination of the canker-stain fungus exceeded 75% within 24 hours. Germ tube growth doubled after 48 hours and globose-shaped appressoria were produced in abundance. Chlamydospores and immature perithecia were observed on leaf surfaces 8 days after inoculation. Germination of both mature and immature conidia, multiple germ tubes, and extensive hyphal branching characterized the sycamore dieback fungus 24 hours after inoculation. Appressoria and fruiting structures were not observed.

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DO PARENTAGE AND EARLY MEASURES OF BASAL AREA AND CANKER DISEASE SERVE AS INDICATORS OF GROWTH POTENTIAL IN A NINE-YEAR OLD HYBRID POPLAR PLANTATION? M. H. Lo, L. P. Abrahamson, P. D. Manion and A. P. Drew, State University of New York College of Environmental Sciences & Forestry, Syracuse, New York, 13210.

Differences in % survival, canker disease rating and growth potential (basal area/ha) were quantified for a plantation of 54 hybrid poplar clones in northern New York at ages three and nine years to test the hypothesis that early growth and canker incidence can indicate future growth. In addition, differences in growth and canker severity among clones with similar parentage were assessed. Five of the seven clones which exhibited high basal areas and low disease ratings at age three years maintained their growth potential at nine years of age. Four of the five clones which had 0% survival at age nine years, had low basal areas at age three years. The growth potential of the remaining clones could not be predicted based on early measures of growth and canker incidence. Extreme variation in basal area and canker rating among clones within the same parentage group made it impossible to use parentage as a predictor of growth potential. Correlations among canker rating and total basal area and % survival increased over time, suggesting that impacts of canker disease became more significant with age. Septoria musiva was suspected to be the primary cause of stem cankers but isolations revealed the presence of many other fungi.

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USING A GEOGRAPHIC INFORMATION SYSTEM TO QUANTIFY VOLUMES OF DEFECT, DECAY AND MERCHANTABLE WOOD IN DISEASED SUGAR MAPLES. D. R. Bergdahl, J.R. Bove*, P. Sendak* and D. R. Tobi. Department of Forestry, University of Vermont, Burlington, 05405 and *USDA Forest Service, NEFES, So. Burlington, VT 05403.

An ARC/INFO geographic information system (GIS) was used to study effects of Eutypella parasitica (eutypella canker) and Glycobius speciosus (sugar maple borer) and associated wood defects on merchantable volume of sugar maples. Fifty-nine trees affected by one of the wounding agents were cut into 4-foot (1.22 m) long bolts, and a 1-inch (2.54 cm) thick cross-section was cut from the proximal end of each bolt. A digital image was produced of each section using a still video camera and image processing software. Digital images were scaled to true size using ARC/INFO. Areas of healthy, discolored and decayed wood were then digitized directly from the monitor. Polygon areas, by wood type, were determined by ARC/INFO and Smalian's formula then used this information to calculate gross tree volume, volume loss due to defect and decay, and merchantable volume per tree. This study uniquely extends the use of digital imaging and GIS to examine spatial information at a non-landscape scale.

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USE OF ISOLATED ROOT CAP CELLS AND CELL CULTURES FROM *PINUS* SPP. IN AN ASSAY FOR TOXIN PRODUCTION BY *SPHAEROPSIS SAPINEA*. W.J. Swart, W.C. Saaiman, and W-M. Botes, Department of Plant Pathology, University of the Orange Free State, Bloemfontein 9300, South Africa.

The range of physiological mechanisms employed by the pine pathogen, *Sphaeropsis sapinea* in symptom expression has never been identified. It is, for example, still unknown whether the fungus produces toxic metabolites which cause the rapid degradation of host tissue. Suspensions of isolated root cap cells and callus cells have been shown to provide a useful tool for reproducible, quantitative *in vitro* assays of plant sensitivity to fungal toxins. The objectives of this study were therefore firstly, to establish whether viable root cap cells and callus cells of various *Pinus* spp. can easily be obtained and secondly, whether these cells can be used as indicators for demonstrating the production of toxic metabolites by *S. sapinea* in liquid culture. The viability of root cap cells isolated from the radicles of germinated pine seeds and cells derived from needle callus was effectively demonstrated by staining cells with fluorescein diacetate (FDA) and examining them under a fluorescent microscope. Significantly greater mortality rates of root cap cells and pine callus cells exposed to culture filtrates of *S. sapinea* compared to control treatments was consistently demonstrated. These preliminary results provide the first evidence of the production of a toxic metabolite by *S. sapinea*.

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GUIDES FOR PREDICTING OAK DECLINE ON UPLAND HARDWOOD SITES IN SOUTHEASTERN UNITED STATES. S. W. Oak, D. A. Starkey, J. G. Williams, and F. H. Tainter. USDA Forest Service, Southern Region, Forest Health, Atlanta, GA 30367 and Department of Forest Resources, Clemson University, Clemson, SC 29634.

Fifteen stand/site variables were analyzed for their possible association with incidence of advanced oak decline on three national forest ranger districts located across the southeastern United States. The most significant variables associated with declined plots were oak composition, stand age, site quality, soil properties, and elevation, but not all variables had equal effects on all districts. Logit equations were used to develop probability tables useful to forest managers in these localities to predict the likelihood of oak decline in individual stands.

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SOYBEAN SUDDEN DEATH SYNDROME: ISOLATION AND IDENTIFICATION OF A NEW PHYTOTOXIN FROM CULTURES OF THE CAUSAL AGENT, *FUSARIUM SOLANI* R. A. Baker, U.S. Citrus and Subtropical Products Laboratory, USDA, ARS, P.O. Box 1909, Winter Haven, FL 33880 and S. Nemec, U.S. Horticultural Research Laboratory, USDA, ARS, 2120 Camden Road, Orlando, FL 32803.

A Sudden Death Syndrome (SDS) isolate of *F. solani* cultured on a mineral salts-glucose medium developed a colorless compound as a major culture filtrate component. Four other cultures of SDS associated *F. solani* also produced this compound, which was not seen in culture extracts of numerous isolates of this species from other hosts. The compound was purified by adsorption and elution from Amberlite XAD-7 resin, separation on a silica gel column with chloroform-acetone gradient, and crystallization from chloroform: Melting point, IR, and mass spectra confirmed its identity as monorden, an antibiotic compound previously isolated from *Monosporium* and *Nectria* cultures. When applied to soybean cuttings as a single dose of 200μg/plant, mature leaves developed interveinal necrosis, curling, and drying. Rooting of cuttings was suppressed by 20μg, and eliminated by 200μg.

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FIRST REPORT OF FUSARIUM WILT OF BUTTONBUSH CAUSED BY FUSARIUM OXYSPORUM AND F. SOLANI IN THE UNITED STATES. Z.L. Liu and M.A. Wells, Agricultural and Environmental Research Center, Formosa Plastics Corp. P.O. Box 69, La Ward, TX 77970-0069.

In 1993, a severe disease of common buttonbush, Cephalanthus occidentalis L., which destroyed over 3,000 plant stock, was observed in a local nursery in Jackson County, Texas. Isolates of Fusarium oxysporum and F. solani (verified by P.E. Nelson, Pennsylvania State University) were recovered from symptomatic tissue and used to complete pathogenicity tests and Koch's postulates. Following host inoculation, infected plants developed a marginal leaf chlorosis that progressed to a brownish tan desiccation. Infected plants showed wilting symptoms under stress and died within four months. Discoloration of cortex and secondary xylem was observed in vertical sections of infected cuttings.

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PATHWAYS FOR INFECTION OF CORN KERNELS BY *FUSARIUM MONILIFORME*. G.P. Munkvold, D.C. McGee, and W.M. Carlton. Dept. of Plant Pathology, Iowa State University, Ames 50011.

Four different strains of *Fusarium moniliforme* were used to inoculate corn seeds, crowns, stalks, or silks. Seeds were planted in the field and greenhouse; remaining inoculations were in the field. Strains recovered from inoculated plants were identified by vegetative compatibility with inoculant strains, as determined by complementation of nitrate non-utilizing (*nit*) mutants on a minimal medium. Seed inoculation resulted in seedling infection in the greenhouse but did not affect emergence or stalk and kernel infection in the field. The seed inoculant was recovered from seedlings in the greenhouse but not from plants in the field. Inoculations in the field significantly increased crown and stalk infection over the noninoculated control. Crown, stalk, and silk inoculants were recovered from crowns, stalks, cobs and kernels. Silk inoculation significantly increased kernel infection to 61% compared to 30% in the noninoculated control, and the silk inoculant was recovered from 87% of mature infected kernels from the silk-inoculated treatment. Crown and stalk inoculations did not significantly increase kernel infection, but the inoculants were recovered from 44% and 53% of mature infected kernels from the crown- and stalk-inoculated treatments, respectively. Plants receiving all four inoculation types had 63% kernel infection; 86%, 3%, and 1% of these kernels were infected with the silk, stalk, and crown inoculants, respectively.

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PROTEINACEOUS ELICITORS FROM *FUSARIUM OXYSPORUM* CULTURE FILTRATES ELICITE SYMPTOMS OF VASCULAR WILT DISEASE IN PLANT TISSUES. Bryan A. Bailey Biocontrol of Plant Diseases Laboratory, ARS/USDA, Beltsville, MD.

Because of their host specificity, isolates of *Fusarium oxysporum* are considered as possible biocontrol agents for the control of many plant species. Studies aimed at understanding how *F. oxysporum* incites disease have identified many factors of possible importance including toxins and vascular occlusion. We have identified components of culture filtrates of *F. oxysporum* that are capable of mimicking symptoms of disease in plant tissues. Plant responses induced by culture filtrates of *F. oxysporum* include ethylene biosynthesis, chlorosis, vascular discoloration and necrosis. The primary proteinaceous component of the filtrates responsible for eliciting these responses has been purified using FPLC gel chromatography. The protein has a molecular weight of approximatly 11 kDa as determined by SDS gel electrophoresis. More extensive studies of this protein are presently being carried out.

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RESISTANCE OF CITRUS ROOTSTOCK SEEDLINGS TO FUSARIUM SOLANI. S. Nemec, USDA-ARS, SAA, U.S. Horticultural Research Laboratory, 2120 Camden Rd., Orlando, FL 32803.

Sixteen citrus rootstocks were grown from seed to 25 to 30 cm tall in steam pasteurized Astatula fine sand and root-dip inoculated in aqueous slurries of F. solani cultures grown 3 to 4 days in liquid Fries media. They were replanted and evaluated in 7 to 10 days for root rot and wilt. All rootstock seedlings became infected; wilt varied more than root rot ratings which, in general, ranged from moderate to moderately severe, but both were used to score resistance. Large flowered trifoliate orange was tolerant. Citrus macrophylla and Calamondin were tolerant to intermediate. Eureka lemon, Key lime, Duncan grapefruit, Rangpur

lime, and Pummelo had intermediate resistance. Carrizo citrange, rough lemon, Volkameriana, and Pineapple sweet orange were intermediate to susceptible and sour orange, Cleopatra mandarin, Orlando tangelo, and Palestine sweet lime had the least resistance. Resistance in juvenile seedlings may not be equivalent to field levels of resistance.

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INFLUENCE OF BLACK ROOT ROT ON COTTON SEEDLING DEVELOPMENT. P.M. Kinney and C.S. Rothrock, Dept. of Plant Pathology, University of Arkansas, Fayetteville, AR 72701.

Black root rot caused by Thielaviopsis basicola is common on cotton seedlings in many fields in the Mississippi Delta. The effects of black root rot on cotton seedling development were examined in microwave-pasteurized soil infested with 0, 100, 250, or 500 chlamydospores/gram. Seedlings were grown 3 weeks at 20, 24, 28, or an increasing temperature (IT) regime from 20 to 28 C. Black root rot reduced seedling height, seedling weight, and number of nodes at 20, 24, and IT, but not at 28 C. Seedling weight reductions over the different inoculum levels ranged from 22-31%, 13-19%, or 18-28% at 20 C, 24 C, or IT, respectively. The number of nodes for the noninfested treatment was 0.6 at 20 C, 1.6 at 24 C, 2.9 at 28 C, and 1.6 for the IT treatment. The number of nodes for seedlings from infested soil was reduced approximately 0.6 nodes. Disease severity was evaluated on a 1-5 scale, with 1 = no symptoms and 5 = >50% of the root system discolored. Disease severity over the 100, 250 and 500 inoculum levels ranged from 4.2-5.0, 3.8-4.7, 1.8-2.8 or 3.2-4.9 at 20 C, 24 C, 28 C or IT, respectively. The percentage of the root system colonized by T. basicola, based on pathogen isolation, was positively correlated with disease severity.

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VIRULENCE GENE EXPRESSION DURING CONIDIAL GERMINATION IN *COCHLIOBOLUS CARBONUM* RACE 1. M.J. Jones and L.D. Dunkle, USDA-ARS, Department of Botany and Plant Pathology, Purdue University, West Lafayette, IN 47907

The fungal pathogen *Cochliobolus carbonum* race 1 produces a host-specific toxin that is responsible for increased virulence on *hm1/hm1* genotypes of maize. The toxin is synthesized by a cyclic peptide synthetase, which is a product of the *HTS1* gene in the *TOX2* locus. Because the toxin is not stored in dormant conidia, early expression of *HTS1* is crucial for extensive colonization of susceptible leaf tissue. To determine the onset of *HTS1* gene expression, we analyzed RNA preparations from ungerminated conidia and from conidia germinated on nutrient medium by reverse transcriptase-polymerase chain reaction using oligonucleotide primers within the 15.7 kb open reading frame. The transcript was detected in RNA from ungerminated conidia, and the quantity increased during germ tube elongation. Digestion with restriction endonucleases confirmed the identity of the amplified product, and the use of primers spanning the coding sequence and the upstream non-transcribed region established that RNA and not DNA was amplified. The *HTS1* mRNA was not detected by Northern analysis, indicating that it is a rare transcript. Its presence in ungerminated conidia suggests that, during sporulation, the conidia are equipped for pathogenicity.

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CLONING CONIDIATION GENES FROM *Colletotrichum graminicola*. Guang-Chen Fang and Robert M. Hanau. Purdue University, West Lafayette, IN 47907.

Colletotrichum graminicola (teleomorph= *Glomerella graminicola*) causes anthracnose disease of maize. This fungus produces two types of conidia, falcate and oval, which differ not only in morphology but in ontogeny. Falcate conidia are associated with dispersal whereas oval may be involved in colonization or movement of the pathogen within infected plant tissues. To gain a better understanding of differentiation and regulation of conidium development, we constructed a cDNA library representing genes expressed during production of oval conidia. Three cDNA clones, oval-5, oval-7 and oval-11 were recovered from the library by differential screening. The oval-5 clone hybridizes to a 2.2 kb transcript, oval-7 to a 1.8 kb transcript, and oval-11 to a 6.0 kb transcript. These three transcripts differ in their temporal expression. Oval-7 is detected early in development when the transcripts of oval-5 and oval-11 are much less abundant. Oval-7 appears to decrease at later stages when levels of oval-5 and oval-11 are more abundant and increasing. Expression of oval-5 is unique among the three transcripts in that it also accumulates in oval conidia. Gene disruptions are in process to address the function(s) of the genes corresponding to these clones.

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IDENTIFICATION OF A CYTOCHROME P450 GENE FROM ASCOCHYTA PISI: CHARACTERIZATION AND FUNCTIONAL ANALYSIS. Kevin McCluskey and Hans VanEtten, Department of Plant Pathology, University of Arizona, Tucson, Arisona 85721

A cytochrome P450 gene and partial cDNA have been identified from a strain of the Ascomycete phytopathogen Ascochyta pisi. This strain is known to degrade the phytoalexins pisatin and maackiain via distinct cytochrome P450 enzymes. This gene was identified using a modified 3' RACE PCR protocol to clone a partial cDNA using degenerate PCR primers for fungal P450 genes. DNA sequence analysis supports the conclusion that this is a P450; it has ca. 40% sequence homology with other fungal P450s, including the P450 gene PDA from Nectria haematococca, another pea pathogen. This cDNA hybridizes preferentially to RNA extracted from a culture induced with pisatin. A cosmid clone homologous to this cDNA was identified from a genomic DNA clone library. Although preliminary indications are that the cloned genomic DNA does not confer PDA activity to Aspergillus nidulans, additional experiments are underway to confirm this finding. While the cDNA was found to have conserved restriction fragment sizes in several Ascochyta strains, a second molecule with homology to the most conserved region of the PDA gene was absent in a strain of A. pisi that lacks the ability to detoxify maackiain.

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KARYOTYPE VARIATION IN ISOLATES OF *GLOMERELLA GRAMINICOLA* AND THEIR F_1 TETRAD PROGENY J. A. Rollins and R. M. Hanau, Department of Botany and Plant Pathology, Purdue University, West Lafayette, In 47907.

Electrophoretic karyotypes were established for six maize isolates of *Glomerella graminicola* (anamorph *Colletotrichum graminicola*) and their tetrad progeny. On the basis of size, two classes of chromosomes, minichromosomes (0.4 to 1.4 Mb) and maxichromosomes (3.0 to >6.0 Mb), were discernible. Among isolates, minichromosomes were variable in size and number (3 to 5 per isolate), whereas maxichromosomes were variable in size but not in number (6 per isolate). When isolates having different karyotypes were crossed, nonparental karyotypes were generated among tetrad progeny. Cloned genes were physically mapped to chromosomes to identify homologous chromosomes among isolates and to investigate the inheritance of chromosome variation in tetrad progeny. Recombination between homologous, size polymorphic chromosomes, ectopic recombination between nonhomologous chromosomes, and the loss of nonessential chromosomes are being explored as possible mechanisms that explain the generation of these nonparental karyotypes.

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MORPHOLOGICAL AND MOLECULAR GENETIC VARIATION AMONG ISOLATES OF SEPTORIA MUSIVA. K. T. Ward, M. E. Ostry, USDA - Forest Service, North Central Forest Experiment Station, 1992 Folwell Ave., St. Paul, MN 55108 and G. R. Furnier, Depts. of Forest Resources and Plant Biology, University of Minnesota, St. Paul, MN 55108.

Many hybrid poplar clones grown for fiber and energy production are susceptible to premature defoliation and stem breakage caused by the fungus Septoria musiva Peck. The population genetic structure of S. musiva is currently unknown, making it difficult to reliably determine whether putatively resistant poplar clones will be resistant over a wide geographic area. We recovered 700 S. musiva isolates from native poplars and from 45 hybrid poplar clones across 16 locations in 5 states (MN, WI, SD, ND and IA). At two locations, collections were made from the same poplar clones in July, August and September in two consecutive years to investigate temporal variability among isolates. A study of 162 isolates revealed substantial variability in growth rate, color, morphology and sporulation. Mean increase in colony diameter at 8 days varied greatly by collection site and by poplar host, ranging from 1.1 to 14.8 mm. Randomly amplified polymorphic DNA (RAPD) assays based on the polymerase chain reaction have revealed a large number of DNA fragments and a large amount of genetic variation among the isolates. Cuttings from selected poplar clones are being propagated to serve as host differentials to determine variation in pathogenicity, aggressiveness and possible host specificity among S. musiva isolates.

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USE OF RAPD AND RIBOSOMAL RNA SPECIFIC PRIMERS IN PCR TO CHARACTERIZE ISOLATES OF GAEUMANNOMYCES SPECIES. H.M. Fouly[1], L.L. Domier[2], H.T. Wilkinson[1], and W. L. Pedersen[1] [1]Department of Plant Pathology, [2]USDA-ARS, University of Illinois, Urbana, IL 61801.

Isolates of *Gaeumannomyces graminis* var. *tritici*, *G.g.* var. *graminis*, *G.g.* var. *avenae*, and *G. incrustans* were characterized using random amplified polymorphic DNA (RAPD) and restriction analysis of amplified nuclear internal transcribed spacer (ITS) and 5.8 s rDNA regions in polymerase chain reaction (PCR). For RAPD, sixty primers were evaluated for their ability to distinguish among species and varieties of *Gaeumannomyces*. Four primers produced DNA amplification patterns that could facilitate distinguishing the three varieties of *G. graminis* and *G. incrustans*. In contrast, analysis of restriction enzyme cutting patterns of the internal transcribed spacers, along with the 5.8 s rDNA, detected few polymorphic sites among the G.g. isolates. The G.i. isolates, however, could be easily distinguished from G.g. varieties. These data suggest that the ITS-5.8 rDNA are highly conserved among the varieties of *G. graminis* but not in *G. incrustans*. The RAPD banding patterns immediately will be useful in identification of Gaeumannomyces isolates. Because of the highly conserved nature of the ITS and 5.8 s rDNA regions, the nucleotide sequence of this region will be determined to identify taxinomically useful characteristics.

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ANTIFUNGAL ACTIVITY OF TOBACCO OSMOTIN ON VARIOUS FUNGI IN VITRO. L.R. Todd, M. Paino D'Urzo, P.M. Hasegawa, and R.A. Bressan, Horticulture Department, Purdue University, West Lafayette, IN 47907.

Various fungi were tested for sensitivity *in vitro* to osmotin, a group 5 pathogenesis-related (PR) protein that accumulates in tobacco under osmotic stress. Fungi were subcultured to fresh potato dextrose medium and allowed to grow to a radius of 2 cm. Sterile filterpaper disks, each loaded with one of the following: water, bovine serum albumin (BSA) (100µg), or osmotin (30, 60 or 100µg) were placed outside the leading edge of the colony. After incubation at room temperature for 3 days, a zone of growth inhibition was evident surrounding the disks containing 30, 60 or 100µg osmotin in *Verticillium dahliae, Fusarium oxysporum* f. sp. *dianthi, F. oxysporum* f. sp. *lycopersici, Bipolaris maydis and Kabatiella zeae.* A growth inhibition zone was evident around disks containing 60 or 100µg osmotin in *Diplodia maydis, Phytophthora infestans* and *Phytophthora parasitica* var. *nicotiana* and around disks containing 100µg osmotin in *Magnaporthe grisea, Botrytis cinerea, Colletotrichum graminicola* and *C. gloeosporioides.* No effect of osmotin was seen on *Rhizoctonia solani* at these concentrations. Neither water nor BSA caused growth inhibition of any of the fungi tested. Disks containing 100µg of thaumatin, a PR-5 protein with 55% homology to osmotin, were found to have no activity when tested on *Fusarium oxysporum* f. sp. *dianthi, F. oxysporum* f. sp. *lycopersici, Verticillium dahliae* or *Rhizoctonia solani*. These results indicate that osmotin inhibits the growth of a wide range of phytopathogenic fungi *in vitro.*

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ELECTROPHORETIC KARYOTYPE ANALYSIS AND MAPPING OF AN ENDOGLUCANASE GENE FROM *MACROPHOMINA PHASEOLINA.* Richard W. Jones and Suzanne Canada, Dept. of Botany & Plant Pathology, Lilly Hall, Purdue Univ., West Lafayette, IN 47907.

Macrophomina phaseolina, causal agent of charcoal rot, is a deuteromycete soilborne fungus with a host range of over 500 plant species. In an effort to begin characterizing the potential diversity in the chromosomal organization of a pathogen with such a broad host range, we have employed CHEF electrophoresis. Chromosomes from a soybean isolate were separated by running the samples for 80 hours at approximately 2.0 V/cm with a switching time ramp from 13 to 25 minutes. These conditions separated 7 chromosomal bands of approximately 1, 2.5, 2.8, 3.0, 3.7, 4.6, and 5.7 Mb as determined from comparison with *Schizosaccharomyces pombe* and *Saccharomyces cerevisiae* standards. To begin mapping potential pathogenicity determinants, probes were prepared from a cloned *M. phaseolina* endoglucanase gene. Hybridization signals were detected at the 4.6 Mb chromosome. Additional cellulase clones are currently being mapped to determine if they are clustered or dispersed in the genome.

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IDENTIFICATION OF THE WHEAT AND BARLEY BIOTYPES OF *STAGONOSPORA NODORUM* USING RESTRICTION FRAGMENT LENGTH POLYMORPHISMS AND BIOLOGICAL CHARACTERISTICS. P.P. Ueng[1], B.M. Cunfer[2], and W. Chen[3]. [1]Plant Molecular Biology Lab., USDA-ARS, BARC-West, Beltsville, MD 20705, [2]Department of Plant Pathology, University of Georgia, Griffin, GA 30223, [3]Illinois Natural History Survey, University of Illinois, Champaign, IL 61820.

Forty-three isolates of *S. nodorum* from wheat, barley, and other hosts were identified as either the wheat or barley biotype based on cultural characters and host range. Identification of the biotypes based on these phenotypic criteria were comparable to results obtained with genetic identification by restriction fragment length polymorphisms (RFLP) analysis. Genomic DNA probes from both biotypes of *S. nodorum* and *S. avenae* f.sp. *avenae* can clearly differentiate these two biotypes of *S. nodorum*, and *S. avenae* f.sp. *triticea* and *S. avenae* f.sp. *avenae*.

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MOLECULAR SYSTEMATICS OF THE PLEOSPORALES OF THE LOCULOASCOMYCETES. J. Dong, W. Chen, and J.L. Crane, Dept. of Plant Pathology, University of Illinois at Urbana-Champaign, and Illinois Natural History Survey, 607 East Peabody Drive, Champaign, IL 61820

The Pleosporales is a large order of the Loculoascomycetes, and the arrangement of its families and genera is controversial. Seven cultures belonging to the families Lophiostomaceae, Leptosphaeriaceae, and Pleosporaceae were used in this study. The 5' end 28S rDNA (about 600 bps) was amplified using polymerase chain reaction. Single-stranded DNA was generated from the purified PCR product with a primer ratio of 1:50. DNA sequences were obtained from the single-stranded DNA and analyzed using the PAUP. The results based on the partial 28S rDNA sequences generally supported the Barr's (1987) separation of the Leptosphaeriaceae from the Pleosporaceae. However, when *Nectria cinnabarina* was used as an outgroup, the placement of the *Pleospora herbarum* (Pleosporaceae) is inconsistent with previous work (Barr, 1987). Additional data are needed in order to better understand the phylogenetic relationships among the families of the Pleosporales. To obtain this, we are analyzing the small-subunit rDNA and expanding genera and families in our study.

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DNA AMPLIFICATION FINGERPRINTING OF ISOLATES FROM DIFFERENT VEGETATIVE COMPATIBILITY GROUPS OF *FUSARIUM MONILIFORME.* D. Zhang, J. Qiu, R.A. Shelby and S. Tuzun, Dept. of Plant Pathology, Auburn University, AL 36849

DNA amplification fingerprinting (DAF), a polymerase chain reaction based technology that can be applied to fungal DNA to produce specific bands, offers a possible alternative to the labor intensive system of Vegetative Compatibility Group (VCG) testing to identify isolates of *Fusarium moniliforme*. We compared DAF banding patterns of 23 isolates from soil and maize plants which belonged to 8 VCG's. Each of several different primers typically resulted in formation of two distinct fingerprints, but within a VCG, the DAF's were identical. No single primer produced a fingerprint which is distinctive for each VCG, however, it is possible to use a series of different primers which would result in a distinctive group of fingerprints for each VCG. This is the first report of using DAF to differentiate VCG's and confirms that VCG's of *Fusarium* have different genomic compositions.

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IDENTIFICATION OF THE *nifHDK* and *ntrC* REGIONS OF *ACETOBACTER DIAZOTROPHICUS* PAL5, A BACTERIAL ENDOPHYTE OF SUGARCANE. Myrna Sevilla[1], Dietmar Meletzus[1], Katia Teixeira[2], Ivo Baldani[2], and Christina Kennedy[1] [1]Department of Plant Pathology, University of Arizona, Tucson, Arizona, USA 85721; [2]EMBRAPA/CNPBS, Seropedica, 23851, Rio de Janeiro, Brazil.

Acetobacter diazotrophicus, a gram-negative N_2 fixing species may be important for sugarcane nutrition. Our immediate goal is to isolate Nif$^-$ mutants of *A. diazotrophicus* to test this hypothesis. Plasmid and phage genomic libraries of *A. diazotrophicus* PAL5 were constructed in *E. coli* using pLAFR3 and EMBL3, respectively, to isolate *nifH* and *ntrC*. A potential *nifHDK* region was identified by heterologous hybridization using a 4.5 kb *Eco*R1 *nifHDK* fragment of *Azospirillum brazilense* as probe. The *ntrC* gene was isolated by complementation of mutant strains *E. coli* ET8556 and *Azotobacter vinelandii* MV103 and MV521 using the pLAFR3 gene library. DNA sequencing results of these regions will be presented. presented. In addition, the putative regulatory *nifA* gene is currently being isolated by similar strategies. Mutations in these genes are being constructed using *uid*A-antibiotic resistance gene cassettes and/or Tn5 derivatives. Nif$^-$ mutants will be used in plant inoculation experiments with sterile sugarcane plants to determine whether or not *A. diazotrophicus* nitrogen fixation contributes to sugarcane biomass production.

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DIFFERENTIATION OF GEOGRAPHIC SOURCES OF *MYCOSPHAERELLA DEARNESSII* ESTIMATED BY RAPD MARKERS. Zhengyu Huang, Eugene B. Smalley and Raymond P. Guries, University of Wisconsin-Madison, Madison, WI 53706.

Randomly amplified polymorphic DNA (RAPD) markers were used to characterize different geographic sources of *Mycosphaerella dearnessii*, the causal agent of brown spot disease of pine. Twenty of 40 10-base oligonucleotide primers detected polymorphism among 43 isolates (14 from the northern United States, 14 from the southern United States and 15 from southeastern China). Northern U.S. isolates comprised one group while southern U.S. and Chinese isolates comprised a second group. Results further supported the existence of several races within *Mycosphaerella dearnessii*. The Chinese isolates appear to have originated from the southern U.S., as suggested by the phylogenetic trees generated using the molecular marker data.

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LOSS OF PISATIN DEMETHYLASE GENES ASSOCIATED WITH LOSS OF CHROMOSOMAL ELEMENTS IN CROSSES WITH *NECTRIA HAEMATOCOCCA*. D. L. Funnell-Baerg, P. S. Matthews and H. D. VanEtten, Department of Plant Pathology, University of Arizona, Tucson, Arizona 85721.

Strains of the fungus *N. haematococca* pathogenic on pea are able to detoxify the pea phytoalexin pisatin, a trait designated Pda^+. Crosses involving the highly virulent Pda^+ isolate, 34-18, with Pda^- isolates, resulted in unusual segregation ratios for Pda. Most of the progeny, regardless of Pda phenotype, had low virulence on pea. Genetic and Southern analyses have shown that there are two active *PDA* genes *(PDA1* and *PDA5)* and one non-functional gene *(Phd)* in 34-18. Each *PDA* gene and *Phd* segregate independently and can be lost during crosses. Analyses of CHEF gels indicate that *PDA1* resides on a 1.5 Megabase (Mb) chromosome that is not present in $PDA1^-$ isolates. *PDA5* and *Phd* are on chromosomes that vary in size from 3.5 to 5.7 Mb in different progeny. Parental chromosome bands that hybridize with *PDA* are absent in progeny that have lost one or more of these *PDA* genes. The loss of virulence, and the unusual segregation of *PDA* genes in the progeny from crosses with 34-18, may result from normal segregation events occurring together with the loss of chromosomal elements during meiosis.

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RFLP VARIATION AMONG 24 ISOLATES OF *GIBBERELLA ZEAE*. S.J. Hogarth and R.L. Bowden, Department of Plant Pathology, Kansas State University, Manhattan, KS 66506.

Twenty-four isolates of *Gibberella zeae* from North America, Africa, Japan, and Australia were compared at 11 loci using probes generated from a random genomic library. The library consists largely of single-copy clones which provide probes that give simple banding patterns when labeled and hybridized to Southern blots of genomic digests of fungal DNA. These banding patterns are easily distinguishable as RFLP alleles. Of 11 probes, 4 were monomorphic and the remaining 7 identified between 2 and 5 alleles. Hybridization of the *G. zeae* probes to the related *G. fujikuroi* was tested using two strains of *G. fujikuroi*. Several of the probes hybridized at normal stringency. This RFLP screening will provide an estimate of genetic variability within the species and probes to identify individuals in field studies. The screening will also be used to identify individuals sufficiently different to serve as parents in a cross to construct an RFLP linkage map.

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PRELIMINARY ANALYSIS OF dsRNA LENGTH POLYMORPHISMS IN CLONAL, ROOT, AND FIELD POPULATIONS OF *MONOSPORASCUS CANNONBALLUS*. B. R. Lovic, V. A. Valadez, D. J. Lofland, R. D. Martyn, and M. E. Miller. Department of Plant Pathology and Microbiology, Texas A&M University, College Station 77843, and Weslaco 78596.

A hierarchical sampling strategy was employed in two 15-acre muskmelon fields to collect isolates of *Monosporascus cannonballus*, causal agent of root rot/vine decline of watermelon and muskmelon. Isolations were performed by plating sections of surface-sterilized roots on streptomycin-amended water agar. The number of isolates obtained from any individual root system varied from 1 to 26. The isolates were grown in liquid medium, nucleic acids were extracted using a standard CTAB protocol, and dsRNA length polymorphisms were visualized following gel electrophoresis of total nucleic acid preparations on ethidium bromide-stained gels. The fragments were identified as dsRNA by the resistance to RNase in high-salt buffer and by a green fluorescence following acridine-orange staining. Of the 300 isolates collected, 65% harbored 1 to 13 dsRNA fragments that varied in length from 1.7 to 3.7 kb relative to a DNA size marker. The dsRNA pattern in any individual isolate (clone) appeared to be stable in subcultures. A total of 16 different patterns were observed among isolates within the entire field. Up to eight different patterns were observed among the dsRNA-containing isolates sampled from a single root system. Analysis of the spatial distribution of polymorphic types revealed no clustering in either of the two fields examined.

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RELATIONSHIP BETWEEN THE SOUTH AMERICAN AND EUROPEAN POPULATIONS OF WHEAT STEM RUST REVEALED BY RAPD, ISOZYME, AND VIRULENCE MARKERS. B.D. McCallum, A.P. Roelfs, and J.V. Groth, Department of Plant Pathology, University of Minnesota, and Cereal Rust Lab, USDA-ARS, St. Paul, MN 55108, USA.

The possible sources of origin for the South American population are; accidental introduction from other wheat growing regions of the world, particularly Europe, or adaptation of native populations to cultivated wheat. The possibility that the South America population of the wheat stem rust fungus, *Puccinia graminis* f. sp. *tritici*, was introduced from Europe was tested by comparing the genetic similarity of collections from South America and Europe. RAPD, isozyme, and virulence markers were used to compare twenty isolates from all over South America and nineteen isolates from all over Europe. The three types of markers showed slightly different similarity groupings of the isolates. Relationship patterns based on RAPD or isozyme markers were more similar to each other than to those based on virulence markers. The South American population was diverse, but consisted of two main groups containing most of the isolates, and a number of isolates which were not closely related to either group. The two main South American groups were distinct from the major European groups. These results indicate that the current South American population probably was not founded by a single introduction from Europe.

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ALTERATIONS IN CELL WALLS OF *POPULUS TREMULOIDES* INFECTED WITH *HYPOXYLON MAMMATUM*. B. Bucciarelli[1], M.E. Ostry[4], N.A. Anderson[1], R.G. Fulcher[2] and C.P. Vance[3]. [1]Dept. of Plant Pathology [2]Dept of Food Science and Nutrition, [3]USDA-ARS, University of Minnesota, St Paul, MN, [4]USFS-NCFES St. Paul, MN.

Green internodal tissue from glasshouse grown plants of putative resistant and susceptible genotypes of *Populus tremuloides* were wounded and/or wound-inoculated with *Hypoxylon mammatum*. Cell walls near the wound margin were analyzed for alterations by fluorescence microscopy (excitation 365 nm, barrier 397 nm). By 96 hrs after wounding a zone of autofluorescent cortical cells developed in both genotypes. Pathogen presence at the wound site triggered an increase in the number of fluorescent cells and their location, with cortical and phloem cells displaying autofluorescence. A major difference between resistant and susceptible genotypes was observed in the number of mycelial aggregates forming and the efficiency by which they were enclosed by autofluorescing pith cells. Wall autofluorescence due to wounding and/or inoculation is identical to that of the xylem and phloem fibers suggesting the deposition of phenolic or lignin-like substances.

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ANALYSIS OF TRANSGENIC ALFALFA PLANTS WHICH EXPRESS GENES CODING FOR A RICE CHITINASE AND AN ALFALFA GLUCANASE. Sameer Masoud[1], Christopher J. Lamb[2] and Richard A. Dixon[1]. [1]The Samuel Roberts Noble Foundation, Plant Biology Division, P. O. Box 2180, Ardmore, OK 73402; [2]The Salk Institute for Biological Studies, Plant Biology Laboratory, 10010 N. Torrey Pines Road, La Jolla, CA 92037.

The hydrolytic enzymes chitinase and β-1,3-glucanase partially digest isolated cell walls of potential pathogens, and synergistically inhibit fungal growth *in vitro*. Constitutive over-expression of several plant chitinase and glucanases in transgenic plants has been shown to enhance resistance against phytopathogenic fungi. We over-expressed an acidic alfalfa glucanase cDNA (Aglu1) and a genomic clone of rice chitinase (RCH10) in transformed alfalfa plants via *Agrobacterium tumefaciens*. Glucanase activities were up to 10 fold higher in leaf extracts of transformed plants compared to leaf extracts of control plants. The gene product of the Aglu1 transgene appears to produce a glucanase activity band present in leaf extracts of some transgenic alfalfa plants but not in leaf extracts of control alfalfa plants. A band of similar mobility was constitutively present in root extracts of non-transformed alfalfa plants and was induced by fungal pathogens in leaves. The presence of an additional chitinase activity band in extracts of chitinase transformed plants suggests that the introduced rice chitinase is being expressed in transgenic alfalfa plants. The transgenic alfalfa plants are being assayed for enhanced resistance to several phytopathogenic fungi.

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COMPLEMENTARY GENETIC INTERACTION IN FUSIFORM RUST DISEASE AND IDENTIFICATION OF MARKERS LINKED TO GENES FOR SPECIFICITY IN CRONARTIUM QUERCUUM F. SP. FUSIFORME. R. L. Doudrick and C. D. Nelson, U. S. D. A. Forest Service, Gulfport, MS.

Nineteen clones of slash pine and two single urediniospore cultures of Cronartium quercuum f. sp. fusiforme were used to identify complementary genetic interactions in fusiform rust disease. The pathogen culture CCA-2 was hypothesized heterozygous at locus *pf3*; when inoculated using basidiospores, galls develop on approximately one-half of the ramets of differential host clones (1h*f3*). The feasibility of using bulked segregant analysis to identify molecular markers linked with *pf3* in C.q.f. was investigated using random primers in PCR. Single drops of pycniospores (0p*f3*) collected on galls on differential clones (1h*f3*) were grouped for comparison with drops of pycniospores (0p*f3* or 1p*f3*) collected on galls on non-selective clones (0h*f3*). 1000 random 10-mer primers were screened for amplification of polymorphic DNA segments on the two pools of genomic DNA. Linkage relationships among 22 polymorphic markers and between the markers and *pf3* were determined by segregation analysis. The linked markers will be converted to sequence tagged sites. Linkage between the sequence tagged sites and *pf3* will be confirmed and quantified by independent segregation analyses.

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A RAPID METHOD FOR EVALUATION OF DEFENSE GENE CONSTRUCTS FOR THEIR UTILITY IN ENHANCING DISEASE RESISTANCE. Amy J. Nelson and William R. Bushnell, Cereal Rust Laboratory, USDA-ARS, and Department of Plant Pathology, University of Minnesota, St. Paul, MN 55108

Plants respond to disease by induction of a large array of host response genes, some of whose gene products are known to play an important role in plant defense. It is widely believed that modification of the expression of these genes could lead to enhanced disease resistance. Unfortunately, the time and labor that must be invested for evaluation of a single gene construct introduced into transformed plants is very substantial. We are developing a rapid method for evaluation of gene constructs by transiently expressing them in barley (*Hordeum vulgare*) coleoptile epidermal cells via microprojectile bombardment with a helium gun, and assessing disease response by immediate inoculation of the coleoptiles with the powdery mildew pathogen (*Erysiphe graminis* f. sp. *hordei*). The R and C1 genes of maize, which confer anthocyanin expression, are being used as reporter genes, providing a nondestructive means for identifying cells that are expressing

the introduced gene. The number of transiently expressing cells per shot is now approaching a frequency where assessment of pathogen development in transformed cells should be feasible.

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PHYTOALEXINS ACCUMULATING DURING INDUCTION AND CHALLENGE INTERACTIONS IN ALFALFA ANTHRACNOSE. Nichole R. O'Neill, USDA, ARS, Beltsville, Maryland, 20705

Defense responses in alfalfa to races of *Colletotrichum trifolii* include limitation of fungal ingress, accumulation of enzymes of the phenylpropanoid pathway, and accumulation of phenolic and fluorescence compounds, principally the phytoalexin medicarpin. Several phytoalexins accumulated in cultivars with the An_1 or An_2 resistance genes during attempted infection by avirulent races. Their role in limiting fungal growth, and the chronology of accumulation during induction and challenge interactions was investigated. During incompatible interactions medicarpin accumulated to levels below the in vitro ED_{50} for inhibition of spore germination, sporling growth, and hyphal growth. However a subsequent compatible challenge with race 2 boosted the medicarpin and sativan levels to nearly twice that of unchallenged levels. Concentrations of vestitol, formononetin, genistein, coumestrol, and daidzein were also determined. The results suggest that protection may not be solely a result of the medicarpin produced in response to an initial incompatible infection attempt. The incompatible interaction may serve to prime the plant for further alterations in the defense response upon challenge by compatible fungi.

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CHARACTERIZATION OF HOST PLANT GENES INDUCED BY *MELOIDOGYNE INCOGNITA* INFESTATION. C. Potenza[1], E. Higgins[2], S. Thomas[2] & C. Sengupta-Gopalan[1,1]Dept. Agronomy and Horticulture, [2]Dept. Entomology, Plant Pathology and Weed Science, New Mexico State University, Las Cruces NM 88003.

Our interest is to characterize host plant genes important in root-knot nematode-plant interaction, including genes controlling giant cell formation in susceptible plants, and resistance mechanisms in resistant plants. Using selected alfalfa cultivars, we have established that *in vitro* translation products of RNA isolated from control and infested roots show quantitative and qualitative differences when separated via 2D SDS-PAGE. Analysis of *in vitro* translation products of RNA isolated from mechanically wounded roots show that these differences cannot be completely accounted for by wounding due to nematode root penetration and migration. DDRT-PCR of products made to RNA from control and infested alfalfa roots supports our 2D SDS-PAGE results. Because phytoalexins have been shown to undergo localized increases in roots infested with nematodes, we have analyzed by Northern blot anaysis the expression pattern of two key enzymes within the phenylpropanoid pathway, chalcone synthase (CHS) and phenylalanine-lyase (PAL). Preliminary results show increased expression of CHS gene(s) in roots of both susceptible and resistant cultivars following invasion.

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TRANSDUCTION OF THE SALICYLIC ACID SIGNAL IN THE ACTIVATION OF PLANT DEFENSE RESPONSES. Uwe Conrath, Z. Chen, P. Sanchez-Casas, J. Ricigliano, H. Silva and D. F. Klessig; Waksman Institute, Rutgers, The State University of New Jersey, P. O. Box 759, Piscataway, N. J. 08855 USA

Salicylic acid (SA) is an endogenous inducer of several plant defense responses including systemic acquired resistance. We have purified and characterized a soluble SA-binding protein (SABP) from tobacco, whose binding specificity and affinity are consistent with a receptor function. The sequence of a cDNA encoding SABP is essentially identical to that of catalase and SABP can readily convert H_2O_2 to H_2O and O_2. A variety of experiments strongly argue that SA's mode of action is to bind SABP/catalase thereby inhibiting its ability to degrade H_2O_2. The resulting rise in H_2O_2 levels then acts as a signal to induce expression of defense-related genes such as pathogenesis-related (PR)-1 gene. For example, increasing H_2O_2 levels by treatment with SA or with the known catalase inhibitor 3-amino triazole induces PR-1 gene expression. PR-1 genes are also induced in transgenic plants expressing an antisense copy of the catalase cDNA which inhibits synthesis of the endogenous catalases.

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RESISTANCE TO FUSARIUM HEAD BLIGHT IN SOFT RED WHEATS. S.E. Penix, K.D. Kephart, A.L. McKendry and G.S. Smith, University of Missouri, Columbia 65201.

Economic losses to Fusarium head blight (FHB), caused by Gibberella zeae, occurred in Missouri and surrounding states in 1982, 1990 and 1991. All commercial soft red winter wheat cultivars are considered susceptible to FHB, and relative differences in resistance are difficult to identify on the basis of field observation. In field and greenhouse experiments at the University of Missouri, 64 soft red winter wheat cultivars were screened for FHB resistance. The middle floret of each wheat head was inoculated at mid-flowering with a 5-µl suspension of 375 macroconidia of F. graminearum. Fusarium head blight index (FHBI), which measures the extent of disease spread from the inoculated floret, was calculated based on the percentage of infected spikelets in the total spikelets examined. Index values ranged from 12 to 20 for the most resistant cultivars 'Freedom' through 'HybriTech Pacer', compared with 64 for the most susceptible cultivar, 'OH 470'. Planting one of the more resistant cultivars, coupled with crop rotation and tillage to maintain lower levels of inoculum, can reduce losses to FHB.

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HERITABILITY OF RESISTANCE TO SUGARCANE RED ROT AND THE EFFECT OF ENVIRONMENTAL STRESS ON DISEASE SEVERITY. Z. Yin, J. W. Hoy, and S. B. Milligan. Dept. Plant Path. and Crop Phys., La. Agr. Exp. Sta., La. State Univ. Agr. Center, Baton Rouge, LA 70803.

Heritability of resistance to red rot, caused by *Colletotrichum falcatum*, was studied in 40 crosses with 24 parents. A traditional method was used to evaluate resistance, in which stalks were inoculated, held under controlled conditions, then assessed for rot symptoms. Heritability estimates determined by mid-parent-offspring regression for disease variables were low, ranging from 0.18 ± 0.04 to 0.32 ± 0.06 and genetic gain in resistance, using a 10% selection intensity, ranged from 13.9 to 26.6%. However, field experiment results suggest that resistance to red rot is affected by drought stress at planting. Stalk counts in plots planted with stressed, inoculated stalks were reduced for each of seven cultivars. Stress alone, due to waterlogging, reduced stalk populations for each of three cultivars. Populations were lowest in the stressed, inoculated treatment, but the further reductions resulting from inoculation were not significant.

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VERTICILLIUM ALBO-ATRUM X LIGHT X CLONE INTERACTION IN ALFALFA. Pennypacker, B.W., Knievel, D.P., Risius, M.L., and Leath, K.T., Dept. of Agronomy, Penn State Univ., and USDA-ARS, Univ. Park, PA 16802

Light (PPFD) was manipulated in the greenhouse to determine whether carbon assimilation regulates resistance to *V. albo-atrum (Vaa)*. Pathogen (*Vaa*, no *Vaa)*, clone (resistant, susceptible), PPFD (100%, 70%, 40% of ambient), and time (3 wks) were the treatments. Clones were inoculated similarly in expt 1 and 6 wks apart in expt 2. Dry weight of plant parts and disease ratings were determined weekly. Vaa x clone x PPFD x wk interactions occurred in expt 1 in all parameters. Growth of the infected susceptible clone was suppressed under all PPFD levels, but the infected resistant clone was symptomatic only under 40% PPFD. In expt 2, the resistant clone was infected 6 wks longer than the susceptible clone, and only *Vaa* x clone interactions occurred. The lack of a differential response to *Vaa* and PPFD in the susceptible clone in expt 1 is evidence that the defense mechanism under investigation is not simply a constitutive part of all alfalfa plants, but is unique to resistant alfalfa.

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CONCURRENT USE OF COMPUTER SIMULATION WITH LABORATORY EXPERIMENTS IN AN INTRODUCTORY PLANT PATHOLOGY COURSE. Guy R. Knudsen, University of Idaho, Moscow, ID 83843.

Epidemiological concepts often are not addressed in laboratory exercises for introductory plant pathology courses, because of time and space constraints. A combined experimental and computer simulation exercise was developed to explore environmental and spatial aspects of postharvest disease spread. Experimentally, arrays (5 x 5) of ripe Bartlett pears were placed in wooden trays, with the center pear wounded and inoculated with a *Penicillium* sp. isolate obtained from orchard fruit. Pears were incubated at 5° or 25° C and ≈ 95% or ≈ 50% RH. Daily for 14 d, students recorded patterns and levels of disease incidence and severity for each treatment. A microcomputer model (written in Visual Basic for Windows) graphically simulated disease development on a similar array of fruit. Model parameters were derived in part from three semesters' repetition of the experiment. Students simulated a variety of environmental conditions, graphed and compared disease progress curves for experiments and simulations, and critically evaluated the model's assumptions and parameters.

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AN AEROPONICS SYSTEM FOR PRELIMINARY SCREENING OF MAIZE GENOTYPES FOR RESISTANCE TO SEEDLING BLIGHT CAUSED BY *FUSARIUM GRAMINEARUM*. L.J. du Toit, H.W. Kirby, and W.L. Pedersen. Department of Plant Pathology, University of Illinois, Urbana, IL 61801.

An aeroponics system was evaluated as a method for rapid screening of maize hybrids for resistance to *Fusarium graminearum* seedling blight/root rot. This system allows for nondestructive, repetitive sampling of seedlings for assessing disease progress and seedling growth. Seven- to ten-day-old seedlings were inoculated by dipping the root

systems in a mycelial suspension of *F. graminearum* for 10 seconds. Root systems were suspended in the aeroponics chamber and received a 1 second root misting of modified Hoagland's solution every 15 minutes. Shoot growth and root rot were assessed at 3 day intervals for 15 days (using a 0 - 6 scale for root rot, with 0 = no disease, and 6 = dead plant). Final shoot and root dry mass were determined at day 15. The data indicated significant differences among hybrids for root rot rating and final root dry mass. These factors may be useful in screening maize seedlings for resistance to *F. graminearum* using an aeroponics technique. Shoot growth did not show significant differences among hybrids.

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FACTORS THAT INFLUENCE THE DETECTION OF *PYTHIUM ULTIMUM* USING A SPECIES-SPECIFIC MONOCLONAL ANTIBODY. F. Avila, G.Y. Yuen. Department of Plant Pathology, Univ. of Nebraska, Lincoln, NE 68583-0722.

We studied factors that influence the detection of *P. ultimum* by ELISA and immunofluorescence using the species-specific monoclonal antibody, MAb E_5. Detection of *P. ultimum* by ELISA depended on culture age, type of liquid medium, carbohydrate source, sucrose concentration, and presence of seed exudates in the media. Storage of mycelium extract for 3 years did not influence the antigen detection. As the cultures aged, antigen accumulation in the filtrates followed a saturation pattern. The mycelium produced antigen only during the first three days of growth. Antigen content was higher in filtrates and mycelia when the fungus was grown in Czapek-Dox Broth, flooded (f) Oat Meal Agar, f-V-8 juce Agar, or f-Lime Bean Agar than when *P. ultimum* was grown in f-Corn Meal Agar or Corn Meal Broth. Antigen production was stimulated by sucrose, glucose, maltose, fructose and mannose, but was inhibited by xylose, ribose and galactose. Low concentrations of seed exudates stimulated antigen production. Detection of antigen by immunofluorescence depended on the stage of fungal development. Immunofluorescence demonstrated that the antigen is present in young, actively-growing structures, but it is not detectable in aged structures.

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FIRST TIME PROPAGATION OF SHIITAKE MUSHROOM PROPAGULES IN ARTIFICIAL CULTURE MEDIA. R. P. Pacumbaba, Department of Plant and Soil Science, Alabama A&M University, Normal, AL 35762.

Shiitake [*Lentinula edodes* (Berk.) Pegler] germinated only on medium # 2, 20 days after axenically plating the propagules. Using mycelial tip transfer from medium # 2 to subsequent artificial culture media, flocculent mycelial growth was obtained only on media # 34, 37, 40, 43, 44, 45, 46, 48, 49, and 50, 10 to 15 days after inoculation. Tiny shiitake mushroom buttons appeared only on media # 43, 44, 45, 46, 48, 49, and 50; and on medium # 34, 20 and 30 days after inoculations, respectively. Flocculent mycelial growth without shiitake mushroom buttons was obtained only on media # 53, 54, 55, and 56. Mycelial growth for spawn production was obtained on substrate vermiculite supplemented with medium # 51, 20 days after inoculation. Using maple and oak sawdust, vermiculite, and various combinations of the substrates supplemented with either medium # 51, 52, or 59, also induced excellent mycelial growth for spawn production 20 days after inoculation. Fruiting bodies started to appear on maple + vermiculite (1:1) supplemented with medium # 59, 99 days or 3.3 months after inoculation; or 79 days or 2.6 months, 20 days after spawn production. Additional fruiting bodies appeared on similar substrate or on various combination of substrates supplemented with either medium # 51 or 52. All the experiments were carried out at room temperature in the laboratory. This study is still going on.

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PRESERVATION OF *PYTHIUM* SPECIES AND *RHIZOCTONIA SOLANI* AT -80 C. R.A. Kuznia and C.E. Windels. Northwest Experiment Station, University of Minnesota, Crookston 56716.

Autoclaved oat and barley grains were added to cultures of *Pythium* spp. and *R. solani*, respectively; incubated for 14-21 days; and placed in cryotubes (1.8 ml). Tubes were cooled at 4C for 24 hr, -15 C for 24 hr, and then stored in an ultra-low (UL) freezer (So-Low, Cincinnati, OH) at -80 C. Tubes were removed from the UL freezer, kernels scattered onto agar, and observed for growth within 24-48 hr; another tube of colonized grain was tested if no growth occurred after the first attempt. Viability was 85% for 29 cultures of *P. ultimum* var. *sporangiiferum* and 82% for 9 of *P. aphanidermatum* stored for 11-24 mo. Single cultures of *P. acanthicum*, *P. intermedium*, *P. irregulare*, *P. parocandrum*, and *P. rostratum* were alive after 6-24 mo storage at -80 C. Viability was 100% for 9 cultures of *R. solani* AG-1, 5 of AG-2-1, 51 of AG-2-2, 2 of AG-3, 69 of AG-4, and 30 of AG-5 stored for 21-26 mo. Storage at -80 C is a reliable and easy method for long-term preservation of *Pythium* spp. and *R. solani*.

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DISTRIBUTION OF MATING TYPE AND METALAXYL INSENSITIVE STRAINS OF PHYTOPHTHORA INFESTANS IN BRITISH COLUMBIA. C.I. Chycoski, P. Morris, and Z.K. Punja, Centre for Pest Management, Simon Fraser University, Burnaby, B.C., Canada.

Potato leaves of 16 cultivars infected by Phytophthora infestans were collected from 49 fields (representing 31 farms) in four different regions of British Columbia during June - September, 1993. Up to 12 leaflets or stems with sporulating lesions were sampled per field. Isolations were made from a single lesion per leaflet, by plating segments onto rye agar containing rifampicin, nystatin, and vancomycin and incubating at 15 C in the dark for 7-14 days. Mating type was determined by pairing tester strains (A1 and A2) with the unknown isolate on clarified V8 agar. After 1-3 wk, the dishes were examined for presence of oospores. Among 163 isolates, 85% were A2, which was recovered from all regions sampled. The A1 mating type was found in 3 of the 4 regions sampled; eight fields had both A1 and A2. Metalaxyl insensitivity (MI) was determined by comparing growth rates on rye agar containing 30 or 50 µg/ml of metalaxyl to unamended controls. Isolates were designated MI if the ratio of growth at 50 µg/ml:0 µg/ml was >0.4. The results showed that 68% of the isolates were MI, and the A1:A2 composition was 15%:85%.

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COMPARISON OF CARPOGENIC AND ASCOSPORE GERMINATION AMONG DIFFERENT CLONES OF SCLEROTINIA SCLEROTIORUM. Deena Errampalli, Tania L. Baker and Linda M. Kohn. Department of Botany, University of Toronto, Erindale College, Mississauga, Ontario L5L 1C6 Canada

Twenty seven isolates belonging to 11 different clones of *S. sclerotiorum* were compared for carpogenic germination of sclerotia produced on potato dextrose agar at 10 C. Preliminary results indicate that 14 isolates belonging to 8 clones germinated carpogenically by producing apothecia. Six out of seven common clones, that occurred at a higher frequency over a wide geographical area, produced apothecia under these experimental conditions. We do not know whether failure to produce apothecia is genetically determined. Ascospores were produced in all of the apothecia. Ascospore germination rates ranged from 95.6 to 99.4%. Single ascospore isolates collected from all of the apothecia established colonies and produced sclerotia. Effect of different temperatures on the ascospore germination will be discussed.

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MOLECULAR VARIABILITY IN *MACROPHOMINA PHASEOLINA*. T.E. Chase[1], Y. Jiang[1], and J. Mihail[2]. [1]Plant Science Dept. South Dakota State University, Brookings, SD 57007 and [2]Dept. Plant Pathology, University of Missouri, Columbia.

Macrophomina phaseolina is recognized as a highly variable fungal pathogen with a very wide host range. PCR-based techniques were applied in order to examine molecular variability among a phenotypically diverse set of *M. phaseolina* isolates. The hypothesis being tested is whether molecular variability can be associated with factors such as host range, geographic distribution, and/or biochemical or morphological features. A fragment of the rDNA repeat unit was amplified by ITS1/ITS4 primers, and the resulting ca. 700 bp fragment was digested with seven restriction enzymes (BfaI, BstUI, DpnII, Fnu4HI, Sau3A1, ScrFI and TaqI). No polymorphisms were detected among forty isolates. The Random Amplified Polymorphic DNA (RAPD) assay was also used to assess variability among *M. phaseolina* isolates. Twenty decameric primers (Operon Technology, Inc., Kit A) were used. Eighteen primers produced polymorphic banding patterns. The results revealed a high degree of molecular variability among *M. phaseolina* isolates from different phenotypic groups.

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GENETIC DIVERSITY AND VARIATION IN SCLEROTIUM ROLFSII AND RELATED SPECIES. C. Harlton, C.A. Lévesque and Z.K. Punja, Department of Biological Sciences, Simon Fraser University, Burnaby, British Columbia, Canada V5A 1S6.

Mycelial compatibility groupings (MCGs) and PCR-amplified regions of the internal transcribed spacer (ITS) of the nuclear rDNA and of the mitochondrial (mt) rDNA regions were used to assess genetic diversity and variation between field isolates of Sclerotium rolfsii and two related species. Among 109 isolates, 42 MCGs have been identified to date. Sixty-nine S. rolfsii, 11 S. delphinii and 2 S. coffeicola isolates from several different hosts and geographic areas have been included in the PCR-RFLP study. In addition, 27 single-basidiospore progeny derived from 4 different S. rolfsii parental isolates and 4 Athelia species (an outgroup) were examined for RFLPs. HpaII and RsaI restriction digests of the ITS region distinguished all three Sclerotium species, while AluI and MboI digests distinguished S. rolfsii and S. delphinii from S. coffeicola. Similarity of banding patterns resulted in restriction enzyme genotypes for each of the species. Distinct RFLP segregation patterns of the ITS region were observed for SB progeny

isolates using MboI. Digestion of mt rDNA with 12 enzymes did not show banding pattern variations in field or SB progeny isolates.

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PRODUCTION OF SCLEROTIA BY THE S STRAIN OF *ASPERGILLUS FLAVUS* DURING INFECTION OF DEVELOPING COTTON BOLLS AND INTERFERENCE WITH SCLEROTIAL PRODUCTION BY AN L STRAIN ISOLATE. R.K. Garber and P.J. Cotty. USDA, ARS, SRRC, P.O. Box 19687, New Orleans, Louisiana 70179.

Over the past five years we observed sclerotia of the S strain of *Aspergillus flavus* on surfaces of tight locks in commercial cotton in western Arizona. Greenhouse experiments assessed variability among S strain isolates in ability to produce sclerotia within seeds and on locule surfaces of developing bolls. All eight S strain isolates produced sclerotia on locule surfaces. However, more variability existed among isolates in ability to produce sclerotia within seeds. One isolate produced no sclerotia within seeds; whereas, other isolates replaced most seed endosperm with sclerotia. Strain interactions greatly affected formation of sclerotia. Coinoculation of developing bolls with an L strain isolate resulted in reductions in both aflatoxin formation and formation of sclerotia in seeds and on locule surfaces. Formation of sclerotia in developing bolls may have both ecological and feed safety implications.

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VARIATION IN A POPULATION OF *PHYTOPHTHORA CAPSICI* FROM SOUTHERN NEW MEXICO BASED ON ISOZYME ANALYSIS. C. M. Liddell, S. E. Drake, K. Blackstone and C. L. Biles. New Mexico State University, Department of Entomology, Plant Pathology, and Weed Science, Box 3BE, Las Cruces, NM 88003, U. S. A.

Variation in isozyme patterns of 56 isolates of *Phytophthora capsici* from southern New Mexico were compared to patterns of 9 isolates of *P. capsici* representing three sub-specific groups (CAP1, CAP2 and CAP3) as defined by Oudemans and Coffey (1991: *Mycological Research* 95:1025-1046). All New Mexico isolates were obtained from pepper plants (*Capsicum annuum* L.) collected at 11 sites throughout southern New Mexico. We examined 27 enzyme systems of which 14 systems were visualized and of the 14, 6 (SOD, MDH, LDH, PGM, ADH and PGI) were chosen for analysis of polymorphisms at 12 loci. The New Mexico isolates fell fairly evenly into 12 electrophoretic types (ET) more than the two ET's reported by Oudemans and Coffey, however, they clearly fit the criteria for the CAP1 group. No correlation of ET with mating type or site of collection was found and different ET's and both mating types were found at each site. The results of this study indicate that *P. capsici* forms a single, relatively homogeneous population in southern New Mexico. The presence of both matings types at each site and the presence of heterozygotes strongly indicates that this population is an actively outcrossing and may show greater variability for characters which are under active selection pressure, such as virulence to peppers, and resistance to fungicides.

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VARIATION IN THE INTERNAL TRANSCRIBED SPACER REGION OF THE NUCLEAR RIBOSOMAL DNA OF *ANISOGRAMMA ANOMALA*. N.K. Osterbauer[1], T.L. Sawyer[1], A. Liston[1], K.B. Johnson[1], and S.A. Mehlenbacher[2], [1]Dept. of Botany & Plant Pathology and [2]Dept. of Horticulture, Oregon State University, Corvallis 97331.

In the Pacific Northwest (PNW), the introduced fungus *Anisogramma anomala* causes eastern filbert blight, a canker disease on European hazelnut (*Corylus avellana*). It has been suggested that the PNW population of *A. anomala* is genetically homogenous. To determine whether genetic variation exists in this population, 60 isolates were collected from five geographically separated infection centers and DNA extracted from ascospores of these isolates. The polymerase chain reaction (PCR) was used to detect length polymorphisms in the internal transcribed spacer (ITS) region of the nuclear ribosomal DNA (nrDNA). The ITS region was amplified with primers homologous to the 3' end of the 16S nrDNA and the 5' end of the 28S nrDNA. A 620 base pair (bp) PCR product corresponding to the ITS region was observed in 59 isolates. An additional 720 bp product was observed in three isolates, JG-2, ZD1-1, and JE1-3. A single product of 520 bp was observed in isolate DU2-5. The presence of these length polymorphisms in the ITS region of the nrDNA of *A. anomala* suggests a higher level of genetic variation in the pathogen than previously believed.

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MATING OF *COLLETOTRICHUM GLOEOSPORIOIDES* ISOLATES WITH DIFFERENT HOST SPECIFICITIES ON A PARENTAL HOST Thornton, A.B., Cisar, C.R., and TeBeest, D.O. Department of Plant Pathology, University of Arkansas, Fayetteville, AR 72701

Colletotrichum gloeosporioides is an agriculturally important fungus pathogenic on many plant species. Many strains of *C. gloeosporioides* are believed to be incapable of completing a sexual cycle, including *C. gloeosporioides* f.sp. *aeschynomene* (Cga) which is pathogenic to the leguminous weed, northern jointvetch (*Aeschynomene virginica*). However, we have recently shown that Cga is sexually compatible with at least one other strain of *C. gloeosporioides* (isolated from pecan) when crossed on agar medium. In this study, two isolates (one from northern jointvetch and the other from pecan) were mated on northern jointvetch. Stems were inoculated with one of the isolates followed by inoculation of the same site one week later with the other sexually compatible isolate. The co-inoculated plants were incubated in a growth chamber for one week after which the inoculated stems were excised and placed on agar plates or moistened filter papers. After 2-3 weeks, perithecia with asci and ascospores typical of *Glomerella cingulata* (teleomorph of *C. gloeosporioides*) formed on the excised stem pieces. Ascospore progeny were isolated from several perithecia. Progeny were analyzed for pathogenicity to parental hosts and several RFLP markers were used to verify outcrossing. Successful mating of isolates of *C. gloeosporioides* with different host specificities on a parental host indicates that sexual recombination may occur in nature.

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USE OF ESTERASE ISOZYMES TO DISTINGUISH *ASPERGILLUS FLAVUS* GROUP SPECIES, STRAINS, AND VEGETATIVE COMPATIBILITY GROUPS. K.S. Elias and P.J. Cotty. USDA, ARS, SRRC, P.O. Box 19687, New Orleans, Louisiana 70179.

Aspergillus flavus group fungi vary widely in many morphological and physiological traits of both economic and ecological importance. To address questions about *A. flavus* group populations, rapid techniques to identify and distinguish population constituents are needed. Isozymes have potential application in this context. In the current study, total proteins were extracted from sixty-five isolates in the *A. flavus* group for isozyme analysis via polyacrylamide gel electrophoresis. Esterases (α and β) were found to be the most useful of 24 enzymes tested for rapid identification of specific members of the *A. flavus* group. The species *A. tamarii*, *A. nomius*, *A. flavus* and *A. parasiticus* could be readily distinquished via esterase polymorphism patterns. Furthermore, the S and L strains of *A. flavus* and several vegetative compatibility groups of the L strain were non-ambiguously identified with esterase banding pattern alone.

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REGULATION OF MYCORRHIZAL DEVELOPMENT AND INHIBITION OF NODULATION BY VAM FUNGI IN ALFALFA. D.D. Douds, Jr., L. Galvez, G. Bécard (USDA-ARS, 600 E. Mermaid Lane, Philadelphia, PA 19118) and Y. Kapulnik (Volcani Center, P.O. Box 6, Bet-Dagan 50250, Israel).

Two cultivars of alfalfa (*Medicago sativa*), Moapa-69 and Gilboa, were inoculated with the VAM fungi *Gigaspora margarita*, *Glomus mosseae*, and *Glomus intraradix* and indigenous *Rhizobium*. Plant growth, development of mycorrhizae, and nodulation were measured over 21 weeks. *Glomus intraradix* inhibited nodulation, plant growth, and N accumulation relative to uncolonized controls; and sporulated and colonized heavily in both genotypes. *Gigaspora margarita* sporulated only on Gilboa and *G. mosseae* sporulated on neither alfalfa genotype. Percentage root length colonized by *G. margarita* was declining by the end of the experiment, while that of *G. mosseae* remained stable. Colonization and sporulation by these fungi on a 'standard' host, *Paspalum notatum*, increased throughout the experiment, indicating an ability of alfalfa to regulate the development of its mycorrhizal symbiont.

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PRODUCTION OF MONOCLONAL ANTIBODIES TO SELECTED ARBUSCULAR MYCORRHIZAL FUNGI. R.S. Strobel, J.E. Kurle, and F.L. Pfleger. Department of Plant Pathology, University of Minnesota, St. Paul, MN 55108.

Arbuscular mycorrhizal fungi (AMF) are ubiquitous root inhabiting symbionts of most terrestrial plants. Currently, AM fungi are identified by morphological characters of the hyphal attachment and spore walls. Our laboratory has a need to rapidly assess what species of AM fungi are present in colonized corn roots. A specific monoclonal antibody designated GM1, was developed for *Glomus mosseae*, an AM fungus commonly associated with roots of corn plants grown in southwestern Minnesota. Mice were immunized with homogenized spores, and their spleenocytes fused to the myeloma line NS1 using standard methods. Resulting hybridomas were screened by dot blot assay for binding to homogenized spores. Although several hybridomas were positive in this screen, only the GM1 line survived subcloning, and remained positive. The GM1 antibody also reacts with hyphae. Screening for binding of this antibody to other species of AMF (*G. occultum* and *Acaulospora spinosa*) have not shown crossreactivity. Similar procedures are being used to raise monoclonal antibodies to *Acaulospora spinosa*. These results indicate that it is possible to immunize mice with AM fungal spores, and raise monoclonal antibodies that react with spores and hyphae.

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CHARACTERIZATION OF THE MYCORRHIZAL COMMUNITY IN A PINE FOREST FOLLOWING DIFFERENT SILVICULTURAL TREATMENTS.

Bradley R. Kropp, Department of Biology, Utah State University, Logan, Utah 84322

The effect of thinning and clearcutting on populations of ectomycorrhizal fungi was studied in a lodgepole pine stand in northern Utah. The forest studied was uniform and had an experimental thinning, a clearcut replanted to lodgepole pine and undisturbed area situated adjacent to one another. The fruiting patterns of the ectomycorrhizal species found in the forest were strongly affected by the silvicultural treatments. At least 29 species of

ectomycorrhizal fungi occurred in the undisturbed forest. Fungal species richness sharply declined after thinning and dropped further after clearcutting. Species evenness was also affected, showing a strong shift towards the Boletaceae as the severity of the treatment increased. Total sporocarp production was roughly equal in the undisturbed and thinned stands but sharply declined in the clearcut.

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EFFECTS OF PREVIOUS SEASON COTTON AND COWPEA ON *STRIGA HERMONTHICA* PARASITISM ON MAIZE . E.S. Ariga, D.K. Berner and J. Chweya. International Institute of Tropical Agriculture, PMB 5320, Oyo Road, Ibadan, Nigeria.

Effects of previous season crop on *S. hermonthica* parasitism on maize was evaluated in the screenhouse. Ridges were infested with 24,000 germinable *S. hermonthica* seeds, and planted to cotton (cv. Abuja Local) or cowpea (cv. TVx 3236) which were then uprooted 10, 40, 70, 100 days after planting. After uprooting the previous crop, susceptible maize (cv. 8338-1) was grown in the same ridge. A control with no previous crop was included. Emerged *S. hermonthica* plants were recorded fortnightly for 12 weeks. *S. hermonthica* damage symptoms on maize were recorded after 10 weeks on a scale of 1=no damage to 9=heavy damage. Maize root, stem and grain yields were recorded. Parasite emergence and maize symptom severity decreased while grain yield and harvest index increased with increasing cotton and cowpea growth duration. Parasite emergence was positively correlated with symptom expression (r= 0.73) and maize root biomass (r= 0.66) but negatively correlated with grain yield (r = -0.78) and harvest index (r= -0.83). Cowpea and cotton performed equally after 40 days growth.

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CHARACTERIZATION OF SOYBEAN CULTIVARS FOR *STRIGA HERMONTHICA* CONTROL. M. O. Alabi, D. K. Berner, and G. O. Olaniyan. International Institute of Tropical Agriculture, PMB 5320, Oyo Road, Ibadan, Nigeria.

Striga hermonthica is one of the most important constraints to cereal production in sub-Saharan Africa. Non-host crops like soybean that stimulate germination of *S. hermonthica* seeds, but are not parasitized, can be used in *S. hermonthica* control. Initial selection of soybean cultivars capable of stimulating *S. hermonthica* germination was done in the laboratory. In the screenhouse, ten selected cultivars of soybeans were planted in pots infested with 10,000 germinable *S. hermonthica* seeds. A susceptible cultivar of maize and a cultivar of cowpea (nonhost) were used as checks. All plants were harvested at maturity and susceptible maize (cv. 8338-1) was then planted in all pots. Emerged *S. hermonthica* plants were recorded weekly. At maturity, maize roots were washed and unemerged *S. hermonthica* plants counted. Average parasitism attributable to different soybean cultivars ranged from 10.2 to 2.2 attached parasites per pot. These results closely paralleled results in the laboratory and indicate laboratory selection can be effectively used to identify soybean cultivars useable in *S. hermonthica* control.

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IDENTIFICATION OF NON-HOSTS FOR CONTROL OF *STRIGA GESNERIOIDES*. A. Moors[1], D. K. Berner[2], and P. Van Damme[1]. [1]University of Gent, Coupure Links 653, B-9000, Gent, Belgium, and [2]International Institute of Tropical Agriculture, PMB 5320, Oyo Road, Ibadan, Nigeria.

Non-hosts that stimulate germination of *Striga* spp. seeds, resulting in the death of the parasites, are valuable controls; but, few have been reported for *S. gesnerioides*, a major pest of cowpea. The objectives of this study were to establish laboratory conditions for *S. gesnerioides* seed germination and then screen different plants for ability to stimulate germination. To do this, *S. gesnerioides* seeds were incubated on moist glass-fiber filter paper in darkness at 23 and 28 C for 7, 14, 21, and 28 days and then exposed to 0.5, 1, 1.5, and 2 g of cut roots of cowpea. The root pieces and parasite seeds were then incubated together at 28-30 C for 5 days, and germination was checked daily. Maximum germination was obtained with incubation at 28 C for 14 days followed by incubation with 1.5 g cut roots for 3 days. Using these conditions, 81 cultivars of 26 species were tested. Most of those with the ability to stimulate germination were indigenous African legumes; but, one cultivar of sorghum, a non-host, consistently stimulated high levels of germination.

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GERMINATION REQUIREMENTS OF *STRIGA ASPERA*. Y. D. Ndirpaya, D.K. Berner and L. J. Musselman. International Institute of Tropical Agriculture, PMB 5320, Oyo Road, Ibadan, Nigeria, and Old Dominion University, Norfolk, VA, U.S.A.

Striga aspera (Willd.) Benth. is widely distributed as a parasite of wild grasses in West Africa, but is not widely distributed on cereal crops. No information is available on *S. aspera* host range or germination requirements which may govern host range and distribution. To study this, germination of *S. aspera* seeds incubated for 7, 14, 21, and 28 days at 18, 23, 28 and 33 C was tested. Incubating *S. aspera* seeds at 28 C for 14 days, introducing a synthetic stimulant and then incubating at 28 C for 24 hr produced maximum germination. Under these conditions, 5 cultivars of maize, 4 of sorghum and 5 of cowpea were tested for their ability to germinate *S. aspera* seeds. To do this, 1 g of cut root pieces of 7-day-old seedlings were placed in a 2-cm-diam. aluminum-foil ring, centered on moistened filter paper in a petri dish. Incubated *S. aspera* seeds were introduced and germination was observed after 24 hr. Cut roots of all cultivars stimulated *S. aspera* seed germination. Maize and sorghum were also readily parasitized in pot studies, suggesting that other unknown factors must be involved in determining *S. aspera* field distribution.

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CROP-SPECIFIC STRAINS OF *STRIGA HERMONTHICA* IN NIGER. D.E. Hess, ICRISAT Sahelian Center, B.P. 12404, Niamey, Niger.

The obligate root hemiparasite *Striga hermonthica* is endemic to the semi-arid savannas of Africa. It is a serious agronomic pest in West Africa, where it attacks principally maize (*Zea mays*), sorghum (*Sorghum bicolor*) and pearl millet (*Pennisetum glaucum*). Crop-specific strains preferentially attacking the pearl millet or sorghum host have been reported in the region. Crop specificity of seven *Striga* populations from five widely separated locations in Niger (Maradi, Birni n'Konni, Bengou, Gueza, and Sadoré) was studied in 1992 and 1993 on four hosts: three pearl millet land races (Zongo Maradi, Heini Kirei Bengou, and Sadoré Local) and one sorghum land race (Bengou Local). Seed of *Striga* parasitizing pearl millet was harvested at each location; *Striga* parasitizing sorghum was obtained at Birni n'Konni and Bengou. Parasite emergence in outdoor pots infested with the sorghum-specific strains was higher on sorghum (21 *Striga* pot^{-1}) than on pearl millet (0.6 *Striga* pot^{-1}). The converse was true in pots infested with the pearl millet-specific strains: 19 *Striga* pot^{-1} on pearl millet and 0.6 *Striga* pot^{-1} on sorghum. Results of the agar gel assay suggested that for sorghum, the difference is attributable to increased germination of seeds of the sorghum-specific strains by the host. Increased infestation of pearl millet by millet-specific strains seems to be attributable to other mechanisms. Therefore at least two distinct *S. hermonthica* strains are present in Niger, one infesting primarily sorghum and the other pearl millet. Biochemical genetic traits of the seven *Striga* populations are being studied in the laboratory.

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CHEMICAL COMPOSITION OF *STRIGA* HERBAGE, AND ITS INTAKE AND DIGESTION BY SHEEP. S. Fernàndez-Rivera and D.E. Hess, ILCA and ICRISAT Sahelian Center, B.P. 12404, Niamey, Niger.

The hemiparasitic weed *Striga hermonthica* is a serious agronomic pest attacking sorghum and pearl millet in Niger. Hand pulling is an effective but little utilized method to reduce seed production and subsequent infestation and might be encouraged if farmers could benefit from pulled *Striga*. This study evaluated its chemical composition and voluntary intake and digestion of fresh *Striga* herbage by six sheep. Levels of whole plant crude protein (18.4 ± 3.8%) were similar to those found in forage legumes and higher in leaf than in stem tissues (23.0 vs. 8.7%). The concentration of N in *Striga* before flowering increased from 18.2 ± 1.9% to 29.8 ± 2.2% in response to moisture stress. *Striga*, compared to other feeds, had an unusually high ash content (20.4 ± 4.8%). High levels of tannins were found in leaf (11.7%), stem (14.6%) and inflorescence (8.7%), whereas high levels of biogenic silica were found only in leaf (6.3%). Cell wall contents, lignocellulose and lignin of ten pot-grown plants were 36.4 ± 4.9%, 27.8 ± 4.6% and 12.7 ± 3.2%, respectively. During the first two days of the feeding trial *Striga* was mostly rejected, but within a week it was readily eaten. The digestibilities of dry and organic matter were 49.3 ± 2.0% and 65.7 ± 2.8%, respectively. The consumption of digestible organic matter was 27.1 ± 5.0 g kg^{-1} $W^{.75}$, which is comparable to that of cereal crop residues. The use of *Striga* as basal feed appears to be limited by anti-nutritional compounds and low energy consumption. However, the high N and P levels in *Striga* warrant further evaluation of its potential as a protein source or for compost.

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PRUNUS TOMENTOSA, BIOINDICATOR FOR PLUM POX VIRUS. V. D. Damsteegt[1], H. E. Waterworth[2], G. I. Mink[3], W. E. Howell[3], and L. Levy[4], [1]USDA, ARS, Frederick, MD 21702, [2]USDA, ARS, Glenn Dale, MD 20769, [3]Washington State University-Prosser, Prosser, WA 99350, [4]USDA, ARS, Beltsville, MD 20705.

The standard bioindicator of plum pox virus (PPV) infection is Prunus persica (L.) Batsch cv GF305 (GF305). Other commonly used indicators are P. persica cv Siberian C (Sib C), and P. tomentosa Thunb. (Nanking Cherry). The efficacy of the three hosts as diagnostic indicators of plum pox infection was evaluated in a series of experiments conducted in the quarantine containment facility at Frederick, MD. Seedlings of the three hosts were graft-inoculated with eight different strains or isolates of PPV. Initial PPV symptoms appeared within 30 days after grafting as chlorotic banding along the main vein spreading along lateral veins from the leaf base upward, giving the appearance of a chlorotic oakleaf. Not all leaves on a branch were symptomatic, symptoms sometimes appearing on only lower leaves, middle leaves, or the newest leaves. Additional symptoms included chlorotic interveinal blotches in association with the oakleaf pattern, chlorotic patterns becoming necrotic on older leaves, and some leaf distortion. Symptoms were most diagnostic in P. tomentosa.

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DETECTION OF A PUTATIVE DIAGNOSTIC PROTEIN FOR OAK WILT DISEASE BY IMMUNOBLOT TECHNIQUE. C. Guggenbühl, J.J. Yu, S.L. Silverman, G.T. Cole, Department of Botany, University of Texas, Austin, TX 78713

Proteins were extracted from the xylem sap of live oaks *(Quercus fusiformis)* which showed the typical symptoms of infection with *Ceratocystis fagacearum* (oak wilt pathogen). The crude mixture was analyzed by silver stained SDS-PAGE (polyacrylamide gel electrophoresis). The protein profile obtained was compared to that of proteins extracted from oaks growing outside the endemic area. Detection of a 35 kilodalton (kDa) protein band correlated in 91.5% of the trees with presence of disease symptoms. The pathogen was cultured from 38% of the symptomatic trees. The 35 kDa protein was electroeluted from SDS-PAGE gels and 5µg aliquots were used to immunize BALB/c mice. The 35 kDa-specific polyclonal antibody obtained was used to detect the protein in xylem sap extracts by immunoblot analysis. Positive immunoblots correlated with disease symptoms in 96% of the tested live oaks. All control trees (outside endemic area) were negative by immunoblot analysis. Detection of the 35 kDa protein in xylem sap of oaks is useful for early diagnosis of *C. fagacearum* infection.

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DEVELOPMENT OF A PCR-BASED ASSAY TO IDENTIFY TILLETIA INDICA, CAUSAL AGENT OF KARNAL BUNT OF WHEAT. O. P. Smith, G. L. Peterson, R. J. Beck, M. R. Bonde, and N. W. Schaad. USDA, ARS, Frederick, MD 21702

A PCR-based assay to differentiate Tilletia indica from the morphologically similar pathogen T. barclayana is being evaluated. The technique uses total DNA extracted from fungal mycelium and a set of oligonucleotide primers developed from sequence analysis of a 1-kbp fragment cloned from a Dra 1 digest of the T. indica mitochondrial (mtDNA) genome. This fragment did not produce a detectable hybridization signal when probed with mtDNA of T. barclayana by Southern-blot hybridization (^{32}P) analysis. Tests have been conducted on 30 isolates each of T. indica and T. barclayana from a geographically diverse collection. Post-PCR analysis by agarose gel electrophoresis and ethidium bromide staining demonstrated that the 800-bp PCR product was consistently amplified from mycelial samples of T. indica but not T. barclayana. The specificity of the product was confirmed by Southern-blot hybridization using the cloned mtDNA fragment. All T. indica samples produced positive hybridization signals, whereas 28 of the T. barclayana samples were negative. Two of the T. barclayana samples produced faint signals indicating a small percentage of T. barclayana populations may contain the sequence in low copy number, or that partial primer sequence homology exists.

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YIELD LOSSES DUE TO LEAF RUST IN NEAR-ISOGENIC WHEATS FOR *Lr34*. J. Huerta-Espino, and R. P. Singh; INIFAP, Apdo. postal 515, Cd. Obregon, Son. 85000; and CIMMYT, Apdo. postal 6-641, 06600, México, D. F.

Leaf rust caused by *Puccinia recondita* f. sp. *tritici* is an important disease of irrigated wheat (*Triticum aestivum* L.) in the north-western México. Jupateco 73R, which carries *Lr34*, and Jupateco 73S, which lacks it, are reselections from the heterogeneous Mexican cultivar Jupateco 73. Gene *Lr34* confers a slow rusting resistance in Jupateco 73R with Mexican *P. r. tritici* pathotype TBD/TM, whereas, Jupateco 73S is highly susceptible. Jupateco 73R and Jupateco 73S were used to estimate the yield loss due to leaf rust in the presence and abscence of *Lr34*. The experiment was established during the 1992-93 growing season with three different planting dates and disease free treatments were included. An artificial leaf rust epidemic was created using race TBD/TM. Leaf rust severity reached up to 100% in Jupateco 73S 30 days after the spreaders were inoculated; whereas, a maximum of 40% leaf rust was recorded for Jupateco 73R close to maturity. Results showed average losses of 16% due to leaf rust in Jupateco 73R and 73% in Jupateco 73S. In the protected plots, Jupateco 73R yielded on an average 5% less, which could be due to the genetic association of *Lr34* with leaf tip necrosis of adult plants.

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NUTRITIONAL CHARACTERIZATION OF NATURAL MICROFLORA OF APPLE AND ITS POSSIBLE ROLE IN SELECTION OF BIOCONTROL AGENTS. W. J. Janisiewicz, USDA-ARS-Appalachian Fruit Research Station, Kearneysville, WV 25430

Microbial colonization of wounded Golden Delicious apple was studied from early August to mid-September (harvest time) in an abandoned orchard. Fruit samples were collected at weekly intervals and the composition of the microflora was determined. In the early weeks, yeast populations predominated with occasional bacteria; however, as harvest approached, yeast were isolated almost exclusively. Diversity of the yeast populations declined toward harvest. Based on utilization of more than 50 carbon sources, the yeasts were grouped into four clusters. Preliminary evaluation of the yeast for biocontrol potential against Penicillium expansum indicated the presence of effective antagonists in each cluster at each sampling time. Combining antagonists from different clusters should facilitate the development of more effective biocontrol by using mixtures of antagonists with a higher level of coexistence.

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BIOLOGICAL CONTROL OF POSTHARVEST PATHOGENS OF APPLE WITH ANTAGONISTIC MICROORGANISMS. P.L. Sholberg, P. Haag, J. Bechard, A. Marchi and R. Utkehede, Agriculture Canada, Research Station, Summerland, B.C., Canada V0H-1Z0.

Ninety-seven bacterial and one yeast isolate were screened against *Penicillium expansum* on mature harvested apple fruit at 5, 10 and 20°C. Ninety-one of the isolates were originally from internal apple fruit tissue and considered to be bacterial endophytes. The remaining isolates consisted of four *Pseudomonas spp.*, *Enterbacter aerogenes* and an unidentified yeast. Fruit replicates were injured and inoculated with the test microorganism followed 2 hr later by fungal conidia and incubated until rot developed in the control fruit. Ten, 4 and 5 endophytes significantly reduced decay caused by *P. expansum* at 5, 10 and 20°C, respectively. *E. agglomerans*, all four *Pseudomonas spp.* and the yeast isolate inhibited decay by *P. expansum* at 10°C and at 5°C the yeast isolate and *Pseudomonas* isolate 1100#6 completely prevented fungal growth.

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BIOLOGICAL CONTROL OF POSTHARVEST DISEASES OF CITRUS WITH ESC-10 AND ESC-11. L. F. Yourman and S. N. Jeffers. EcoScience Corporation, 377 Plantation Street, Worcester, MA 01605.

ESC-10 and ESC-11 are naturally occurring, epiphytic bacteria being developed as biocontrol agents for postharvest disease management. Experiments were conducted to determine if these agents were effective against the postharvest pathogens *Penicillium digitatum* (*Pd*), *P. italicum* (*Pi*), and *Geotrichum candidum* (*Gc*) on citrus fruit. Inoculum of each species was conidia, usually from several different isolates. Field-run citrus fruit (free from postharvest treatment) was obtained from Florida, California, and the Bahamas. Individual fruits were wounded, inoculated with 10^4-10^6 conidia/ml, and treated with 10^7-10^9 (usually 10^8) cfu/ml of bacteria. Fruit was incubated at 15 or 25 C for 1-4 wk. Both ESC-10 and ESC-11 significantly controlled postharvest citrus diseases. On Valencia oranges inoculated with a mixture of *Pd* and *Pi*, disease incidence was 78, 36, and 25% on untreated fruit or fruit treated with ESC-11 or ESC-10, respectively. On lemons inoculated with *Pd* + *Pi*, disease incidence was 75, 33, and 18% on untreated fruit or fruit treated with ESC-11 or ESC-10, respectively. On lemons, ESC-10 reduced disease incidence due to *Pd* from 100% to 1%, that due to *Gc* from 75% to 45%, and that due to *Pd* + *Gc* from 60% to 15%. Wettable powder formulations of ESC-10 and ESC-11 have been produced and are being evaluated in citrus packing houses in Florida and California.

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REDUCTION OF STORAGE ROTS OF FRUITS AND VEGETABLES BY COMBINING UV-C APPLICATION AND BIOCONTROL STRATEGIES. C. Stevens[1] V. A. Khan,[1] J. Y. Lu,[1] C. L. Wilson,[2] P. L. Pusey,[3] M. K. Kabwe,[1] Y. Mafolo,[1] J. Liu,[1] E. Chalutz[4], and S. Droby[4]. [1] GWC Agric. Expt. Station Tuskegee University AL 36088. [2]USDA/ARS, NAA Appalachian Fruit Research Station, Kearneyville WV 25430. [3]USDA/ARS,

Southeastern Fruit and Tree Nut Research Lab., Byron GA 31008. [4]Volcani Center, Bet Dagan Israel.

Application of low dosages of ultraviolet light (254 nm, UV-C) has been shown to reduce the incidence of brown rot (*Monilinia fructicola*) of peaches, green mold (*Penicillium digitatum*) of tangerines and Rhizopus soft rot (*Rhizopus stolonifer*) of tomatoes and sweetpotatoes, resulting from both field infection and artificial inoculation. In most studies, application of postharvest fungicide (PF) was better than UV-C treatment. In this study effectiveness of combining a biocontrol agent, *Debaryomyces hansenii* (BC) with low UV-C dose for postharvest disease control was investigated. When these commodities were treated with BC 3 days after UV-C treatment the reduction of storage rots was more effective than when UV-C was used alone. For example, the percent brown rot infection of artificially inoculated Elberta peaches 36 hr after inoculation of the untreated control, UV-C treated, BC treated, UV-C + BC and benlate treated peaches were 100, 55, 67, 12 and 12 %, respectively. The efficacy of UV-C + BC was similar to when PF were used alone, indicating that an integration of UV-C treatment and BC can reduce storage rot to the levels of commercial PF treatment.

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EFFECTS OF POLYMERIC SEED COATING ON STAND ESTABLISHMENT AND SEED MAIZE DECAY CAUSED BY *PYTHIUM* SPP. B. Arias, D.C. McGee, and J.S. Burris, Seed Science Center and Department of Plant Pathology and Agronomy, Iowa State University, Ames, IA. 50011.

A field study was conducted to determine the effects of seed coating polymers either alone, or in combination with the standard captan seed treatment on stand establishment and infection of non-germinated maize seeds by soilborne *Pythium* spp. The polymers Sacrust, Certop and Chitosan in combination with captan were as effective as captan alone in reducing seed infection. Average stands at 12, 16, 20 and 25 days after planting (DAP) for Certop + captan, captan and Sacrust + captan were 81, 78 and 75% respectively, and significantly greater (P=0.05) than those in the rest of the treatments. Polymer alone had no effect in reducing *Pythium* spp. population in the soil when compared with the untreated seeds at 4, 8, and 15 DAP. However, *Pythium* growth was reduced on water agar medium amended with 2% Chitosan. The addition of Chitosan to captan seed treatment has the potential to improve the efficacy of captan under field conditions and provide a mechanism for reducing fungicide dosage in maize seed treatment.

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Assessing risk of seed transmission of *Erwinia stewartii* in maize.
C. C. Block, D. C. McGee, and J. H. Hill. North Central Regional Plant Introduction Station, Seed Science Center, and Department of Plant Pathology, Iowa State University, Ames, IA 50011.

Many countries permit import of maize seed only if certified free of *E. stewartii* (ES). Risk of ES transmission through seed was assessed by correlating data on percent infected seed with that on rates of seed transmission. Transmission data were collected by assaying plants grown from seed lots with incidence of infected seed ranging from <1% to 80%. No positive plants were detected among 3,000 tested from low (<5%) infection seed lots. One positive plant was found among 8,600 tested from moderate (10-15%) infection seed lots. Twenty-eight positives were found among 42,000 tested from high (25-80%) infection seed lots. In 1992, the correlation between percent diseased leaf tissue and percent infected seed produced by those plants was examined. Seed was harvested from plants in 6 severity classes: 0, 5, 10, 25, 35 and 50% leaf area killed. No seed infection was detected in the 0, 5, and 10% severity classes. Incidence of infected seed was low in the higher classes with 0.2%, 1.2%, and 1.8% in the 25, 35, and 50% classes, respectively. Examination of 25 additional maize genotypes, with similar results, suggests that the risk of spreading ES through maize seed is essentially zero for seed obtained from plants with <50% diseased leaf tissue.

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EFFECTS OF SEED COATING FORMULATION NO. 17 ON THE DEVELOPMENT OF POWDERY MILDEW IN WHEAT. Jin-Yu Li and Zhen-Sheng Kang, Seed Coating Chemicals Centre, Beijing Agricultural University and Department of Plant Protection, Northwest Agricultural University, respectively, Beijing (PEK) 100094, P. R. China

Seedlings from wheat seeds coated with seed coating formulation (SCF) No. 17 which contains fungicide triadimenol and other chemicals were inoculated with a virulent isolate of *Erysiphe graminis* f. sp. *tritici*. Observations by light and electron microscopy showed that SCF No. 17 increased frequency of malformed appressorium and decreased successful infection considerably. Only primary haustorium was formed in each infection site and usually became malformed. There were some electron-dense materials in extrahaustorial matrix. Mycelial growth and branching were strongly inhibited and mycelial tips were swollen obviously. Accumulation of small vesicles between mycelial and haustorial plasmalemma and their walls made their walls thicken irregularly. Necrotic host cells in infection sites were similar to hypersensitive necrosis of resistant cultivar.

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TEST METHODS Emily J. A. Blakemore and James C. Reeves, Molecular Biology & Diagnostics Section, National Institute of Agricultural Botany, Cambridge CB3 OLE, UK.

Erwinia stewartii (Dye) causes stewart's wilt, a seed transmitted bacterial disease of maize. This pathogen is of quarantine importance and responsible for serious crop losses in many tropical countries. At present, seed health tests are time consuming and lack sensitivity. This paper describes the development of a new, sensitive PCR test to detect E. stewartii and its comparison with two other methods (Selective agar medium and ELISA) for detecting E. stewartii in maize seed.

The polymerase chain reaction (PCR) with arbitrary primers was used to identify a DNA fragment for use as a probe to detect E. stewartii in maize seed. Specific nested primers for E. stewartii were designed from the partial sequence of the E. stewartii DNA probe. These primers were highly specific and did not cross-react with other erwinias or a wide range of bacterial isolates obtained from maize seed samples. This test takes approximately 3-4 hours, starting with bacterial cells to detecting an amplified product on an agarose gel. The sensitivity of the nested PCR is 50 fg of E. stewartii DNA which is equivalent to 12.5 bacterial cells.

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THE DETECTION OF ASYMPTOMATIC SOYBEAN SEEDS INFECTED WITH *ASPERGILLUS AND PENICILLIUM* SPP USING ULTRASOUND ANALYSIS. Ron Walcott, M.K Misra, and D.C. McGee, Iowa State University, Ames, IA.

Ultrasound analysis, which utilizes the impact response method to generate sound waves representing the seed's acoustic properties, was used to detect diseased soybean seeds. Soybean seeds from the same lot were incubated at 90% relative humidity and 25° C for different periods of time, in order to generate different degrees of infection with *Aspergillus and Penicillium* spp. The seeds were dropped 10 cm onto a piezoelectric transducer and the sound waves were measured electronically. Analysis of the slope, width and peak values of the sound waves consistently distinguished between seeds with different levels of fungal infection: the peak values decreased while the slope and width values increased with increasing infection levels. Germination percentages of the seedlots also decreased as infection levels increased, and consequently, a significant correlation was observed between the sound wave parameters and the germination percentages. This study demonstrates the potential for ultrasound analysis to be a valuable tool in the seed industry, for the detection and elimination of asymptomatic diseased seeds.

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EFFECTS OF SOIL AMENDMENT WITH ORGANIC WASTES ON PYTHIUM ROOT ROT AND GROWTH OF SUGARCANE. N. Dissanayake, J. W. Hoy, and G. A. Breitenbeck. Dept. Plant Path. and Crop Phys., La Agric. Exp. Sta., La. State Univ. Agric. Center, Baton Rouge, LA 70803.

Municipal solid waste and cotton gin trash composts, sugar mill filtercake, and sewage sludge were evaluated as soil amendments to suppress Pythium root rot in sugarcane. Sewage sludge reduced Pythium infection and disease severity and generally increased plant growth in pathogenicity tests and in Pythium infested soils. Gin trash compost and filtercake enhanced plant growth but generally did not reduce infection. Effects of yard waste composts were inconsistent. Municipal waste composts did not increase plant growth or reduce disease severity. Beneficial effects were not evident with steam sterilized organic wastes suggesting the involvement of biological agents or heat labile compounds in disease suppression. Enhanced plant growth resulting from organic waste amendments was not caused by changes in soil physical properties or nutritional effects.

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RELEASE OF BIOLOGICALLY ACTIVE CHEMICALS BY ROOT BORDER CELLS. Zhu Yanmin & Martha C. Hawes, Departments of Plant Pathology & Molecular & Cellular Biology, UA, Tucson AZ 85721

Plants of most species produce large numbers of root "border" cells which are released from the root tip into the rhizosphere. Border cells exhibit selectivity in their interactions with fungal and bacterial pathogens and symbionts, and chemotactic recognition of border cells by *Agrobacterium tumefaciens* is important in pathogenesis of soil grown pea plants. Based on such observations, we have proposed that border cells function to regulate the balance of beneficial and pathogenic microorganisms in the vicinity of the growing root. One way this could occur is by the production by border cells of biologically active chemicals needed for microbial pathogenesis and symbiosis. The objective of this study was to test the hypothesis that border cells are a source of chemicals required to induce *A. tumefaciens* virulence (*vir*) genes and *Rhizobium leguminosarum* nodulation (*nod*) genes. Living border cells from pea roots were washed and incubated with strains carrying *virE*::*lacZ* or *nodD*::*lacZ* reporter genes. *NodD* from *R. leguminosarum*, but not from *R. meliloti*, was induced by chemicals released by border cells of pea, in a time- and dosage-dependent manner. Induction by border cells was 3 fold higher at 10°C than at 28°C, and the presence of bacteria stimulated release of *nod* inducing chemicals by border cells. Pea border cells also released chemicals that induce *A. tumefaciens vir* genes.

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POPULATION DYNAMICS OF ENTEROBACTER CLOACAE AUXOTROPHS IN SPERMOSPHERE. D. P. Roberts, USDA, ARS, Biocontrol of Plant Diseases Laboratory, Beltsville, MD 20705.

Auxotrophs of Enterobacter cloacae strain 501R3 were screened to find strains capable of suppressing Pythium ultimum on cucumber but were non-persistent in soil. Auxotrophs of E. cloacae were generally capable of proliferation in spermosphere. However, populations of one E. cloacae auxotroph, strain A-46, did not increase in any spermosphere when approximately 10^4 colony-forming units (cfu) were applied to the seed. Strain 501R3 showed significant increases ($P = 0.05$) in all the spermospheres tested. In addition, strain A-46 decreased to undetectable levels within 30 days after applying 10^8 cfu to cucumber seeds. However, strain 501R3 was detected at 10^5 cfu greater than 30 days after applying 10^8 cfu to cucumber seed. Strain A-46 was an effective biocontrol agent of P. ultimum on cucumber in preliminary biocontrol experiments. Enterobacter cloacae strain A-46 shows promise for use in environmental containment strategies where occupancy of the spermosphere by the biocontrol agent for short durations is required.

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SPATIAL DESCRIPTION OF ENCYSTED ZOOSPORES ON ROOTS: INFLUENCE OF SAMPLING INTENSITY. L. M. Dandurand & G. R. Knudsen, Plant Pathology Division, University of Idaho, Moscow, ID 83843.

Zoospores of *Pythium ultimum* var. *sporangiiferum* were applied to pea roots in sand, at three densities (500, 2000, 8000 zoospores/g). After 3 hr, roots were removed, excised, and stained with Trypan blue. Encysted zoospores on the visible root surface were completely enumerated in 83-µm x 83-µm sample units (approximately 2500 units per root). Mean and variance of cysts/sample unit were calculated, and geostatistics was used to describe spatial structure of cysts. A computer program randomly selected samples of 63, 125, 250, 500, 1000, or 2000 sample units/root. The same statistics were calculated. For 500 or 2000 zoospores/g, encystment was random or uniform at all levels of sampling intensity. For 8,000 zoospores/g, when 250 or fewer units were sampled, aggregation of cysts was not apparent. Accuracy and reliability of estimates of spatial structure increased as sample numbers increased. For 500 or 1000 units sampled, mean and variance were accurately estimated, but the amount of variation attributed to spatial structure was either underestimated or overestimated. Not until 2000 units were sampled was an aggregated spatial structure evident. At this scale, with random sampling, a sample size of 2000 (approximately 80% intensity) was necessary to adequately describe the spatial structure of encysted zoospores.

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HORIZONTAL AND VERTICAL DISTRIBUTION OF *MONOSPORASCUS CANNONBALLUS* IN CULTIVATED AND NATIVE SOILS IN ARIZONA. M.E. Stanghellini, S.L. Rasmussen, and D. H. Kim. Department of Plant Pathology, University of Arizona, Tucson, AZ 85721.

A soil extraction technique (Phytopathology 82:1115) was used to estimate the population and distribution of ascospores of *Monosporascus cannonballus*, a destructive root pathogen of cantaloupes, in native desert and cultivated soils. Populations, in 43 commercial cantaloupe fields, ranged from 1 to 4.5 ascospores/g of soil (0 to 10 cm soil depth) and were uniformly distributed both horizontally and vertically (0 to 25 cm soil depth). These results support the findings of a previous study (Plant Dis. 76:766-771). In native desert soils (0 to 10 cm soil depth), populations ranged from 0.15 to 1.9 ascospores/g of soil. Populations in dry washes ranged from 0.35 to 3.4 ascospores/g of soil and were uniformly distributed within the 0 to 25 cm soil depth. These results indicate that the fungus is indigenous in Arizona and the uniform distribution of the fungus in soil may account for the uniform distribution of the disease in problem fields.

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SELECTION OF STABLE ANTIBIOTIC-TOLERANT *PYTHIUM* SP. AND FUNGICIDE-TOLERANT *THIELAVIOPSIS BASICOLA* STRAINS FOR SOIL ECOLOGICAL STUDIES. B. L. Candole and C. S. Rothrock, Department of Plant Pathology, Univ. of Arkansas, Fayetteville, AR 72701.

Indicator strains facilitate the monitoring of soil-inhabiting pathogens in ecological studies. Mutant strains of *Pythium* sp. with stable tolerance to kanamycin A were selected by means of sublethal enrichment. UV-irradiation treatment of *Thielaviopsis basicola* endoconidia at 254 nm was used to select mutant strains with stable tolerance to benomyl. The stability of these tolerant strains was determined by dilution plating of soils naturally infested with *Pythium* spp. or *T. basicola* which also had been artificially infested with their respective kanamycin-tolerant and benomyl-tolerant strains. Background populations of *Pythium* spp. and *T. basicola* were inhibited but not the mutant strains when plated on the appropriate selective media, P_5ARP + kanamycin and TB-CEN + benomyl, respectively. The usefulness of these marked strains in the study of soil suppressiveness associated with sustainable agriculture practices will be discussed.

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A NEW VIRUS INFECTING SORGHUM IN KANSAS. D. L. Seifers, Kansas State University, Agricultural Research Center-Hays, Hays, Kansas 67601

Sorghum plants from Ellis County Kansas, having severe mosaic symptoms, were infected with a mechanically transmissible virus. The virus was negative in enzyme-linked immunosorbent assay against antiserum to maize dwarf mosaic virus, sugarcane mosaic virus strain MDMV-B, Johnsongrass mosaic virus, wheat streak mosaic virus, maize chlorotic mottle virus, Agropyron mosaic virus, and brome mosaic virus. Electron microscope observation of virions, in leaf dips from sorghum infected 9 days previously, revealed flexuous rods of approximately 10 nm in diameter and a modal length of 422 nm. The capsid from purified virus had a relative molecular weight of 27 KDa by sodium dodecyl sulfate polyacrylamide electrophoresis. The glyoxal modified viral nucleic acid comigrated with a 7.40 kb RNA marker (2.5×10^6 Da) in 1% agarose gels. The taxonomic position of the virus and its potential threat to sorghum production remain to be determined.

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PSOROSIS-LIKE AGENT FOUND IN HEALTHY AND RIO GRANDE GUMMOSIS AFFECTED TREES IN THE INDIAN RIVER CITRUS AREA. R.F. Lee[1], C.A. Powell[2], R.R. Pelosi[2] and R.M. Sonoda[2]. [1]Univ. of FL, CREC, Lake Alfred, FL 33850 and [2]AREC, Ft. Pierce, FL 33454.

Rio Grande Gummosis (RGG)-affected trees start gumming and bark scaling anytime after 3 yr of age. The cause of the disorder is unknown. Budwood was collected from healthy-appearing and RGG-affected 13-40-yr old registered grapefruit trees from five locations in the Indian River area. Madam Vinous sweet orange indicator seedlings grown under a cool-night warm-day regime, produced oakleaf patterns and slight vein-clearing after inoculation with budwood from both healthy and RGG-affected trees. These data raise concern about the role of psorosis in RGG, its avoidance of detection in the Florida budwood certification program, and its possible field spread.

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SEASONAL DETECTION OF GRAPEVINE LEAFROLL ASSOCIATED VIRUSES IN GREENHOUSE AND TISSUE CULTURE GROWN GRAPEVINES Judit Monis*, Richard K. Bestwick*, and James A. Stamp**. * Agritope, Inc., Research and Development Department; ** Vinifera, Inc., 8505 SW Creekside Place, Beaverton, OR 97005

The best plant tissue and season for the detection of grapevine leafroll associated viruses (GLRaV) I, II, and III in greenhouse and tissue culture grown infected material was investigated using ELISA. The results indicate that the bottom portion of vegetatively growing stems, and petioles have the highest concentration of virus. Old and symptomatic leaves have higher titers of virus than young leaves. The difference in the virus concentration between young and old tissue prompted us to design experiments to determine the distribution of these viruses in vegetatively grown and dormant tissue. Our data indicates that GLRaV I, II, and III are distributed unevenly in infected tissue,

although the highest titers of virus are generally found in the lower portion of the plant. When explants from individual nodes were propagated *in vitro*, high virus titers were detected in every sample, but the distribution of the virus was similar to the originally tested plant. An experiment is in progress to determine the effect of different culture media in the titer of GLRaV I, II, and III in *in vitro* grown plants.

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SURVEY TO DETERMINE THE OVERWINTERING HOSTS OF CUCUMBER MOSAIC VIRUS IN ALABAMA. M. Andrianifahanana and E. J. Sikora. Department of Plant Pathology, Auburn University, AL 36849.

During 1992 and 1993, a multivirus epidemic significantly reduced fresh market tomato production in North Alabama. Cucumber mosaic virus (CMV) alone, or in combination with potato virus Y (PVY) and/or tobacco etch virus (TEV), was responsible for the crop failure. In the spring of 1994, a survey was conducted to determine the overwintering hosts of CMV in this tomato production region of the state. Three tomato fields that sustained damage due to viruses in 1993 were selected for the study. Fields were sampled on February 21, 28 or March 14, 1994. Plants were collected randomly along a 100 meter long, 10 meter wide, area bordering each field. Over 20 potential viral hosts were collected among the three fields. Samples were tested for CMV using the enzymed-linked immunosorbent assay technique (ELISA). CMV was detected in *Cardamine hirsuta* (hairy bittercrest), *Geranium carolinianum* (Carolina geranium), *Rumex crispus* (curly dock), *Allium vineale* (wild garlic), *Lamnium amplexicaule* (common henbit), *Lactuca serriola* (wild prickly lettuce), *Conyza* canadensis (marestail), *Capsella bursa-pastoris* (shepardspurse) and *Stellaria media* (common chickweed).

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A NEW DISEASE OF DIAKON RADISH (RAPHANUS SATIVUS cv. LONGIPINNATUS) CAUSED BY TURNIP CRINKLE VIRUS. M.L. Putnam and B.B. Reddick, Oregon State University, Corvallis, OR; and University of Tennessee, Knoxville, TN.

In 1993, diakon radish plants from three fields in two different counties in Oregon exhibited stunting, foliar distortion, mosaic, and black spots on petioles, leaves, and stems. When examined with transmission electron microscopy sap from affected plants appeared to contain icosahedral virus-like particles. Inoculation to Brassica campestris var perviridis resulted in severe stunting, mottling and eventual death of the plants. Sap from symptomatic plants reacted positively with antiserum to turnip crinkle virus (TCV). There were no apparent differences in serological reaction with immunoblotting or ELISA between isolates obtained from radishes from two different fields. However, differences were observed in host symptomatology. This appears to be the first report of the occurrence of TCV in naturally infected crucifers in the United States.

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SCREENING FOR RESISTANCE TO TOBACCO ETCH VIRUS IN PEPPER. I. Ariyaratne, H.A. Hobbs, R.A. Valverde, and L.L. Black. Department of Plant Pathology and Crop Physiology, Louisiana Agricultural Experiment Station, Louisiana State University Agricultural Center, Baton Rouge, 70803

Screening trials were conducted using 20 isolates of tobacco etch virus (TEV) collected from different geographic locations throughout the United States, Central America and the Caribbean. Fifteen pepper (Capsicum spp) cultivars and lines were selected for mechanical inoculation experiments in the greenhouse. These cultivars and lines have been reported to have resistance to some isolates of TEV and potato virus Y. Cultivars Casca Dura, and Yolo Wonder were susceptible to all TEV isolates tested. However, Delray Bell and Agronomico peppers were resistant to 13 isolates showing no symptoms upon inoculation. Symptoms of susceptible lines ranged from mild mosaic to severe leaf deformation and necrosis. Symptomless plants were tested by the enzyme-linked immunosorbent assay (ELISA) for the presence of TEV.

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EFFECTS OF MIXED INFECTIONS OF TABASCO MOTTLE VIRUS AND POTYVIRUSES IN PEPPER. D.J. DUFRESNE, R.A. Valverde and H.A. Hobbs. Department of Plant Pathology and Crop Physiology, Louisiana Agricultural Experiment Station, Louisiana State University Agricultural Center, Baton Rouge 70803.

Tabasco mottle virus (TaMV) is a beetle transmitted comovirus found in tabasco pepper in Central America and the Caribbean. This virus is often found in plants infected with pepper mottle virus (PeMV) or tobacco etch virus (TEV). Mechanical inoculations to Capsicum annuum and C. frutescens cultivars were conducted to determine the effects of mixed infections. Single infections of Yolo Wonder by TaMV were symptomless. PeMV or TEV alone induced mosaic only. However, TaMV in combination with either PeMV or TEV induced severe mosaic. Similarly, single infections of tabasco pepper (cvs. Tabasco and Greenleaf Tabasco) with TaMV consisted of mild mosaic. PeMV or TEV alone induced mosaic. However, TaMV in combination with either PeMV or TEV induced severe mosaic and systemic necrosis. TaMV could become a limiting factor in tabasco pepper production in these regions.

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CUCUMBER MOSAIC VIRUS (CARNA-5) ASSOCIATED WITH RINGSPOT AND MOSAIC DISEASE OF AJUGA REPTANS. J. R. Fisher and S. T. Nameth, Department of Plant Pathology, The Ohio State University, Columbus, OH 43210.

Bugleweed (Ajuga reptans L.) is a perennial ground cover commonly grown throughout temperate climates. In the summer of 1993 Bugleweed samples displaying ringspot and mosaic symptoms were analyzed for virus infection using viral-associated double stranded ribonucleic acid analysis (dsRNA). Results indicated the presence of cucumber mosaic virus (CMV) with an associated satellite RNA. Indicator hosts, serology and dsRNA analysis with a known isolate of CMV (CARNA-5) confirmed that the virus associated with the ringspot and mosaic disease of Bugleweed was indeed CMV (CARNA-5). Results indicated that the satellite RNA, CARNA-5, may only be expressed in selected Bugleweed cultivars, eg. 'Royalty'. This is the first report of CMV associated with Bugleweed in the United States and the first report worldwide of CMV (CARNA-5) associated with Bugleweed.

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TWO GEMINIVIRUSES ASSOCIATED WITH TOMATOES IN CENTRAL AMERICA. M. K. Nakhla[1], M. D. Maxwell[1], S. H. Hidayat[1], D. R. Lange[1], A. O. Loniello[1], M. R. Rojas[1], D. P. Maxwell[1], E. W. Kitajima[2], A. Rojas[3], P. Anderson[3], and R. L. Gilbertson[4]. [1]Univ. of Wisconsin, Madison, WI 53706, [2]Univ. de Brasília, Brasilia, DF, Brasil, [3]Univ. Agricola Nacional, Managua, Nicaragua, and [4]Univ. of California, Davis, CA 95616.

Tomatoes in Central America often have severe virus-like symptoms of yellow mottle and leaf crumple. Geminivirus DNA-A and -B fragments were amplified from tomatoes with these symptoms from Costa Rica (CR), Guatemala (GA), Honduras (HON), and Nicaragua (NIC) with degenerate PCR primers for whitefly-transmitted geminiviruses (Plant Dis. 77:340-347). The DNA-A PCR fragments were cloned and the common region and part of the AC1 and AV1 ORFs were sequenced. The common region sequence identities among the tomato geminiviruses (TGV) from GA, HON, and NIC were >92%; and the common region sequence of TGV-GA was 64%, 68%, 59%, and 86% identical to those of TGV-CR, tomato mottle virus from Florida, tomato leaf crumple virus from Mexico, and squash leaf curl virus (SqLCV-R) from CA. TGV-GA, -HON, and -NIC are considered isolates of one virus and members of the SqLCV phylogenetic cluster, whereas TGV-CR is distinct from these isolates and not closely related to other characterized TGV's.

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ABNORMAL VIRION MORPHOLOGY IN TWO PARTIALLY DEFECTIVE ISOLATES OF IMPATIENS NECROTIC SPOT VIRUS. R.H. Lawson, M.M. Dienelt, and H.T. Hsu. USDA, ARS, USNA, FNPRU, Beltsville, MD 20705.

Infections of non-defective Tospoviruses contain double membrane-bounded virions (DMV) in the cytoplasm that fuse to form mature, single membrane-bounded virions (SMV) in swollen membraneous cisternae. Two partially defective variants of impatiens necrotic spot virus, INSV HT-1 and INSV HT-2, produced abnormal virions that failed to fully mature. Infections of INSV HT-1 and HT-2 in *Impatiens* 'Accent Salmon' were compared to those of a non-defective isolate, INSV B. In INSV HT-1 infections, only a single membrane could be clearly distinguished enveloping cytoplasmic virions whereas membranes were not distinguished around virions in cisternae. INSV HT-1 infections contained electron-dense masses but not striated nucleocapsids characteristic of INSV infections. The former did not react with antiserum to nucleocapsid (N) protein of INSV in immunogold labelling tests and may be another manifestation of the defects in INSV HT-1. In INSV HT-2 infections, mature SMVs were formed, but DMVs were observed more frequently. Many DMVs had diffuse fibrillar cores and apparently degenerated before maturation. In immunogold labelling tests, antiserum to N protein of INSV reacted with striated nucleocapsids and SMVs in INSV HT-2 and INSV B infections. DMVs were erratically labelled. Variants of INSV show different membrane (INSV HT-1) and core (INSV HT-2) abnormalities that appear characteristic of specific isolates.

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CELLULAR MECHANISMS REGULATING CIRCULATIVE TRANSMISSION AND APHID VECTOR SPECIFICITY OF SOYBEAN DWARF LUTEOVIRUSES. F.E. Gildow, V.D. Damsteegt, O.P. Smith, and S.M. Gray, Dept. Plant Pathology, Penn State University, University Park, PA 16802; USDA-ARS, Bldg. 1301, Fort Detrick, Frederick, MD 21702; and USDA-ARS, Dept. Plant Pathology, Cornell University, Ithaca, NY 14853.

Three aphid species, *Aulacorthum solani* (As), *Acyrthosiphum pisum* (Ap), and *Myzus persicae* (Mp) were compared for their ability to transmit a Japanese isolate of soybean dwarf virus (SbDV-Y), and a similar SbDV-like luteovirus (Va20) isolated from clover in Virginia. Aphids acquired virus by a 24 hr feeding on membranes of each purified virus at 100 µg/ml. This was followed by a 24 hr inoculation feeding on clover or soybean. The percentage of single aphids of As, Ap, and Mp transmitting SbDV-Y following membrane feeding was 70, 0, and 0 %, respectively. The percentage of As, Ap, and Mp transmitting Va20 was 0, 60, and 80 %. Ultrastructural examination of hindgut tissues of the 3 aphid species verified acquisition of SbDV-Y and Va20 by all 3 species. Sites determining vector-specificity were identified at the accessory salivary gland where only the transmitted virus was observed to penetrate the surrounding basal lamina and cell membrane, and be transported to the salivary duct.

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A NOVEL METHOD OF TOSPOVIRUS ACQUISITION BY THRIPS. W. B. Hunter and H. T. Hsu. USDA, ARS, USNA, Floral and Nursery Plants Research Unit, Beltsville, Maryland 20705.

A feeding method for improved acquisition of tospoviruses by thrips vectors has been developed. Three day old larvae of the western flower thrips, Frankliniella occidentalis (Pergande), were caged in a cylindrical clear plastic tube with Parafilm covering both ends. Virus preparations extracted from infected Nicotiana benthamiana leaf tissues were given one cycle of differential centrifugation and were placed above the surface of a membrane on the cage and covered with Parafilm. After a 24 hr acquisition access period at room temperature, thrips were placed on green bean pods in a container until they became adults. Ten day old adults were tested. Thrips that fed on sap from infected N. benthamiana, had a significantly greater number of ELISA positive individuals ranging from 1.3 to 2.5 fold increase over groups fed on symptomatic leaves or sap of infected Datura stramonium. Acquisition access feeding on infected N. benthamiana leaves resulted in no thrips acquiring virus from the plant and high insect mortality.

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BACILLIFORM DNA VIRUSES OF YAMS (*DIOSCOREA* SPP.) R.W.Briddon*, S.Phillips@, A.Brunt@ and R.Hull*. *Dept. of Virus Research, John Innes Institute, Colney Lane, Norwich, NR4 7UH, UK. @ Horticultural Research International, Littlehampton, West Sussex, BN17 6LP, UK.

Dioscorea species are cultivated for their underground tubers and are a staple food crop in the Caribbean, West Africa and Pacific regions. Bacilliform virus particles have been identified in yams and are associated with interveinal leaf chlorosis and discoloration of yam tubers. Evidence will be presented which identifies these viruses as members of the recently established bacilliform DNA (BADNA) virus group. The genetic variability of the viruses and their relationship to previously characterised BADNA viruses will be discussed.

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SYMPTOM DEVELOPMENT IN RELATION TO SEED TRANSMISSION OF BARLEY STRIPE MOSAIC VIRUS. Michael C. Edwards. USDA-ARS Cereal Crops Research Unit, Northern Crop Science Lab., Fargo, N.D. 58105.

Non-seed-transmissible and poorly seed-transmissible isolates of BSMV exist despite the fact that BSMV relies exclusively on seed transmission for its survival in nature. Using recombinants constructed from strains ND18 (efficiently seed-transmitted) and CV17 (poorly seed-transmitted), we recently demonstrated that seed transmission of BSMV in barley is a complex phenotype determined primarily by RNAγ. Seed transmissibility was influenced by the RNAγ 5' UTR, the presence or absence of a repeat in the γa gene, and the γb gene. Symptoms exhibited by plants infected with recombinant and pseudorecombinant viruses varied from extremely mild to severe streaking and mosaic. Some recombinants induced symptoms quite distinct from those induced by either parent strain. Although the effects on symptom type and severity were predominantly attributable to RNAγ, RNAs α and β also contributed to the phenotype. Relationships between the symptomology and seed transmissibility phenotypes as well as the possible contributions of RNAs α and β to these phenotypes will be presented.

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CITRUS TRISTEZA VIRUS COAT PROTEIN GENE DELETIONS AS A TOOL FOR MAPPING MONOCLONAL ANTIBODIES. O.V. Nikolaeva[1], A.V. Karasev[1], D.J. Gumpf[2], R.F. Lee[1] and K.S. Derrick[1]. [1]*University of Florida, CREC, Lake Alfred, FL 33850;* [2]*Department of Plant Pathology, University of California, Riverside, CA 92521.*

Citrus tristeza virus (CTV) coat protein (CP) has been recently expressed as a fusion protein in *E.coli* cells. We created two deletion mutants of the original construction lacking 53 and 103 C-terminal amino acids of the CTV CP. Eleven CTV-specific monoclonal antibodies (MCAs) were screened with the use of these mutants in order to extend the panel of MCAs suitable for detection of the severe stem-pitting CTV isolates. The data obtained indicate that CP may have at least two epitopes characteristic of these CTV isolates.

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ANALYSIS OF TOMATO PSEUDO-CURLY TOP GEMINIVIRUS R.W.Briddon, J.H.Tsai*, I.Bedford and P.G.Markham. Dept. of Virus Research, John Innes Institute, Colney Lane, Norwich, NR4 7UH, UK.
* Institute of Food and Agricultural Sciences, University of Florida, Fort Lauderdale 33314.

Tomato pseudo-curly top virus (TPCTV) is the only member of the Geminiviridae group transmitted by a treehopper (*Micrutalis malleifera*); all other members are transmitted by either a leafhopper or a whitefly vector. A full-length, infectious insect-transmissible clone of this virus has been obtained. Sequence evidence will be presented which confirms the inclusion of this virus in the Geminiviridae group. The relationship of TPCTV to other characterised geminiviruses, in particular beet curly top and tobacco yellow dwarf viruses, will be discussed.

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BEET YELLOW STUNT AND CARNATION NECROTIC FLECK VIRUSES REPRESENT TWO RELATED VIRUS GROUPS WITHIN THE FAMILY *CLOSTEROVIRIDAE*. A.V. Karasev[1,2], O.V. Nikolaeva[1,2], D.J. Gumpf[1], S.M. Garnsey[3], and W.O. Dawson[2]. [1]*Dept. of Plant Pathology, University of California, Riverside, CA 92521;* [2]*University of Florida, CREC, Lake Alfred, FL 33850;* [3]*USDA-ARS, Horticultural Res. Lab., Orlando, FL 32803.*

Beet yellow stunt (BYSV) and carnation necrotic fleck (CNFV) viruses have very flexible filamentous particles containing a single molecule of positive-strand RNA, and one species of the coat protein. Both viruses were classified as definitive members of the heterogeneous closterovirus group. To clarify genetic relationships among different closteroviruses, we cloned large genome fragments including and adjacent to the polymerase gene of BYSV and CNFV. Both viruses were demonstrated to encode the HSP70 gene, a hallmark of closteroviruses. Phylogenetic analysis of the BYSV helicase, polymerase, small hydrophobic protein and HSP70 suggests a common evolutionary origin of these genes for all closteroviruses studied so far. BYSV, however, contains an additional open reading frame (ORF) downstream of the polymerase gene, which is absent from the CNFV genome. The data obtained confirm the proposed splitting of closteroviruses into separate groups and suggest classification of BYSV with citrus tristeza virus, and CNFV with beet yellows virus into a higher taxon, probably into a family *CLOSTEROVIRIDAE*.

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PRODUCTION OF POLYCLONAL ANTISERA TO COAT PROTEIN OF CITRUS TRISTEZA VIRUS OVER EXPRESSED IN *E. COLI:* APPLICATION FOR IMMUNODIAGNOSIS. O. V. Nikolaeva[1,2], A. V. Karasev[1,2], D. J. Gumpf[1] and S. M. Garnsey[3]. [1]Dept. of Plant Pathology, University of California, Riverside, CA 92521; [2]University of Florida, CREC, Lake Alfred, FL 33850; [3]USDA\ARS, Horticultural Res. Lab, Orlando, FL 32803.

The coat protein (CP) gene of the severe SY 568 strain of citrus tristeza virus (CTV) was cloned, sequenced and subcloned into an expression vector under the control of the *lacZ* promoter. The fusion product contained the maltose binding protein (MBP) plus the complete CTV CP and was produced at rates of 40 mg per 500 ml of *E. coli* within 3 days. Following purification by affinity chromatography, the MBP-CP fusion product was demonstrated by immunoblotting to retain antigenic properties of the CTV. The purified MBP-CP protein was injected in rabbits and CTV-specific antisera with titers to 10^{-6} were obtained. These antisera reacted with a single major protein of 25 kDa in immunoblots of CTV-infected plant tissues. The antisera were used successfully as a source of unlabeled intermediate antibody for double antibody sandwich indirect ELISA to detect CTV in the field.

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DOES THE CAULIFLOWER MOSAIC VIRUS (CaMV) TRANSLATIONAL TRANSACTIVATOR FUNCTION PROPERLY IN YEAST (*SACCHAROMYCES CEREVISIAE*)? Yehsiung Sha, John Cannon*, and James Schoelz. Dept. of Plant Pathology and *Dept. of Molecular Microbiology and Immunology, University of Missouri, Columbia MO 65211.

The gene VI product of CaMV is necessary for the posttranscriptional expression of CaMV genes I - V, which are translated from the polycistronic 35S RNA. As a preliminary step towards identifying host proteins that interact with the gene VI product, we are investigating whether the gene VI product can transactivate expression of the reporter gene chloramphenicol acetyltransferase (CAT) in yeast. A yeast reporter plasmid was constructed in which the CAT gene was inserted in frame into CaMV gene II. Transcription in yeast cells was driven by the CaMV 35S promoter, resulting in a polycistronic mRNA that was identical to the 35S RNA up to the CAT reporter gene present within gene II. Northern blot analysis demonstrated that the CAT gene was transcribed, but no CAT activity was detected in yeast cells that contained only this plasmid. A low level of CAT activity was detected in yeast cells when the gene VI coding region was present *in cis* on the yeast plasmid, and a greater level of CAT activity was detected when gene VI was supplied both *in cis* and *in trans*. We are now investigating whether CAT expression is dependent on the presence of the CaMV gene VI product.

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Two revertants of turnip crinkle carmovirus mutant TCV-M18 are competent for systemic movement in *Nicotiana benthamiana*. **M. H. Walter, L. A. Heaton, Department of Plant Pathology, Kansas State University, Manhattan, KS 66506-5502.**

Turnip crinkle carmovirus mutant TCV-M18 has asparagine residues substituted for Asp155 and Asp157 which probably interact with calcium ions in assembled particles. TCV M18 is assembly-competent in protoplasts and movement-deficient in plants. Transcripts of TCV-M18 were used to inoculate *Nicotiana benthamiana*. While symptoms were evident on leaves inoculated with wild type transcripts by 5 days, no symptoms appeared on leaves inoculated with M18 transcript. Symptoms on plants inoculated with TCV-M18 transcripts appeared on non-inoculated leaves 11 days after similar symptoms appeared on plants inoculated with wtTCV. Two revertants regained systemic movement and were called M18-r10 and M18-r11 respectively. M18-r11 produced stunting atypical of wtTCV. Virus yields from M18-r10 were approximately 1/10 of yields from M18-r11, which, in turn, were similar to those of wtTCV (approx.200 ug/g.l.t.). Both revertants appeared more porous to ethidium bromide in non-denaturing virus gels, but r11 migrated slightly faster than wtTCV and -r10. M18-r11 coat protein migrated slightly faster than wtTCV and M18-r10 coat protein in denaturing polyacrylamide gels, suggesting a deletion in the M18-r11 coat protein. Nucleic acid sequence analysis of r10 suggested that reversion to the movement phenotype was due to a second-site mutation(s).

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SINGLE TUBE IMMUNOCAPTURE/PCR DETECTION FOR FOUR POTATO VIRUSES. **J.H.Weston[1], P.R.Mills[2], R.B.Copeland[1]. [1] The Queen's University of Belfast, Department of Applied Plant Science, Newforge Lane, Belfast BT9 5PX, Northern Ireland, UK. [2] Horticulture Research International, Wellesbourne, Warwick CV35 9EF, UK.**

Immunocapture/PCR (IC/PCR) detection of plant RNA viruses is a sensitive assay based on the amplification of cDNA produced from the reverse transcription of antibody captured virus particles. As virus specific antisera and sequence data have become more readily available so it has become possible to use IC/PCR to detect a wide range of plant viruses. Here we describe the use of IC/PCR to detect the potato viruses PVX, PLRV, PVY and PVS in a single tube from both infected foliage and tuber material. Antisera raised to the purified particles of each virus are mixed and used to coat a 0.5 ml microcentrifuge tube. Infected sap from haulm or tubers is added to the coated tube. Reverse transcription and PCR (RT/PCR) follows incubation and washing. The primers for RT/PCR have been designed to produce a ladder of cDNA products, ranging from 180bp for PVS, 450bp for PLRV, 650bp for PVX and 1.2kb for PVY when visualised on an agarose gel. Therefore, IC/PCR can be applied to detect a number of different viruses that infect the same host.