Abstracts of Presentations at the 1989 Annual Meeting of The American Phytopathological Society

August 20-24, 1989
Richmond Marriott Hotel and Richmond Convention Centre
Richmond, Virginia

The number above an abstract corresponds to its designation in the program of the 1989 APS Annual Meeting in Richmond, VA, August 20-24. If a presentation was not given at the meeting, the abstract is not printed among the following pages.

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INFLUENCE OF TILLAGE SYSTEMS ON THE INCIDENCE AND SPATIAL PATTERN OF TAN SPOT OF WHEAT. Wolgang Schuh, Department of Plant Pathology, The Pennsylvania State University, University Park, PA 16802.

The incidence and spatial pattern of Pyrenophora tritici-repentis was assessed at four locations (two conventional & two conservation tillage) two times during 1987 and three times during 1988. Tan Spot was detected earlier in 1988 and had a higher final disease incidence in both years under conservation tillage. Spatial pattern analysis, using Morisita's index of dispersion, revealed a shift from clumped to random distribution over time, indicating the importance of residueborne inoculum under conservation tillage systems. Spatial patterns under conventional tillage were random, indicating airborne inoculum as the source of infection. Results from this experiment suggest that straw residue on the field surface will lead to increased levels of Pyrenophora tritici-repentis, thereby counterbalancing advantages associated with the use of reduced tillage systems.

2

TOBACCO GROWTH AS AFFECTED BY MELOIDOGYNE INCOGNITA AND SOIL MOISTURE. T. A. Wheeler, K. R. Barker, and S. M. Schneider, Department of Plant Pathology, North Carolina State University, Raleigh, NC 27695-7616.

Flue-cured tobacco (var. NC27-NF) was grown in microplots in a RCB design with a factorial arrangement of treatments. The main effects were initial nematode population levels (0 to 20000 eggs/500 cm^3 soil) and irrigation rates (none, low, moderate and high). Plants were destructively sampled throughout the growing season. All stages of M. incognita were removed from the roots and soil at each sampling date. Tobacco with a moderate irrigation rate had the highest growth rate (r=0.00899), followed by low and no irrigation (r=0.00709 and 0.00679, respectively). The r parameter is from the logistic equation: $dH/dt=r*H_t(1-H_t)$, where H=host leaf weight and time is in degree-days. Tobacco yield potential was greatly impacted by irrigation rate. Yield suppression by M. incognita was adequately fitted by quadratic regression.

3

DISEASE.PRO: A COMPUTER PROGRAM FOR EVALUATING AND IMPROVING A PERSONS ABILITY TO ASSESS DISEASE PROPORTION. F. W. Nutter, Jr. and O. Worawitlikit, Department of Plant Pathology, University of Georgia, Athens 30602.

A computer program written in BASIC was developed for the purpose of evaluating a persons ability to estimate disease proportions. The program is similar to DISTRAIN but allows greater flexibility in the selection of lesion size (small, medium, large, random mixture) and allows for regression analysis of rating performance. A color monitor is used to display a simulated peanut leaf and disease proportions are generated for early leafspot, late leafspot, both leafspots mixed or peanut rust. Pre-testing, drill and practice, and post-testing sessions were conducted in a 1-hr time period for introductory plant pathology students. Twenty of 22 students significantly improved their ability to estimate disease proportions of late leafspot (random lesion sizes) as measured by (i) a higher coefficient of determination relating the actual proportion (X) to the estimated value (Y) and/or (ii) a regression coefficient (slope) which was closer to 1.0 in post-tests compared to pre-tests.

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EFFECTS OF MAIZE INTERCROPS ON ANGULAR LEAF SPOT OF BEANS. M. A. Boudreau, Crop Science Dept., University of Nairobi, Kenya.

Angular leaf spot (ALS) severity was evaluated on common beans which had been planted alone and simultaneously with maize at Kabete, Kenya in November 1986, and at Kabete and Thika in April 1987. Intercropping reduced the area under the disease progress curve (AUDPC) by 26% in 1986 and at Thika in 1987 by 24%, but did not significantly reduce AUDPC at Kabete in 1987. Additional treatments evaluating bean density and row versus random planting in 1986 had no effect on AUDPC, but fertilizer amendment in 1987 increased AUDPC by 64% at Kabete and 22% at Thika. Modifications in temperature, relative humidity, and wind velocity in the bean canopy when maize was present suggest conflicting influences on ALS development. The results corroborate other data indicating a significant but variable effect of maize intercrops on reducing ALS.

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A GENERALIZED STOCHASTIC SIMULATION MODEL OF THE INCREASE AND SPREAD OF ARTHROPOD-VECTORED PLANT VIRUSES.
R. S. Ferriss and P. H. Berger. University of Kentucky, Lexington, KY 40546 and University of Idaho, Moscow, ID 83843.

A theoretical model was developed for illustration of the effects of virus transmission characteristics and vector activity on virus disease dynamics. The model simulates spread from a source plant in a 425-plant field. Input parameters include probabilities of virus acquisition and inoculation, lengths of latent periods in the plant and vector, length of the vector infectious period, number of vectors per plant, and three options for the type of simulation of vector movement. Decisions about acquisition, inoculation and vector movement are based on pseudo-random values. Output includes numbers of viruliferous vectors and positions of infected plants at each iteration. Model predictions are generally consistent with expected natural spread. The model illustrates the great effect that vector movement can have on disease dynamics and spatial distribution, particularly for diseases transmitted in a non-persistent manner. Although the model will be difficult to fully validate, it provides a logically rigorous way of integrating knowledge about the many processes that affect virus disease epidemics.

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A CLIMATOLOGY OF AIR PARCEL TRAJECTORIES RELATED TO THE ATMOSPHERIC TRANSPORT OF PERONOSPORA TABACINA. J.M. Davis and J.F. Monahan, North Carolina State University, Raleigh, NC 27695

Temperature and wind data from 5-yr of atmospheric soundings were used in conjunction with an atmospheric transport model to construct a trajectory climatology for the transport of the sporangia of P. tabacina. Daily receptor-

oriented trajectories, with the receptor point located in the mountains of North Carolina, were calculated for April through August and for the annual period. The 12-, 24-, and 48-hr air parcel positions prior to arrival at the receptor point were analyzed by examining the plots of the probability density function (pdf). By integrating the pdf over a region of interest the probability that the given receptor site will receive air parcels from this region can be obtained. Results indicate that the probability of the receptor point receiving air parcels from the tobacco producing region of south Georgia/north Florida, which is a potential inoculum source region, increases during the April to August period. This increase can be attributed to the strengthening of the Bermuda high pressure system.

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GENETIC DIVERSITY AND POPULATION STRUCTURE OF THE RICE BACTERIAL BLIGHT PATHOGEN. J. Leach, M. Rhoads, C. Vera Cruz[1], F. White, T. Mew[1], and H. Leung[1]. Dept. of Plant Pathology, Kansas State University, Manhattan, Kansas and [1]International Rice Research Institute, Los Baños, Philippines.

A repetitive DNA element pJEL101 isolated from Xanthomonas campestris pv. oryzae (Xco) was used to determine the population structure of Xco in the Philippines. pJEL101, when used to probe EcoRI-digested genomic DNA of Xco isolates, revealed race-specific restriction fragment length polymorphisms (RFLP). Of 94 isolates examined, 26 RFLP types were identified. Most of the variability (20 RFLP types) was found in race 1, 2, and 3, which were isolated from lowland areas. Three RFLP types were found among isolates from traditional cultivars grown at high elevation. The genetic diversity of the total Xco population was 0.93, of which 44% was due to genetic differentiation between races. The genetic diversities of isolates collected in 1972-1976, 1977-1981, and 1982-1986, were 0.89, 0.89, and 0.91, respectively, suggesting a consistently high level of variability in the pathogen population over the past 15 years.

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CULTURAL CONTROL OF SCLEROTINIA CROWN AND STEM ROT IN ALFALFA SEED FIELDS. R. G. Gilbert, USDA-Agricultural Research Service, P. O. Box 30, Prosser, WA 99350.

Cultural control of *Sclerotinia* in broadcast-planted alfalfa seed fields were: 1) "fall-burn" of harvest residue; 2) "winter-burn"; and 3) "spring-beat-back." Fall-burn reduced sclerotia in the field by >95%. Sclerotia were killed in the residue and surface soil. Only 14% of the surface soil sclerotia were viable after the fall-burn in November and during the next 6 months the number of surface soil sclerotia declined from 246/m^2 to 7/m^2, and the viability of sclerotia was reduced from 12 to 0%. Winter-burn reduced surface residue and sclerotia numbers, but did not reduce viability and survival of surface soil sclerotia. Spring-beat-back dried the soil surface and reduced the canopy relative humidity, which established conditions that were unfavorable for *Sclerotinia* activity. Alfalfa seed yields in the fall-burn and spring-beat-back treatments were 862 and 716 Kg/ha for an increase of 43 and 13%, respectively, over winter-burn.

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CONTROL OF PHYTOPHTHORA ROOT ROT ON CITRUS WITH SODIUM TETRATHIOCARBONATE. M.E. Matheron and J.C. Matejka, University of Arizona, Yuma Agric. Center, Yuma, AZ 85364

Greenhouse studies were initiated to determine the efficacy of sodium tetrathiocarbonate (STTC) for control of root rot on citrus caused by Phytophthora citrophthora and P. parasitica. When roots of rough lemon seedlings were inoculated with zoospores of these two fungi in the presence of STTC at 245 µg/ml or metalaxyl at 10 µg/ml, resultant fresh weights of shoots and roots did not differ from noninoculated plants, whereas inoculated plants without chemical treatment suffered significant loss of shoot and root weights. Treatment with STTC at 4900 µg/ml was necessary to inactivate propagules of P. parasitica in naturally infested soil. Rough lemon seedlings planted 7 days after treatment of soil to avoid phytotoxic levels of STTC had root and shoot weights equivalent to those grown in soil treated with 10 µg/ml metalaxyl. Apparently, STTC can reduce the severity of root rot on citrus caused by P. citrophthora and P. parasitica.

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CONTROL OF FUNGAL DISEASES OF WINTER OILSEED RAPE IN SWITZERLAND. M. W. Andres and W. E. Winter, Swiss Federal Research Station for Agronomy, Reckenholzstr. 191, 8046 Zuerich, Switzerland.

Winter oilseed rape (Brassica napus) grown on 17'000 ha is the predominant edible-oil crop in Switzerland. The most important fungal diseases are caused by Phoma lingam, Alternaria sp., Botrytis cinerea and Sclerotinia sclerotiorum. Cylindrosporium concentricum, Verticillium dahliae and Pseudocercosporella capsellae are rarely found but have the potential to become more prevalent.
Integrated crop management is employed to try to control these diseases. It involves measures such as use of resistant cultivars, crop rotation and removal of infected residue. Fungicides are considered a last resort. None are registered at present. The identification of resistant cultivars is a top priority in our research. Special nurseries have been established. The potential use of biotechnology for this purpose will be investigated.

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INFECTION OF STRAWBERRY ROOTS BY COLLETOTRICHUM FRAGARIAE AND GLOMERELLA CINGULATA. C. M. Howard, C. K. Chandler, and E. E. Albregts. Univ. of Fla., AREC, Dover, FL 33527.

Large roots ca 14 cm long were excised from rhizomes of strawberry plants that had wilted in Florida fruiting fields because of anthracnose crown rot. Isolations from the rhizomes yielded primarily C. fragariae from seedlings that previously had been grown in a Florida nursery and G. cingulata (= C. gloeosporioides) from Chandler plants that previously had been grown in a Tennessee nursery. The roots were cut into 1 cm sections and every other section beginning with the basal section was plated on PDA. Colletotrichum spp. or G. cingulata (primarily C. fragariae from seedlings, and G. cingulata and C. acutatum from Chandler) were isolated from 45% of the basal root sections and from 11% of the third sections. These fungi were isolated from 1.4% to 2.3% of sections 5, 7, 9, 11, and 13.

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A 72KD PROTEIN ASSOCIATED WITH CERCOSPORIN SYNTHESIS IN CERCOSPORA KIKUCHII TOXIGENIC ISOLATE PR. Jeffery A. Rollins and Robert G. Upchurch. Department of Plant Pathology, N.C. State University, Raleigh, NC 27695-7616.

Comparative SDS-PAGE analysis of the protein extracts of C. kikuchii parental isolate PR and cercosporin-minus mutants derived by UV mutagenesis from this isolate revealed that a prominent 72kD protein present in the parental isolate was absent in the mutants. Two-dimensional gel electrophoresis indicates that the protein is basic. Gel scans indicate that the protein composes as much as 12% of the total extractable protein of PR, but that it is present in lower concentration in other Cercospora species. Temporal analysis of toxin and protein synthesis in PR shows that the 72kD protein is positively correlated with the synthesis of cercosporin. We are interested in identifying this protein by cloning and characterizing its encoding gene because of the possibility of its involvement in cercosporin synthesis and/or regulation.

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CHARACTERIZATION OF HYDROXAMATE SIDEROPHORES AND SIDEROPHORE--MEDIATED IRON IN GAUMANNOMYCES GRAMINIS VAR TRITICI. S. Dori[1], S. Solel[3], Y. Kashman[2] and I. Barash[1], Dept. of [3]Plant Pathology, ARO, The Volcani Center, Israel.

Under iron-deficient conditions Gaumannomyces graminis var tritici (Ggt) produces hydroxamate siderophores designated as A, B and C according to their electrophoretic mobility. Siderophores B and C were identified as dimerum acid and coprogen B respectively. Siderophore A exhibited and identical NMR spectra to dimerum acid but was chromatographically distinct. The system of siderophores mediating uptake of iron was characterized. It exhibited an active transport and Michaelis kineties. Ggt could utilize iron effectively from siderophores produced by other fungi. Competition experiments between coprogen B., dimerum acid and hydroxamate siderophores from other structural classes suggest a common transport system. Wheat seedlings were capable of utilizing iron from coprogen B and dimerum acid. The role of siderophores in survival and virulence of Ggt will be discussed.

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Highly virulent isolates of *Fusarium solani* from soybeans produce the *Nectria* ascoma stage. T. S. Abney, T. L. Richards, A. J. Ivanovich, & D. H. Scott. USDA-ARS and Purdue University, W. Lafayette, IN 47907.

Twenty *Fusarium solani* (Mart.) Sacc. isolates from soybeans with symptoms of sudden death syndrome (SDS) were compared in culture and evaluated for virulence on Spencer & Ripley soybeans. Initial colonies growing from soybean root and crown tissues onto agar had macro- and micro-conidia typical of *F. solani* but did not consistently express a blue color. With subculture, the blue pigmentation within fungal colonies was characteristic of isolates in a predominately macroconidial (versus microconidial) 'form' or state of growth. All isolates expressed a bluish color with subculturing. However, the nature and extent of the bluish color varied among isolates and with subculturing. Perithecia and ascospores developed in subculture with two of the *F. solani* isolates. Although, ascospores were not required for hypocotyl infection, virulence of the isolates that produced *Nectria* in PDA culture was higher than that of other isolates. Typical SDS foliage symptoms developed 3 weeks after hypocotyl inoculation with conidial (macro and micro) and mycelial inoculum only with isolates that produced the ascoma stage in culture. Hypocotyl inoculations with ascospore inoculum also produced typical SDS foliage symptoms.

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FULFILLING IPM STRATEGIES FOR CACAO BLACK POD CONTROL IN THE TROPICS. R. H. Fulton, American Cocoa Research Institute, 7900 Westpark Drive, McLean, Virginia, 22102

Cacao, Theobroma cacao, is cultivated mainly on farms of 2 to 6 acres where cash - flow is critical. In the Americas, Phytophthora palmivora, is considered the major pod destroyer. For six decades a host of fungicides and varied spray schedules have failed to give consistent Black Pod control. Formerly in - depth etiological studies confirmed that the soil - tree root systems served as the massive inoculum reservoir. Today, IPM cultural practices for lowering this inoculum source entails soil modifications via drainage and liming; while studies on pod surface characteristics, influence of canopy climate on infection has "reduced" the number of sprays. Trials employing surfactants and oil adjuvants proved that growers could spray "less" copper fungicide with positive control results.

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LEAF REMOVAL FOR MANAGEMENT OF DISEASES AND PESTS OF WINE GRAPES IN THE SAN JOAQUIN VALLEY. J. J. Stapleton#+, W. W. Barnett#+, K. M. Kelley+, G. M. Leavitt+, M. VK. Norton+, and P. S. Verdegaal+, Statewide IPM Project#, and Cooperative Extension+, University of California, Modesto, CA 95355.

Leaves surrounding clusters of several wine grape (Vitis vinifera) varieties were removed after late bloom in 6 San Joaquin Valley vineyards in 1988. Comparisons of treated vs. control, bilateral cordon-trained vines showed that incidence and/or severity of Botrytis bunch rot, sour rot, or "total" rot at harvest was reduced (P=0.05 or P=0.01) after leaf removal in 5 of the 6 experimental vineyards. A trend toward reduced canopy populations of leafhoppers and mites, and reduced cluster damage due to insect pests also was observed following leaf removal. The treatment did not significantly affect grape yield or quality parameters.

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DEFINING THE SOCIO-BIOLOGICAL ENVIRONMENT FOR RICE PEST MANAGEMENT USING SAMPLE-SURVEYS. P. S. Teng, F. A. Elazegui, B. M. Shepard, K. M. Moody, International Rice Research Institute, P.O. Box 933, Manila, Philippines.

An "Integrated Pest Survey" was initiated in the wet season of 1987 to determine existing farmers'pest control practices, farmers' perceptions of pests and the actual pest distribution and intensity. Farmers who applied pesticides did not have significantly higher yield compared with farmers who did not (5.3 t/ha vs 4.5 t/ha). Pest intensity and diversity were low. The 3 most prevalent insects were cutworm, leaffolder, and rice whorl maggot; diseases were sheath blight, narrow brown leaf spot, and stem rot; and weeds were Echinochloa glabrescens, Monochoria vaginalis, and Paspalum distichum. A first record of the nematode, Tylenchorynchus sp. was made. Rice tungro virus was detected in 24.4% of fields using an ELISA test, even though symptoms were not observed. Farmers did not perceive any pest to be a major yield constraint but considered pesticide application a necessary production practice. Farmers generally followed a calendar spraying program without considering pest populations.

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NUCLEIC ACID HYBRIDIZATIONS DISTINGUISH MLO STRAINS TRANSMITTED BY MACROSTELES SPP., VECTORS OF ASTER YELLOWS AGENT. I.-M. Lee, R.E. Davis, T.-A. Chen, L.N. Chiykowski, J. Fletcher, and C. Hiruki. Microbiol. and Pl. Pathol. Lab., Agricultural Research Service, USDA, Beltsville, MD 20705; Dept. of Pl. Pathol., Cook College, Rutgers University, New Brunswick, NJ 08903; Agriculture Canada, Ontario, Canada; Dept. of Pl. Pathol., Oklahoma State University, Stillwater, OK 74078; and Dept. of Plant Science, Univ. of Alberta, Alberta, Canada.

Strains of mycoplasmalike organisms (MLOs) transmitted by aster yellows (AY) insect vectors (Macrosteles spp.) were analyzed by nucleic acid hybridizations with biotin-labeled cloned DNA fragments from AY and tomato big bud (BB) MLOs. All strains examined were genetically related at the level of chromosome, but differential hybridizations distinguished among the strains. Distinctions did not completely coincide with concepts of "eastern" and "western" AY. Some of the data indicated that certain of the strains examined may be more closely related genetically to BB MLO than to AY MLO. The results underscore ambiguities involved in classifying MLOs as strains of AY MLO on the basis of biological properties.

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LOCALIZATION OF SPIROPLASMA CITRI SURFACE PROTEINS USING INDIRECT IMMUNOFLUORESCENCE. M. G. Morley and J. Fletcher. Department of Plant Pathology, Oklahoma State University, Stillwater, OK 74078-9947.

The relative distribution of spiroplasma citri surface proteins (p29, p58, p77, and p89) (Phytopathology 77:1726) was determined using antisera made against specific surface proteins in indirect immuno-fluorescence. Glutaraldehyde-fixed S. citri cells were incubated with antiserum and then treated with fluorescein-conjugated anti-rabbit serum. Cells treated with antiserum prepared against whole cells, membranes, and proteins p29, p77, or p89 showed fluorescence which appeared evenly distributed along the spiroplasma cells; however, cells treated with preimmune or anti-p58 sera did not fluoresce. Fluorescence of entire spiroplasma cells with antisera to p29, p77, and p89 indicated that these proteins are not clustered in one area of the S. citri membrane but are distributed over the cell length.

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PRODUCTION OF MONOSPECIFIC POLYCLONAL ANTIBODIES AGAINST THE ASTER YELLOWS MYCOPLASMA-LIKE ORGANISMS (AYMLO) OF OKLAHOMA. D. Errampalli, J. Fletcher and J. L. Sherwood, Dept. of Plant Pathology, Oklahoma State Univ., Stillwater, OK 74078-9947.

Polyclonal antibodies prepared against partially purified AYMLO from infected periwinkle tested positive with AYMLO infected plants but also showed cross reactivity with healthy controls. AYMLO antibodies were further purified using electrophoretically separated and electroblotted 23K, 55K, and 70K AYMLO-specific proteins as specific immunoabsorbents. The nitrocellulose strips, each containing a specific AYMLO protein, were used repeatedly to trap the specific antibodies, which were then eluted from the strips by changing the pH. Such antibodies reacted positively on the blots of periwinkle infected with Oklahoma AYMLO, but not with healthy controls. The monospecific antibodies were used to screen plants infected with several isolates of presumed AY from Oklahoma and other geographical areas.

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BIOCHEMICAL AND BIOPHYSICAL FACTORS AFFECTING FEEDING OF HOMALODISCA COAGULATA, A VECTOR OF DISEASES CAUSED BY XYLELLA FASTIDIOSA. P. C. Andersen, R. F. Mizell, B. V. Brodbeck and A. B. Gould. University of Florida, AREC-Monticello, Rt. 4 Box 63, Monticello, FL 32344.

Homalodisca coagulata is a polyphagous leafhopper vector of many diseases induced by Xylella fastidiosa, although little is known concerning factors that influence feeding. H. coagulata, confined to stems of host plant species produced copious quantities (1-2 ml/h) of dilute exudate (8-25mM) consisting mainly of inorganic ions, characteristic of a xylem fluid-feeder. Total amino acids, organic acids and sugars were metabolized with > 99% efficiency; major organic compounds (glutamine, asparagine, malic acid and succinic acid) with > 99.9% efficiency. Biochemical and biophysical plant factors, and leafhopper responses were manipulated by altering plant nutrient status and moisture stress. Feeding was not reduced at a specific threshold xylem fluid tension.

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THE PRODUCTION OF A POLYCLONAL ANTISERA TO THE BEET LEAFHOPPER TRANSMITTED VIRESCENCE AGENT. D. A. Golino*, B. C. Kirkpatrick, and G. A. Fisher. USDA-ARS*, Department of Plant Pathology, University of California, Davis, CA 95616.

Antisera specific for the beet leafhopper transmitted virescence agent (BLTVA-MLO) were prepared by injecting rabbits with MLO-enriched extracts prepared from infected leafhoppers, Circulifer tenellus (Baker). When used in a F(ab)2/Protein A ELISA system this antisera reacted positively with roots and leaves of symptomatic BLTVA-infected periwinkle (Catharanthus roseus), but not with periwinkles infected with Spiroplasma citri, X-disease or three strains of western aster yellows. ELISA also detected the BLTVA-MLO in infected radish (Raphinus sativus) and plantain (Plantago major). Western blot analysis of soluble proteins from BLTVA-MLO-infected periwinkle and C. tenellus revealed a single dominant antigen of approximately 40 kd.

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PHYLOGENETIC RELATIONSHIPS OF THE WESTERN X-DISEASE MYCOPLASMA-LIKE ORGANISM (X-MLO) AS ESTABLISHED BY 16S rRNA SEQUENCE. B. C. Kirkpatrick and J. D. Fraser. Department of Plant Pathology, University of California, Davis, CA 95616.

Two cloned DNA fragments, which together contain 97% of the X-MLO 16S ribosomal RNA gene (rRNA), were sequenced. One cloned fragment contains two putative promoters that are very similar to the 16S rRNA promoters of Bacillus subtilis, and 673 bp of the 5' end of the 16S rRNA. The second cloned fragment contains 796 bp of the 3' end of the 16S rRNA gene. The G+C content of the 16S rRNA gene is 45.5%, while the 5' flanking sequence is 23%. These G+C values are among the lowest reported for prokaryotes. The X-MLO 16S rRNA gene is 78% homologous to the 16S rRNA genes of B. subtilus and Mycoplasma capricolum, and 73% homologous to E. coli. These results suggest that the X-MLO is phylogenetically related to, but distinct from, other gram-positive prokaryotes.

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CLONING AND SEQUENCE ANALYSIS OF THE 16S RIBOSOMAL RNA GENE OF WESTERN ASTER YELLOWS MLO (AY-MLO). C. R. Kuske and B. C. Kirkpatrick, Department of Plant Pathology, University of California, Davis, CA 95616.

The 16S ribosomal RNA gene (16S rDNA) of the AY-MLO was identified by Southern blot analyses, using cloned fragments of the Western X-MLO 16S rDNA as heterologous probes. A 1.5 kbp EcoRI/HindIII fragment, unique to AY-MLO DNA, hybridized with a probe containing the 3' region of the X-MLO 16S rDNA. Two AY-MLO-specific fragments (2.5 and 1.0 kbp) hybridized with a probe containing the 5' end and upstream flanking sequences of the X-MLO 16S rDNA. The 2.5 kbp and 1.5 kbp fragments were cloned in M13mp vectors and sequenced. The 2.5 kbp fragment contains about 670 bp of the 5' region of the 16S rDNA and about 1.9 kbp of A:T rich DNA upstream. The 1.5 kbp fragment contains 863 bp of the 3' region of the 16S rDNA, followed by a spacer region containing a tRNA gene and the 5' end of the 23S rDNA. Sequence comparisons with the 16S rDNA of other prokaryotes and plant organelles indicate the AY-MLO is most closely related to Bacillus subtilis and Mycoplasma capricolum.

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THE EFFECT OF ROOT AGE AND POSITION OF MYCORRHIZAL INOCULUM ON COLONIZATION OF COTTON, ONION AND PEPPER. U. Afek, E. Rinaldelli, J. A. Menge and E. L. V. Johnson. Dept. of Plant Pathology, Univ. of California, Riverside, CA 92521.

Onion seedlings which were grown in a greenhouse were inoculated with VAM fungi by placing inoculum on the entire root system after the seedlings had grown for different periods of time. Seedling age had a great effect on mycorrhizal colonization. VAM colonization took place rapidly in 3-day-old onion and reached 51% after one week. Older plants were far less responsive. VAM colonization of onion and pepper reached a maximum two weeks after inoculation and of cotton at 3 weeks after inoculation. In the field, comparisons of VAM colonization efficiency with respect to placement of the inoculum were studied. Inoculum was placed 3 cm below seeds at planting and also 5 cm deep by 3 cm from one side of the root system and 5 cm deep by 3 cm from both sides of the root system 2 weeks after planting. Maximum VAM colonization was achieved when inoculum was applied 3 cm below seeds at planting.

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EFFECTS OF OZONE EXPOSURE AND SOIL WATER DEFICIT ON GROWTH, ECTOMYCORRHIZAE AND NON-STRUCTURAL CARBOHYDRATES OF LOBLOLLY PINE SEEDLINGS. S. Meier and L. F. Grand, Department of Plant Pathology, North Carolina State University, Raleigh 27695.

Loblolly pine (Pinus taeda) seedlings from three full-sib families were exposed to 0, 50, 100, or 150 ppb ozone (5 h/d, 5 d/week for 6 or 12 wk). Soil water potential was maintained near pot capacity (-0.03 MPa) or soil was allowed to dry to approximately -1.0 MPa and resaturated. Exposure to ozone and soil water deficit each resulted in less seedling volume growth and total dry weight. Exposure to increasing ozone concentrations resulted in a linear reduction in foliar starch but did not affect hexose or sucrose production. Soil water deficit lessened starch and sugar content in above- and below-ground plant parts. Soil water deficit did not affect numbers of ectomycorrhizal tips or percentages of roots that formed ectomycorrhizae. A linear dose-relationship between ozone and ectomycorrhizae was observed. The number of ectomycorrhizal tips/cm long root and the percentage of feeder roots that formed ectomycorrhizae were lower as ozone concentration increased.

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CHARACTERIZATION OF PROTEIN/ISOZYME PATTERNS IN A TRIPARTITE SYMBIOSIS. J. S. Neck, J. B. Szerszen, and R. A. Taber, Department of Plant Pathology and Microbiology, Agricultural Experiment Station, Texas A&M University, College Station 77843.

Cosymbionts, *Arachis hypogaea* L. cv. Tamnut, *Bradyrhizobium* spp. (3 strain mixture), and *Glomus etunicatum* Becker and Gerd. were grown in all combinations to assess potential plant (peanut) metabolic responses to colonization by the rhizobial and vesicular arbuscular mycorrhizal (fungal) components. Extractable host leaf/root tissue (50 day old plants) samples and pure culture rhizobial and fungal spore samples were electrophoretically (IEF-PAGE) separated and stained for buffer soluble general protein, glycoprotein, and isozyme patterns. Preliminary results indicate some symbiotic combinations showed differential band patterns and/or band intensities.

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PRODUCTION OF VA MYCORRHIZAL SPORES IN A SAND-VERMICULITE MEDIUM. H. D. Liyanage and N. C. Schenck, Department of Plant Pathology, University of Florida, Gainesville, FL 32611.

A 0.1 strength Long Ashton (nitrate type) nutrient solution of varying P levels (0.05 to 20 mg P/l) was evaluated in a sand-vermiculite (3:1, v/v) mixture as a substitute for a soil pot culture medium. Two species of VA mycorrhizal fungi, Glomus etunicatum (isolate LETC 329) and G. mosseae (isolate LMSS 336) were evaluated on alfalfa, bahiagrass and onion; in addition LETC 329 was evaluated on wheat. Colonized root lengths were maximum at 2 mg P/l for G. etunicatum and G. mosseae at 12 and 15 weeks, respectively. Increasing levels of P caused a more rapid decline in root colonization by G. etunicatum than by G. mosseae. At 0.5 mg P/l, G. etunicatum produced maximum spore numbers per plant (39,500-43,400) on all hosts except wheat. Glomus mosseae produced maximum spores on alfalfa and bahiagrass (990 and 4800) at 0.5 mg P/l and on onion (1381) at 10 mg P/l. Thus, sand -vermiculite was an effective pot culture medium for these two species of VA mycorrhizal fungi.

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ACRONYMS FOR SPECIES OF VA MYCORRHIZAL FUNGI. Y. Pérez and N.C. Schenck, Plant Pathology Dept., Univ. of Florida, Gainesville, FL 32611.

A unique four-letter acronym for each species is proposed which can be used in lieu of epithets in computer databases, publication graphics, and experimental notations. Other abbreviations occur in the literature, but the

means used to derive them were not described. Species can be assigned a unique acronym by following guidelines. The first letter of the acronym represents the genus and should not be one used previously to represent a genus, e.g. *Acaulospora=A, Entrophospora=E, Gigaspora=G, Glomus=L, Sclerocystis=S, Scutellospora=C.* The last three letters are from the species epithet. The second letter of the acronym is the initial letter of the species. The third and fourth letters are consonants, whenever possible, occurring in the species name. The acronyms are written in upper case letters and without punctuation, e.g. *ANCS=Acaulospora nicolsonii, ECLB=Entrophospora colombiana, GMRG=Gigaspora margarita, LDST=Glomus deserticola, SSNS = Sclerocystis sinuosa, CHTG=Scutellospora heterogama.* The complete set of guidelines and acronyms for presently recognized species will be published elsewhere.

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DISCOVERY OF A SPORE OF *SCUTELLOSPORA HETEROGAMA* WITH TWO SUSPENSORS. Rogerio T. Almeida and N. C. Schenck. Plant Pathology Dept., University of Florida, Gainesville, FL 32611.

Nicolson and Gerdemann in 1968 (Mycologia 60:313–325) reported the occasional occurrence of spores of *S. heterogama* with two suspensors. Koske and Walker in 1985. (Mycologia 77:702–720) suggested that this observation could have resulted from a spore formed within a dead spore giving the appearance of a single spore with two suspensors. According to Koske (1984. Mycologia 76:853–862), spores forming within other spore "shells" is a common occurrence. A single spore of *S. heterogama* was observed in a soil sample from Alachua, Florida with a suspensor on opposite poles. The appearance of the spore was that of a zygospore of a typical zygomycetous fungus. The spore and suspensor contents appeared normal. No mycorrhizal pot culture resulted from this single spore, but pot cultures with *S. heterogama* spores from the same sample were established. The observation of a *S. heterogama* spore with two suspensors verifies the report of Nicolson and Gerdemann and indicates that not all 2–suspensor spores result from a spore–within–a–spore phenomenon.

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STIMULATION OF VESICULAR-ARBUSCULAR MYCORRHIZAL SPORE GERMINATION BY *STREPTOMYCES ORIENTALIS*. G. L. Tylka, R. S. Hussey, and R.W. Roncadori, University of Georgia, Department of Plant Pathology, Athens, GA 30602

Surface sterilized spores of *Gigaspora margarita* (GMG) and *Glomus mosseae* (GMS) were plated on 1.5 % Noble water agar (WA), WA covering a layer of *Streptomyces orientalis* (SO) colonies suspended in WA (SO/WA), or SO/WA prepared with autoclaved SO colonies (autoclaved SO/WA). GMG and GMS spores plated on SO/WA exhibited 10 to 70% greater germination than spores on autoclaved SO/WA or WA beginning 5 days after plating. GMG and GMS spore germination was also more frequent when spores were plated on SO/WA or WA in separate compartments of divided Petri plates versus germination of spores in plates containing WA alone. GMS germination was greater when plated on WA separated from SO/WA than when placed directly onto SO/WA. Thus, the stimulatory effect of SO on GMG and GMS appears to be volatile. Conversely, preliminary experiments indicate that *Scutellospora heterogama* spore germination is inhibited by SO.

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USE OF ELISA KITS FOR DIAGNOSIS OF PHYTOPHTHORA CROWN AND ROOT ROT OF AZALEA. J. M. Mullen & A. K. Hagan, Department of Plant Pathology, Auburn University, AL 36849-5409.

During 1988 and 1989, azalea container plants with crown and root rot symptoms submitted to the Plant Diagnostic Lab at Auburn University were checked for Phytophthora spp. infection using apple baiting, isolations on a selective pimaricin-vancomycin-Terraclor 75WP medium ($P_{10}VP$), and serological assays with ELISA multi-well, dip-stick, and rapid assay kits (Agri-Diagnostics Associates). Very good agreement was obtained with results of the apple baiting, culture, and ELISA multi-well kits. The dip-stick and rapid assays did not appear to be as sensitive as the multi-well assay. Isolations indicated that the apple bait and multi-well assay consistently detected P. cinnamomi, P. citricola and P. parasitica.

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DEVELOPMENT OF ENZYME-LINKED IMMUNOSORBENT ASSAY FOR Verticillium spp. IN POTATO PLANTS AND TUBERS. S. Sundaram and E. E. Banttari, Department of Plant Pathology, University of Minnesota, St. Paul, MN 55108.

Specific proteins, obtained by PAGE (polyacrylamide gel electrophoresis) of mycelial extracts, were used to prepare a polyclonal antiserum against Verticillium dahliae. An antiserum obtained 28 days after first immunization reacted well with V. dahliae and to a lesser extent with V. albo-atrum but not with V. nigrescens; V. lateritium; Fusarium oxysporum; Rhizoctonia solani and Colletotrichum sp. Double antibody sandwich enzyme-linked immunosorbent assays (DAS-ELISA) were developed for detection and quantification of Verticillium spp. in potato tubers and plant tissue samples. The results of this study suggest that a relatively specific fungal antiserum can be produced using a soluble protein extract. The double antibody sandwich technique will be evaluated as a potential screening technique for identifying resistance to Verticillium in potato breeding lines.

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DEVELOPMENT OF A DIRECT IMMUNOASSAY TO DETECT PHYTOPHTHORA MEGASPERMA F.SP. GLYCINEA IN SOIL. S. M. Miller, F. P. Petersen, S. A. Miller, J. H. Rittenburg, S.C. Wood and G. D. Grothaus. Agri-Diagnostics Associates, 2611 Branch Pike, Cinnaminson, NJ 08077

A simple, rapid technique has been developed to detect and quantitate Phytophthora megasperma f.sp. glycinea (Pmg) in soybean soil. Pmg oospore and mycelial components are extracted from the soil by a rapid water- flotation technique and fungal antigen is solubilized by grinding prior to analysis by enzyme-linked immunosorbant assay (ELISA). The ELISA incorporates monoclonal antibodies specific for Pmg soluble internal oospore antigen and soluble mycelial antigens. Relative levels of Pmg in soil samples are estimated by comparing the reactivity of the solubilized extracts to that of laboratory-prepared oospore standards. The presence of Pmg in samples which gave positive results in the direct soil immunoassay has been confirmed using a bioassay method. This method directly measures Pmg antigen in the soil and does not require the germination of oospores for their detection. It has been possible to detect other soil-borne plant pathogens, including other Phytophthora spp., Pythium spp. and Rhizoctonia spp. by use of this technique.

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DETECTION AND QUANTITATION OF PHYTOPHTHORA MEGASPERMA F. SP. GLYCINEA IN FIELD SOIL BY IMMUNOASSAY. S.A. Miller, V. Korjagin, S.M. Miller, F.P.Petersen, M. Klopmeyer, R.K. Lankow, and G.D. Grothaus. Agri-Diagnostics Associates, 2611 Branch Pike, Cinnaminson, NJ 08077.

A rapid, direct technique for detection and quantitation of Phytophthora megasperma f. sp. glycinea (Pmg) in soil was evaluated and compared with a baiting/immunoassay method (Phytopathology 78:1576). Samples were collected post-harvest from 198 fields, air-dried, pulverized, mixed thoroughly and subsampled for analysis. Both methods were effective in detecting and quantifying Pmg in the samples. Pmg was detected in soybean fields and fields in which a rotational crop such as corn or wheat had grown immediately prior to sampling. The baiting/immunoassay method was time-consuming (10 days) due to the requirement for oospore germination. Direct tests of Pmg in soil were completed in less than one hour, and provided a quantitative measure of the total amount of Pmg antigen in the sample. Studies are currently underway to determine the relative risk of disease occurrence in fields with various levels of Pmg as determined by these methods.

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DOUBLE-STRANDED RNA ASSOCIATED WITH MULTIFLORA ROSE EXPRESSING SYMPTOMS OF ROSE ROSETTE DISEASE. R. Di, A. H. Epstein, J.H. Hill, Dept. of Plant Pathology, Iowa State University, Ames, IA 50011-1020.

Rose "rosette", a disease of unknown etiology which affects multiflora rose (Rosa multiflora, Thunb.) has been found in several areas of the United States. It is vectored by an eriophyid mite (Phylocoptes fructiphilus). Speculation is that the disease is caused by a virus; however, no virus-like particles have been observed in infected tissue. We have demonstrated the presence of double-stranded RNA (dsRNA) in extracts of diseased multiflora rose leaves using polyacrylamide gel electrophoresis. After digestion with DNase and RNase in high salt solution, three distinct dsRNAs were identified in diseased samples. Similar dsRNAs were not detected in extracts from healthy control rose tissue. Rosette disease can be lethal to rose and is being considered for development as a biological control for multiflora rose, a serious pest on non-cultivated lands.

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USE OF DNA PROBES TO DETERMINE PATHOGENICITY IN AGROBACTERIUM ISOLATES FROM MUSCADINE. C-W. J. Sun, D. S. Luthe, and C. H. Graves. Dept. of Biochemistry and Molecular Biology, and Dept.

of Plant Pathology and Weed Science, Mississippi State Univ., Miss. State, MS 39762.

Cloned fragments of T-DNA from a wide host range Ti plasmid, pTi-A6, and from a limited host range plasmid, pTi-Ag63, have been used as probes in DNA hybridization experiments to determine the pathogenicity of isolates of Agrobacterium species from stems, roots and galls of muscadine. Several hybridization techniques including colony hybridization, slot blot, and Southern hybridization have been used. These techniques consistently identify known grape isolates, however, they are less sensitive when used to probe unknown samples from muscadine isolates. This suggests that the muscadine isolates may differ from Ag63 in DNA sequence homology. Attempts are being made to use the polymerase chain reaction as a more sensitive detection technique.

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COMPARISON OF THE ISOLATION OF XANTHOMONAS CAMPESTRIS PV. CAMPESTRIS FROM CRUCIFER SEEDS IN FOUR SEMISELECTIVE MEDIA. C. J. Chang[1], R. Donaldson[1], M. Crowley[2], and D. Pinnow[2]. [1]Department of Plant Pathology, University of Georgia and [2]Seed Laboratory, Georgia Department of Agriculture, Griffin, GA 30223.

Four semiselective media, CS20ABN, NSCA, NSCAA, and F-S, were compared for their efficacy in isolating Xanthomonas campestris pv. campestris (Xcc) from crucifer seeds. Samples of 50,000 seeds per lot were washed for 2 hr at room temp in saline. The cheesecloth-filtered washings were centrifuged, and the resuspended pellets were diluted and pipetted onto the media. Saprophytic bacteria overgrew Xcc on NSCA and NSCAA but not on F-S or CS20ABN. Colony-forming units (CFUs) of Xcc ranged from 66-97 per plate at 10^{-1} or 37-259 at 10^{-2} on CS20ABN. Moreover, 59-100% of recovered colonies on CS20ABN were Xcc, whereas only 4-29% were Xcc on F-S. Colony size of Xcc on CS20ABN was 2-4 times that on NSCAA or NSCA, but was 4-7 times that on F-S.

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MODIFIED TWEEN MEDIUM FOR DETECTING XANTHOMONAS CAMPESTRIS PV. VESICATORIA IN TOMATO SEEDS. H.M.Kim and N.W. Schaad. Chonbuk Nat. Univ.,Chonju, Korea and Harris Moran Seed Co., San Juan Bautista, Ca. 95045.

Xanthomonas campestris pv.vesicatoria (Xv) is seedborne in tomato. Tween medium A has been described for isolating Xv from fruit and leaves (McGuire,R.G. etal. Pl. Dis. 70: 887-891). The authors describe the same medium plus boric acid and increased cephalexin(Tween B) for assaying seeds. Dilution platings on test media with Xv and tomato seed washings were done in comparison to KMB. Three of 8 strains failed to grow on Tween B. All strains grew on Tween B only after reducing cephalexin, tobramycin and boric acid by 46,50 and 67 %, respectively. The recovery in cfu of 8 strains on modified Tween in comparison to KMB ranged from 50-92% (mean of 73). Reduction in seed flora on Tween B and modified Tween was similar (98 &97%).

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EVALUATION OF SEED BIOPSY METHODS FOR NONDESTRUCTIVE SEED HEALTH TESTING. P. M. Higley, D. C. McGee, J. S. Burris, Seed Science Center and Dept. Plant Pathology, Iowa State Univ., Ames, IA

A previous report described methods for extraction and detection of pathogens from seeds for the purpose of developing nondestructive seed health tests. Of particular value was the biopsy method of coring tissue from dry seeds. The present work focuses on optimizing coring techniques to minimize damage to seed germination. Partial imbibition of corn, soybean, and Phaseolus seeds facilitated removal of cores, but the combined effect of imbibing and coring reduced germination compared to that of seeds that were imbibed only. Treatment of wounded tissue with a paraffin sealant was an ineffective means of correcting deleterious effects of coring on germination. However, reduction of the rate of dry-down by adjusting temperature and humidity improved germination in cored and imbibed soybean and Phaseolus seeds. Because corn germination was unaffected by coring treatments in this experiment, effects of these corrective actions were not detected.

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INCIDENCE AND SURVIVAL OF SCLEROTINIA MINOR IN PEANUT SEED. D. M. Porter, R. A. Taber, and D. H. Smith. USDA, ARS, Tidewater Agric. Expt. Sta., Suffolk, VA 23437; Dept. Plant Path. and Microbiology, Texas A&M Univ., College Sta., TX 77843.

The potential for seed transmission of Sclerotinia minor was assessed in peanut seed harvested from fields with Sclerotinia blight. The incidence of S. minor from 'VA 81B' peanut seed harvested and dried in Virginia according to recommended procedures was 4.2%. Seed with brown testa were colonized more frequently (5.7%) than seed with tan or normal colored testa (2.7%). The incidence of S. minor from seed with brown testa from five field sites was 3.6, 4.6, 5.0, 7.6, and 7.8%. The incidence from seed with tan testa from the same field sites was 1.2, 2.8, 2.8, 3.0, and 3.6%. The incidence of S. minor from seed testa averaged 3.4% while the incidence from cotyledonary tissues averaged 0.1%. A recommended seed treatment (Botec 4 oz./cwt) reduced the incidence of S. minor to 0.1%.

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MOISTURE VARIABILITY AMONG INDIVIDUAL SEEDS OF SOYBEAN FROM THE SAME POD BEFORE HARVESTING. F. A. Lazzari and R. A. Meronuck, Department of Plant Pathology, University of Minnesota, St. Paul, MN 55108.

Moisture is important in storability of soybeans. Variability in moisture content (MC) among single seeds in stored soybeans may affect susceptibility to invasion by storage fungi.

The moisture variability among single seeds of soybean from different positions inside the same pod was determined using oven-dried single seeds at 103 C for 72 hours in copper cups. The average MC of individual seeds from the respective position were: 13.20% (seed near pedicel), 12.60% (middle seed), and 11.10% (farthest seed from pedicel).

The fresh weight of single seeds was inversely proportional to the MC, i.e., 0.1363 g (seed near pedicel), 0.1430 g (middle seed), and 0.1451 g (farthest seed from pedicel). While the moisture content was highest in seeds nearest the pedicel, the fresh weight was lowest.

We conclude that variation in MC exists in mature seeds inside the pod before harvest.

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SUPPRESSION OF STORAGE FUNGI IN GRAIN WITH SOYBEAN OIL. D. C. McGee, A. Iles and M. K. Misra, Seed Science Center, Iowa State University, Ames, Iowa.

Effects of soybean oil, applied to grain at rates used to reduce dust in elevators, on storage fungi, were examined. Corn and soybeans at 17% and 15% moisture content, respectively, were treated with soybean oil at 200 ppm, alone or in combination with thiabendazole at 20 ppm, then stored on-farm in aerated metal bins, with a capacity of 250 kg. After 12 months, kernel infection by Penicillium and Aspergillus spp was 83.0% and 63.7%, respectively, in untreated corn, compared to 60.0% and 46.2%, in soybean oil-treated corn, and 24.7% and 17.0%, in soybean oil + thiabendazole-treated corn. After 12 months, soybean seed infection by Penicillium and Aspergillus spp. was 45.7% and 39.2% , respectively in untreated seeds, 17.7% and 8.2% in soybean oil-treated seeds, and 1.7% and 2.0%, in soybean oil + thiabendazole-treated seeds. The mechanism for reduction in storage fungal growth by soybean oil was not fungicidal.

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A COMPARISON OF THE EPIPRE AND KENTUCKY DECISION GUIDE PREDICTIVE SYSTEMS FOR SCHEDULING FUNGICIDE APPLICATIONS TO CONTROL LEAF DISEASES OF WHEAT IN THE NETHERLANDS AND IN KENTUCKY. R.E. Stuckey, J.C. Zadoks, D.E. Hershman, and W.P. Clinton. University of Kentucky, Lexington, KY 40546-0091 and Agricultural University, Wageningen, The Netherlands.

Predictive decision guides for scheduling fungicide application to control leaf diseases of wheat were developed in Kentucky (KDG) and in The Netherlands (EPIPRE) in response to grower interest in intensive wheat management. In The Netherlands, treatment yields in replicated plots were highest in the traditional protective (PROT) fungicide applications, followed by KDG, EPIPRE, and non-sprayed control, respectively. Dollar return per há above control plots were 151, 126, and 79 for KDG, EPIPRE, and PROT, respectively. At Lexington, Kentucky, increased yields and economic returns for all fungicide treatments occurred such that: KDG and EPIPRE > PROT > non-sprayed control. The effectiveness and limitations of deploying computerized and in-field predictive systems in foreign countries are discussed.

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EFFECT OF TILLAGE AND ROTATION ON YIELD AND STALK ROT OF CORN GROWN IN POORLY DRAINED SOIL IN NORTHWEST OHIO. P. E. Lipps and I. W. Deep, Dept. of Plant Pathology, Ohio State Univ., Wooster, OH 44691.

A field plot was established in 1985 on Hoytville silty clay loam soil in Northwest, OH, to determine the effect of tillage (fall plow vs. no tillage) and crop rotation (continuous corn vs. corn-soybean rotation) on yield and stalk rot of corn during 1987 and 1988. Analysis of variance indicated that rotation, but not tillage, was significant ($P = 0.05$) for yield and tillage, but not rotation, was significant for incidence of stalk rot. Stalk rot incidence was positively ($r=0.73$, $P < 0.01$) and negatively ($r=-0.21$, $P=0.06$) correlated with yield in 1987 and 1988, respectively. Fusarium graminearium and F. moniliforme were the primary stalk rot pathogens in 1987 and 1988, respectively. Results indicated that stalk rot was less in no tillage and was not responsible for the yield depression associated with continuous corn in poorly drained soil.

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EFFECTS OF TILLAGE AND CROP ROTATIONS ON COMMON ROOT ROT OF WHEAT IN TEXAS. J. T. Mathieson, C. M. Rush, D. Bordovsky, and E. Clark, Texas Agric. Exp. Station, and O. R. Jones, U.S.D.A.-A.R.S., Bushland, Texas.

Effect of tillage and crop rotation on the soil population of Bipolaris sorokiniana in the soil and the development of common root rot were studied at three locations. Spore populations were determined at 10-cm increments. Significantly more spores were found in the top 10-cm of soil. Severity and incidence of disease were evaluated by rating discoloration of the subcrown internodes. At Bushland, the cropping sequence had no effect on the measured parameters, but tillage did. Conventional till plots generally had significantly more spores, diseased plants, and higher disease indices compared to reduced till plots. A similar pattern existed at Munday. At Chillicothe, the crop rotation scheme had a greater effect on the measured parameters than tillage. Continuous wheat plots had a significantly higher number of diseased plants and disease index in the fall of 1988 as did plots with the wheat sorghum fallow rotation.

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CULTIVAR RESISTANCE TO RICE KERNEL SMUT. Fleet N. Lee and G.S. Jones. University of Arkansas, P.O. Box 351, Stuttgart, AR 72160

Rice cultivars Mars, Lemont, Lebonnet and Bond appeared resistant and cultivars Tebonnet, Newbonnet and Labelle were susceptible to kernel smut (Tilletia barclayana (Bref.) Sacc. & Syd.) in a 3 year survey. Rough rice samples of the cultivars averaged 4, 5, 6, 7, 20, 58, and 77 smutted kernels per kg, respectively. Selected cultivars were inoculated with spore suspensions either by injection into the flag leaf whorl at three-inch panicle development (stage 1), early swollen boot (stage 2), 1-2 days prior to panicle exsertion (stage 3); or by misting the exposed panicle prior to anthesis (stage 4) or during anthesis (stage 5). Inoculated panicles had 12, 27, 28, 0.6, and 0.2 % smutted kernels for growth stages 1 through 5, respectively. Smut incidence in the inoculated tests did not correspond with cultivar resistance observed in the survey.

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PRIMARY INOCULUM SOURCES OF RICE BLAST (PYRICULARIA ORYZAE, CAV.) IN ARKANSAS. Fleet N. Lee and G.S. Jones, University of Arkansas, P.O. Box 351, Stuttgart, AR 72160

International races IB-49 and IC-17 of Pyricularia oryzae Cav. became widespread during 1986 and 1987. Seedling blast occurrence in 1987 suggested that primary inoculum originated within Arkansas. Random samples of crop stubble from fields, research plots and nearby native grasses were collected during the winter and early spring of 1987 and 1988. Sixteen of 58 grower stubble samples and 34 of 70 research plot stubble samples assayed positive on susceptible rice plants. Assays of 116 grass samples were negative. Commercial Newbonnet seed rice for 1988 was obtained for assay. An average of 1.8×10^4 spores were flushed from the dry seed with water in 61 of the 68 samples. A maximum of 13.5×10^4 spores were found in a single sample. Spore suspensions from 65 of 71 of dry, non-sterilized rice seed induced blast lesions on assay plants. Sixty-five percent of the same samples assayed positive following surface sterilization with sodium hypochlorite, and 72 hours incubation.

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EFFECT OF SOIL FERTILITY LEVELS OF NITROGEN, PHOSPHORUS AND POTASSIUM ON CELL DEATH AND SPREAD OF COLLETOTRICHUM GRAMINICOLA IN SORGHUM STALK TISSUE. R. A. Katsanos, Natural Sciences and Mathematics, Medgar Evers College, Brooklyn, New York 11225.

One variety of sorghum (Sorghum bicolor (L) Moench.) was planted in the greenhouse in 3 replications, each replication divided in 9 nitrogen, phosphorus and potassium combination treatment plots of normal, high and low N,P,K. Certain plants of all treatments were inoculated with the stalk rot pathogen Colletotrichum graminicola (Cesati) Wilson. Four times during the growing period, uninoculated plants were rated for stalk cell death, and inoculated ones for spread of the fungus and the two ratings were compared. The low N-P high K treatment produced the least stalk tissue death rate and also the least spread of the fungus in the inoculated plants, followed by the normal N-P-K. The high N-P low K treatment produced the highest death rate and fungal spread. (Supported, in part, by PSC/CUNY grant # 6-67205.)

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PRESENCE AND ROLE OF MYCOSPHAERELLA GRAMINICOLA IN CALIFORNIA WHEAT. R. B. Madariaga, D. G. Gilchrist, and A. N. Martensen. Department of Plant Pathology, University of California, Davis, CA 95616.

Septoria leaf blotch (SLB) is one of the most important limitations to yield of wheat in California. Pseudothecia of Mycosphaerella graminicola were first found in California in the late fall of 1987. Field stubble, heavily infected by SLB in the previous season, was monitored weekly during the fall of 1987 and 1988 for M. graminicola. Ascospores discharge was recorded under controlled conditions as ascospores/pseudothecia/per leaf/ per week. Samples collected in December 1987 and November 1988 produced a bicellular ascospore (13 X 5µm) which germinated by budding without formation of a germ tube. Monoascosporic isolates produced lesions typical of SLB, contained pycnidia and viable pycnidiospores of Septoria tritici, the anamorph of M. graminicola. Variation in virulence among the monoascospore isolates, biological parameters of the teleomorph and the role of the bud-conidia in the disease cycle will be discussed. This is the second report of the teleomorph in U.S.

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YIELD CONSTRAINTS IN WHEAT DUE TO SOILBORNE PATHOGENS. E.A. Milus and C.S. Rothrock, Dept. of Plant Pathology, University of Arkansas, Fayetteville, AR 72701.

The importance of soilborne pathogens as constraints to wheat yield and quality in Arkansas was determined in a split, strip plot experiment. Main plots were raised or conventional seed beds. Subplots were fumigated (Bromogas 67), metalaxyl-treated seed, and a nontreated check. Sub-subplots were planted with wheat (cv. Keiser, Florida 302, or Caldwell) or oats (cv. Bob). In January plants were dug from the ends of each plot, growth parameters were measured, and the entire crown and root system plated onto water agar. Fungal colonies were transferred to PDA, identified to genus, and representative isolates were tested for pathogenicity. Bedded plots had twice the number of plants infected with Helminthosporium sativum. Plants from fumigated plots had more tillers/plant, greater top weight, and fewer plants infected with Pythium, Helminthosporium sativum, and Rhizoctonia. Pythium was the most common genus isolated.

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RECOVERY OF WHITE AND GREEN ISOLATES OF ASPERGILLUS FLAVUS FROM INDIVIDUAL KERNELS FROM DOUBLE-INOCULATED MAIZE EARS. Natale Zummo, USDA-ARS, Dept. of Plant Pathology and Weed Science, Mississippi State, MS 39762.

Maize ears were inoculated in the field with a white isolate of Aspergillus flavus at 2 days after midsilk in the shank or through the husk in the base of the cob and re-inoculated 4 days later with a green isolate of the fungus through the husk at the top of the ear or in the silk channel. Reciprocal inoculations were made. Ears were harvested at 60 days after midsilk, dried, and shelled. When kernels from these double-inoculated ears were assayed, the white isolate was recovered singly from 8% of kernels, the green isolate was recovered singly from 11.2% of kernels, while both isolates were isolated from 3% of kernels. The infection data indicate that there may be multiple pathways for infection of maize kernels by A. flavus in the field.

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Influence of Pythium spp. on survival and agronomic traits of winter oats. S. Leath and J. P. Murphy. USDA-ARS and Depts. of Plant Pathology and Crop Science, respectively, North Carolina State University, Raleigh 27695.

Field plots were established in early October in 1987 and 1988 in the piedmont and mountain regions of North Carolina. Ten winter oat cultivars varying in winter hardiness and four chemical treatments, metalaxyl seed treatment and drench, benomyl drench, methyl bromide fumigation and a untreated control, were arranged in a factorial treatment design in 5 complete blocks at both locations. Data were recorded on emergence, winter damage, tillers/m, height, grain yield, 500 kernel weight, and seeds/head. Data from 1988 indicated that kernel weight was reduced, but height and seeds/head increased in metalaxyl treated plots with regard to the untreated check. Root and crown isolations indicated that Pythium spp. colonized winter oats within a week of sowing at high frequency.

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Effects of crop rotation, tillage and straw management on common root rot of wheat. Lawn, D. A. and Sayre, K. D. CIMMYT, Apdo. Postal 6-641, Deleg. Cuauhtemoc, 06600 MEXICO D.F.

Incidence and severity of common root rot were evaluated on bread wheat variety Kauz "S" at El Batan, MEXICO. Treatment design was a split-split plot; conventional/reduced tillage as main plots, sub plots of 4 different rotation schemes, and burn straw/chop and incorporate straw as sub-sub plots. Rotations were Summer86/Winter/Summer87/Winter/Summer88 and included: #1 bread wheat (BW);oats(O);durum wheat(DW);(O);(BW); #2 (BW);rape(R);(DW);(R);(BW); #3 (BW);vetch(V);(DW);(V);(BW); #4 (BW);(V);maize;(V);(BW). Data from the Summer of 88 indicated that Bipolaris sorokiniana was the predominant root pathogen at 6 and 12 weeks after planting (WAP). Fusarium graminearum and F. culmorum were isolated from <10% of sampled plants. There were no significant differences on root rot or yield due to tillage or straw management. Rotation #4 had a significantly lower incidence of subcrown internode infections at 6 and 12 WAP and the highest yield, grain test weight and number of spikes/m^2.

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PERSPECTIVES OF DISEASE THREAT IN LARGE-SCALE PINUS RADIATA MONOCULTURE —THE NEW ZEALAND EXPERIENCE. C.K.S.Chou, Forest Research Institute, Rotorua, New Zealand.

About 95% of New Zealand wood production today comes from exotic plantations (mainly *Pinus radiata*). For over 6 decades this forestry monoculture, now 1.1 million ha, has withstood the threat of disease. In the late 1940's 25-35% of 20-25-year-old trees (unthinned stands) were killed in a *Sirex-Amylostereum* epidemic. However the economic loss was insignificant. Aerial spraying to control Dothistroma needle blight since 1966 has cost around $14.5 million. Losses caused by other diseases, i.e., *Sphaeropsis sapinea*, Armillaria root-rot, and Cyclaneusma needle-cast, have been locally severe but tolerable overall. The current practice of waste thinning 75-85% of initial stockings before age 10-12 allows a high level of acceptable loss for diseases which mainly attack young stands, while clear-felling at age 25-30 obviates diseases that affect older stands. However, New Zealand still has only a few pine pathogens and with the trend towards clonal forestry, genetic uniformity, and reduction of initial/final crop stocking ratios, the history of disease in this country is no way near its end.

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HYPOVIRULENT STRAINS OF CRYPHONECTRIA PARASITICA IN NEW JERSEY. P.J. Bedker. Department of Horticulture and Forestry, Rutgers University, New Brunswick, NJ 08903.

Approximately 30 isolates of C. parasitica were collected from cankers on American chestnut at 4 locations. The virulence of each was determined by inoculating "Granny Smith" apple fruits. Mean radial growth (MRG) was determined by measuring the length and width of lesions at 17 days after inoculation for 8 replications / isolate. Each apple was also inoculated with strain EP155 from Connecticut. The MRG for isolates collected from sites in Howell and Millstone Twps. (Monmouth Co.), Plumsted Twp. (Ocean Co.), and Sandyston Twp. (Sussex Co.) were 18.2, 19.1, 17.3, and 18.4 mm, respectively. The MRG for 37.5 % (45/120) of the isolates was significantly ($P<0.05$) less than EP155. In relation to EP155, 7 isolates had reduced MRG values greater than 1.0 cm. Currently, dsRNA has been extracted from two of these strains. Studies are underway to identify the relationship of mean difference in radial growth of C. parasitica on apple fruits and he occurrence of dsRNA.

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CONTROL OF RHIZOCTONIA SEEDLING BLIGHT OF LONGLEAF PINE. G.B. Runion, W.D. Kelley and D.H. Land, School of Forestry, Auburn University, AL 36849.

Eight fungicides with known activity against Rhizoctonia spp. were effective in restricting growth of one loblolly and three longleaf pine isolates of a binucleate Rhizoctonia sp. (CAG-3). Growth inhibition was determined in the laboratory on potato dextrose agar amended with appropriate test fungicide at concentrations of 1, 10 and 100 ug a.i./ml. Subsequently, three fungicides (benodanil, diniconazole, and SN-84364) were tested in a preliminary field study at rates of 140 and 280 g a.i./ha applied on a three-week spray schedule. At lifting time, average groundline diameter was significantly lower and percentage of cull seedlings was significantly higher for seedlings from plots that received diniconazole sprays than for other seedlings. No significant differences among treatments were observed for any other seedling size parameter or for the number of seedlings exhibiting symptoms of Rhizoctonia infection at any disease evaluation date.

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INVESTIGATION OF DECLINE AND MORTALITY OF SUGAR MAPLE AND OTHER NORTHERN HARDWOODS ACROSS A RAIN PH GRADIENT IN WISCONSIN. M.E. Mielke[1], J. Cummings Carlson[2], C.L. Rezabek[2], and B.B. Eav[3], U.S. Forest Service, St. Paul, MN[1] 55108 and Fort Collins, CO[3] 80524, and Dept. of Nat. Res., 3911 Fish Hatchery Road, Madison, WI[2] 53711. Long term wet deposition monitoring has established the presence of an annual weighted mean pH gradient (≤4.4 to ≥4.8) over the range of sugar maple in Wisconsin. Multistage sampling with color infrared aerial photography and ground surveys was conducted to determine the number and volume of healthy, declining and dead sugar maple and associated hardwoods on four million acres across the pH gradient. Based on the ground survey, there were .4 to 1.1 dead or declining sugar maples per acre. The number of dead and declining sugar maples was not related to the pH gradient. Of the 171 dead or declining sugar maples examined, 64% had recognizable insect or disease pests. Armillaria root rot (Armillaria mellea), Eutypella canker (Eutypella parasitica) and sapstreak (Ceratocystis coerulescens) were the most common pests observed.

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THE INCIDENCE AND IMPACT OF SPHAEROPSIS SAPINEA ON PINES IN SOUTH AFRICA. W.J. Swart and M.J. Wingfield, Departments of Plant Pathology and Microbiology, University of the Orange Free State, Bloemfontein 9300, South Africa.

Surveys were conducted over a period of four years to determine the occurrence of Sphaeropsis sapinea diseases in plantations of exotic Pinus spp. in South Africa. The pathogen was associated with shoot blight, canker, root disease and blue stain. Dieback, resulting from shoot blight was the most common cause of tree death, with more than 70% of cases associated with hail damage and moisture stress. Infection associated with moisture stress was more common than with hail damage, but the latter had a far greater impact. The most extensive outbreak of dieback occurred after hail damage in a severely drought stressed plantation of P. radiata. Timber losses due to premature clearfelling in this instance were 28% of the volume and 54.5% of the value of potential production. Inoculation trials with S. sapinea indicated that P. radiata was the most susceptible species, followed by P. pinaster and P. patula. The most resistant species was P. taeda followed by P. elliottii. Our observations indicate that S. sapinea is the most important pathogen of Pinus spp. in South Africa.

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PREPARATION OF CELL-WALL-FREE PROTOPLASTS FROM THE CHESTNUT BLIGHT FUNGUS. F. H. Tainter, J. C. Jang, Clemson University, Clemson, SC 29634-1003, and W. L. MacDonald, West Virginia University, Morgantown, WV 26506-6057.

Certain isolates of Cryphonectria parasitica (Murr.) Barr contain cytoplasmically transmitted dsRNA which is believed to render mycelium hypovirulent. Practical utilization of hypovirulence in fungi is limited because of the general presence of mating incompatibility factors. A method of forcing vegetative fusion, and hence possibly transfection of the dsRNA, involves use of electromanipulation. This research reports the protocol necessary to produce cell-wall-free protoplasts. Two strains were used. E7 is white in culture and is a hypovirulent strain which produces large titers of dsRNA. Strain 591 is virulent, is dsRNA free, and produces a yellowish-brown culture. The cell walls of the E7 isolate were easy to remove enzymatically. E7 yielded approximately 10 times as many protoplasts as did 591 under identical growth conditions. Protoplasts from 591, however, were larger in overall size and had larger vacuoles than did E7. The cytoplasm of E7 appeared much denser.

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EFFECT OF STAND CONDITIONS ON ADVANCE OF PHELLINUS WEIRII IN DOUGLAS-FIR PLANTATIONS. W.J. Bloomberg, Pacific Forestry Centre, Forestry Canada, 506 W. Burnside Rd., Victoria, B.C. Canada, V8Z 1M5.

Advance of Phellinus weirii in 62 infection centers during five consecutive 5-6 yr periods was studied in relation to stand conditions in three Douglas-fir plantations. Stocking level (number of trees/ha) and average tree diameter were higher in areas in which the fungus advanced than in those in which it did not. Advances were more strongly related to tree diameter, whereas failures to advance were more strongly related to stocking level. Presence of other tree species or trees dead from other causes was also associated with failure to advance. The fungus advanced unevenly among 45° sectors of centers. Only 1-3% of advances in a sector followed an advance during the previous period, and 76-96% of non-advances followed non-advances in the previous period. There were significant differences in advances among plantations, centers and stand ages. Incidence of P. weirii in plantations was related to both stocking level and degree of tree aggregation.

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OAK DECLINE: GROWTH RING INDICATORS FROM A SOUTHERN BOTTOMLAND HARDWOOD SITE. F. I. McCracken and Ray Wolf*, USDA/Forest Service, Stoneville, MS 38776; *NOAA/National Weather Service, Stoneville, MS 38776

Radial growth of Quercus phellos L. and Q. nuttallii Palm. in decline and control plots near Stoneville, MS were evaluated and related to weather data. Trees were 54 to 89 years old in a mixed hardwood stand on a Sharkey clay site. Between 1915 and 1986, 18 years showed decreased radial growth and 16 of these years had below average annual rainfall. Radial growth was low in 1973-74 following extensive top breakage by ice, although rainfall was above average in both years. Radial growth was average or above in 5 years that had below average rainfall; however, precipitation was near normal during April-September. The mean basal area increment in decline plots was lower than controls from 1930 to 1986. Data shows short term weather effects on radial growth and effects of unidentified long term factor(s).

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FUNGI ISOLATED FROM NEEDLES OF BLIGHTED EASTERN WHITE PINE IN MAINE. T. Dreisbach, W. Merrill, Dept. of Plant Pathology, Penn State Univ. University Park, PA 16802.

A blight of current-year needles on Pinus strobus L. was documented in Acadia National Park, Maine, in 1983. From June 4 to August 4, 1988 three predominant symptom types were noted: chlorotic spots, necrotic tips, and chlorotic spots with necrotic centers. From June 13 to August 4, fungi were isolated from all symptom types as well as from green, asymptomatic needles. A black yeast was recovered throughout the period. A previously undescribed hysteriaceous fungus and a Leptostroma sp. were recovered from July 8-July 25 and July 8-July 29, respectively. A white, nonsporulating hyphomycete was recovered beginning July 1, Hendersonia pinicola beginning July 25, Truncatella truncata and a Septoria sp. beginning July 29, all in increasing amounts up to August 4. Thus, a succession of fungal species appeared to colonize needles during the isolation period.

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THE RELATIONSHIP OF DOUBLE-STRANDED RNA TO VIRULENCE AND MORPHOLOGY IN TYPE A AND TYPE B SPHAEROPSIS SAPINEA. Nyan-Tsz Wu[1], M. Palmer[2], and G. Adams[1]. [1]Michigan State University, Department of Botany and Plant Pathology, E. Lansing, MI 48824 and [2]Pacific NW Research Station, Corvallis, OR 97331.

Sphaeropsis sapinea causes Diplodia shoot blight and canker of many conifers. Two populations, type A and type B have been reported that differ in morphology and virulence. Our objective was to determine whether the slower growth rate, appressed colony morphology and low virulence of type B was correlated to the presence of dsRNA. We examined 40 isolates from pines for differences in morphology and virulence on four species of pine. Of these, ten of 20 isolates of type A and six of 20 isolates of type B contained dsRNA. In both groups, a portion of dsRNA-containing isolates were low in virulence whereas others were not. Single-spore cultures yielded a small portion of dsRNA-free conidia. The cured subcultures of dsRNA-containing isolates maintained group-specific colony morphology.

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Relationship of seedling height and dolomitic lime application to black cherry leafspot severity in northern Pennsylvania. G. Stanosz, PA Bur. For., 34 Airport Dr., Middletown, PA 17057, and L. Auchmoody, USDA For. Serv., Box 928, Warren, PA 16365.

Leafspot, caused by Blumeriella jaapii (Rehm.) v. Arx, causes defoliation and death of black cherry (Prunus serotina Ehrh.) seedlings. Terminal (≤15 cm) foliage of seedlings, (collectively) by height class (<15, 15-30, or >30 cm) in 227, 141, or 96 (respectively) previously limed (22.4 Mg ha-1) or unlimed milacre forest plots, was estimated visually as ≤10%, 11-50%, or ≥51% leafspot-affected in 1987. Percentages of plots with the most severe rating (by height) were 45, 27, and 10 (respectively). Percentages of plots with the most severe rating (limed: unlimed by height) were 26:66, 14:42, and 4:17 (respectively). Low severity on higher leaves may result from infrequent spore

deposition or unfavorable conditions for spore germination and infection. Low severity in limed plots may result from reduction of primary inoculum from leaf litter or nutrient-related differences in susceptibility. Results suggest little disease impact on regeneration after seedlings achieve heights >30 cm.

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ALLOZYME DIFFERENTIATION AMONG INTERSTERILITY GROUPS OF HETEROBASIDION ANNOSUM ISOLATES FROM EUROPE. W.J. Otrosina, T.E. Chase, F.W. Cobb, Jr. and K. Korhonen. USDA Forest Service, Pacific Southwest Forest and Range Experiment Station, P.O. Box 245, Berkeley, CA 94701; Department of Plant Pathology, University of California, Berkeley; Finnish Forestry Institute, Helsinki, Finland.

Starch gel electrophoresis was conducted for isolates from Finland, Germany, and Italy representing "P", "S", and "F" intersterility groups. No clear differences in mobilities were found between the "S" and "F" groups in any of the 12 loci analyzed. In general, mobilities for the MDH-2 locus for all European isolates were indentical to the North American "S" group. The ADH, IDH, and GDH locus showed consistent differences between the "P" and "S" or "F" groups. Other loci exhibit shared alleles among the intersterility groups, which is in contrast to the nearly total fixation present in most loci betweeen the "S" and "P" groups in western North America. Within the "S" and "P" groups, allele frequency differences are apparent between isolates from both continents.

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SEEDLING RESPONSE OF TWO FOREST TREE SPECIES TO VARYING DOSES OF OZONE USING OPEN-TOP CHAMBERS IN NORTHCENTRAL PENNSYLVANIA. M. Simini, J.M. Skelly, and D. D. Davis, Dept. of Plant Pathology, The Pennsylvania State University, University Park, PA 16802

Open-top chambers were established at three sites in northern PA. Four 2-year-old seedlings each of Prunus serotina Ehrh. and Liriodendron tulipifera L. were planted in 1987 in each of 16 plots in a randomized complete block design at each site. In 1988, the seedlings were exposed to ambient air (no chambers) or to air in filtered chambers which contained 94%, 59% or 42% of the ozone (O_3) in ambient air. The number of hours which O_3 exceeded 60 ppb at the three sites was 600, 646, and 451 hrs, respectively. Ozone-induced stippling occurred on the adaxial leaf surface of both species at all sites. Differential sensitivity was found among treatments and sites. Injury was correlated positively with ozone dose. Total height and basal diameter growth were correlated negatively with O_3 dose.

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DISTRIBUTION AND INSECTICIDAL ACTIVITY OF ALKALOIDS IN FUNGAL ENDOPHYTE-INFECTED GRASSES. M. R. Siegel, L.P Bush, N. Fannin, G.C.M. Latch, D.D. Rowan, and B.A. Tapper. Plant Pathology, University of Kentucky, Lexington, Ky. 40546; DSIR Palmerston North, New Zealand.

The distribution of N-formyl and N-acetyl lolines, peramine, lolitrem and ergovaline was determined in *Acremonium* spp.-and *Epichloe typhina*-infected cultivars and species of *Festuca, Lolium* and other grass genera. thirty of 34 host-fungus combinations produced alkaloids, peramine and ergovaline were the most common found alkaloids, while lolines and lolitrem were the least common. Three alkaloids (lolines, peramine, and ergovaline) were recovered from *A. coenophialum*-infected tall fescue and perennial ryegrass, while peramine, lolitrem, and ergovaline were present in *A. lolii* infected perennial ryegrass, tall fescue, and in *E. typhina* infected *F. longifolia*. Other host-fungus combinations produces only one or two of these alkaloid types. Aphid bioassays, using infected grass stems, indicated that loline alkaloids were toxic to *Rhopalosiphum padi* (Rp) and *Schizaphis graminum* (Sg); peramine was toxic only to Sg and ergovaline was not toxic to either aphid. Lolitrem was not toxic to Rp, but it could not be determined if the alkaloid was toxic to Sg as peramine was always present in the same infected grasses. The relationship of alkaloid synthesis in infected grasses and resistance to herbivory will be discussed.

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PHOTOSYNTHESIS AND STOMATAL RESPONSE IN A SUSCEPTIBLE ALFALFA CLONE INFECTED WITH VERTICILLIUM ALBO-ATRUM. B.W. Pennypacker, D.P. Knievel, K.T. Leath and E.J. Pell, Penn State Univ. and USDA-ARS, U.S. Regional Pasture Lab, Univ. Park, Pa. 16802.

Photosynthesis was measured on a susceptible alfalfa clone infected with V. albo-atrum and compared to that of control plants. Infected plants had symptoms of Verticillium wilt, but photosynthesis was determined only on asymptomatic leaves. The leaf being measured was placed in a sampling chamber and exposed to ca. 750 ppm CO_2. Sequential measurements were taken at 15 sec. intervals for 20 min. as CO_2 was depleted by photosynthesis. A/Ci response curve analysis was used, which allowed in vivo assessment of the amount of active RUBISCO in the leaf. Leaves on infected plants had significantly lower photosynthetic rates and lower stomatal conductances at ambient CO_2 (340 ppm), and significantly reduced levels of active RUBISCO. Protein extraction and subsequent total activation of extracted RUBISCO with ^{14}C indicated a significant reduction in the total amount of RUBISCO in infected leaves.

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EFFECTS OF PLANT GROWTH REGULATORS ON ACREMONIUM COENOPHIALUM GROWTH IN CULTURE. G. E. Huff, K. D. Gwinn, and C. E. Sams. Departments of Entomology and Plant Pathology and Plant and Soil Science, University of Tennessee, P.O. Box 1071, Knoxville, TN 37901.

Growth of the fungal endophyte (Acremonium coenophialum Morgan-Jones and Gams) is stimulated during the transition of tall fescue (Festuca arundinacea Schreb.) from the vegetative to reproductive state. This study was initiated to determine if plant growth regulators elicit a growth response by the endophyte. Plant growth regulators, dicamba and gibberellic acid, had no significant effect on the growth rate of the endophyte in culture. Kinetin completely inhibited growth of the endophyte at a concentration of 100 µM, and reduced endophyte growth by 75-80% at 80 µM. Other plant growth regulators are currently being examined to determine in vitro effects on endophyte growth.

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A cultivar-specific elicitor of the hypersensitive response in soybean has been identified which is associated with expression of the avirulence gene, avrD. Mark M. Stayton*, Stanley J. Tamaki**, and Noel Keen§ *Department of Molecular Biology, University of Wyoming, Laramie, WY 82071; **Cleargene, Inc., University of California-Richmond Field Station, Richmond, CA 94804-4698; §Department of Plant Pathology, University of California at Riverside, Riverside, CA 92521-0122.

A bacterially produced, low molecular weight compound, has been idenitified which is a cultivar specific elicitor (SE) of the hypersensitive response in soybean. The presence of the SE compound is associated with the expression of the avirulence gene, avrD, originally cloned from Pseudomonas syringe pv. tomato (Kobayashi et. al. PNAS 86:157, 1989). Expression of the avrD gene in P.s. pv. glycinea R4 alters the interaction of the bacteria with the cultivars 'Harosoy' and 'Norchief' from compatible to incompatible. The presence of the avirulence gene, however, does not alter the compatible interaction with the cultivar 'Acme'. Furthermore, E. coli cells overexpressing the avrD gene elicit the hypersensitive response on exactly the same set of cultivars as does P.s. pv. glycinea R4-avrD. The SE compound can be isolated from cell-free culture supernatants of E. coli, P.s. pv. tomato and P.s. pv. glycinea R4 which express the avrD gene. In partially purified extracts, the SE compound causes a systemic necrosis only in cultivars which are incompatible with bacterial strains expressing the avrD gene. We have purified, by reverse-phase HPLC, a biologically active SE and have initiated an analysis of its chemical structure.

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A model for the gene-for-gene interaction between P.syringae and soybean. Stanley J. Tamaki*, Mark M. Stayton** and Donald Kobayashi§ *Cleargene, Inc., University of California-Richmond Field Station, Richmond, CA 94804-4698; **Department of Molecular Biology, University of Wyoming, Laramie, WY 82071; §Department of Plant Pathology, University of California at Riverside, Riverside, CA 92521-0122.

In gene-for-gene systems the interaction between a dominant resistance gene in the host and a corresponding avirulence gene in the pathogen results in an incompatible interaction characterized by the hypersensitive response (HR). We have isolated a low molecular weight, cultivar-specific elicitor (SE) from a phytopathogenic bacterium; suggesting that the primary gene product of the avirulence gene is not directly involved in the recognitional event. Rather, the avirulence protein is predicted to possess a catalytic activity involved in the synthesis of the specific elicitor. The current models hypothesize the presence of a host receptor, the resistance gene product, which interacts directly with the specific elicitor and triggers the cascade of events manifested as an HR. Alternatively, the target within the plant for the specific elicitor may not be a dedicated receptor but may play other roles in plant metabolism. The biological effect of the SE shows parallels those of host-specific toxins produced by various Helminthosporium spp. and Alternaria spp. We will discuss the similarities in biological activity between the specific elicitor and various host-specific toxins and propose a working hypothesis which may account for both types of specificity.

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OXALIC ACID, K_2HPO_4 AND K_3PO_4 AS INDUCERS OF SYSTEMIC RESISTANCE AGAINST DISEASES CAUSED BY FUNGI, BACTERIA, AND VIRUSES IN CUCUMBER. Mucharromah and J. Kuc, Dept. of Plant Pathology, University of Kentucky, Lexington, Kentucky 40546

Oxalic acid, K_2HPO_4 and K_3PO_4 induce systemic resistance to cucumber anthracnose caused by Colletotrichum lagenarium as effectively as induction with C. lagenarium. Spraying with either oxalic acid, K_2HPO_4 or K_3PO_4 on the abaxial surface of $leaf_1$ also induced systemic resistance against Cladosporium cucumerinum, tobacco necrosis virus, Sphaerotheca fuliginea, Pseudomonas lachrymans, Mycosphaerella melonis, cucumber mosaic virus and Erwinia tracheiphila. This evidence supports the hypothesis that plants have the potential for resistance to many pathogens, and that defense mechanisms can be induced by stress as well as by infection.

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DYNAMICS OF THE INTERACTION OF THE TERPENOID PHYTOALEXIN DESOXYHEMIGOSSYPOL AND VERTICILLIUM DAHLIAE. M. E. Mace, R. D. Stipanovic, M. H. Elissalde, and A. A. Bell, USDA, ARS, Southern Crops, and Veterinary Toxicology and Entomology Research Labs, Rt. 5, Box 805, College Station, TX 77840.

Desoxyhemigossypol (dHG) is the principal phytoalexin in Verticillium dahliae-infected Gossypium barbadense cotton. About 75% of dHG is absorbed from nutrient solution by V. dahliae conidia in 1 min. Metallic trace elements such as Fe^{3+} and Mn^{2+} increase the rate of oxidation of dHG to its aldehyde product hemigossypol. The toxicity of dHG, assayed by a tetrazolium technique, to a defoliating isolate of V. dahliae was not significantly changed by the addition of trace elements. Evidence for the presence of the toxic hydroxyl free radical, a putative decomposition product of dHG, is equivocal.

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EFFECTS OF VOLATILE LEAF SURFACE COMPOUNDS ON GERMINATION OF PERONOSPORA TABACINA SPORANGIA. Mary L. Menetrez, Dept. of Plant Pathology, David A. Danehower, Dept. of Crop Science, N.C. State University, Raleigh, NC 27695 and Harvey W. Spurr, Jr., USDA-ARS, Crops Research Lab., Oxford, NC 27565.

In vitro microbial bioassays were done to determine the influence of plant volatile oils on germination of P. tabacina sporangia. Aqueous spore suspensions were exposed to vapors from authentic compounds of major components of the volatile mixture emitted by tobacco. Germination was completely inhibited when freshly harvested spores were exposed to volatile ketones and aldehydes for 4 hours. Short exposures (30 sec-15 mins) to 3-octanone, nicotine, 1-methyl naphthalene or 2-ethyl-1-hexanol resulted in slight stimulation of germination. Significant stimulation of germination occurred when spores which were not freshly harvested were exposed to beta ionone, methyl salicylate or nicotine. Leaf surface interactions appear more complex since observing that leaf surface components may alter germination by pathogens.

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EVIDENCE AGAINST POTATO AND TOMATO HOST SPECIFICITY IN *PHYTOPHTHORA INFESTANS*. L. J. Spielman, B. J. McMaster, and W. E. Fry, Cornell University, Ithaca, NY 14853.

We have investigated host specialization in *P. infestans* by comparing pathogenicity on susceptible potato and tomato cultivars, using detached-leaflet tests. Of 54 isolates from potato cultivars and wild *Solanum* species (tested less than 1 year after collection), 47 were pathogenic on both hosts, 3 were pathogenic on potato but showed low pathogenicity on tomato, and 4 showed low pathogenicity on both hosts. Three isolates from tomato were highly pathogenic on both potato and tomato. Progeny of sexual crosses between parents pathogenic on both potato and tomato were also tested. The coincidence of pathogenicity on potato with pathogenicity on tomato ranged from 92-96% for three separate crosses. This evidence does not support the hypothesis of host specialization in *P. infestans*, and indicates that potato and tomato pathogenicities are largely controlled by the same genes.

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SELECTION AND USE OF OXALATE MINUS MUTANTS TO STUDY PATHOGENICITY OF SCLEROTINIA SCLEROTIORUM, CAUSE OF BEAN WHITE MOLD DISEASE. G. Godoy, J.R. Steadman, R. Dam, and M. Dickman, Univ. of Nebraska, Dept. of Plant Pathology, Lincoln, NE 68583-0722.

Mutants of a Nebraska isolate of S. sclerotiorum were derived from UV irradiation of ascospores. Deficiency in oxalic acid production (OA-) was screeened by color change on potato dextrose (PD) agar containing 50 mg/l bromophenol blue. Determinations by enzymatic method, GC and HPLC indicate that the five selected mutants do not produce oxalic acid when grown on PD broth, nutrient agar, bean agar, or bean blossoms. In growth chamber experiments using leaves and stems on Phaseolus vulgaris plants, and in tests using detached leaves and pods, these OA- mutants were non-pathogenic. Lack of pathogenicity remained after over 30 generations of laboratory subculturing while the wild type remained pathogenic. Although the mutants and wild type were phenotypically alike, none of the mutants produced sclerotia. The role of oxalic acid in white mold disease will be discussed.

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STUDIES ON HOST-PARASITE RELATIONS IN OPHIODOTHELLA VACCINII. R.T. Hanlin and C.W. Mims, Department of Plant Pathology, University of Georgia, Athens, GA 30602.

Ophiodothella vaccinii causes a leafspot disease of Vaccinium arboreum, an understory shrub in the southeastern United States. Infection occurs in early summer, and although not observed, penetration appears to occur directly through epidermal cells. Thin-walled fungal hyphae spread throughout the leaf, forming a loose network of inter- and intra-cellular mycelium. When a hypha reaches a cell wall, it passes through by means of a slender penetration peg, which expands to normal size on the other side of the wall. Invaded epidermal cells overlying conidiomata become somewhat flattened and the hyphae in them secrete a dark pigment, forming a shiny black clypeus. As the season progresses, hyphae proliferate to completely fill the leaf tissues. These hyphae develop thick walls and many lipid globules, and serve to carry the fungus through overwintering and development of ascomata the following spring.

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INDENTIFICATION OF THE GENE FOR INDOLE ACETIC ACID LYSINE CONJUGATION FROM PSEUDOMONAS SYRINGAE PV. SAVASTANOI. F. F. White, H. J. Klee, R. Nordeen, and F. Roberto. Department of Plant Pathology, Kansas State University, Manhattan, KS 66506 U.S.A.

The open reading frame (orf) for the gene directing the conjugation of indole acetic acid to the epsilon nitrogen of lysine was identified. The region conferring activity was identified in pLG87 and sequenced. A 1185 bp orf was functional for conjugation activity and directed the synthesis of a 40 kd protein when introduced into the T7 promoter expression plasmid pT7-7. The gene is in the process of being introduced into tobacco plants under the control of the 35S CaMV promoter. The presence of the iaaL gene as well as auxin biosynthetic genes in various Pseudomonas pathovars will be discussed.

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EFFECT OF CULTURE FILTRATE AND MYCELIAL HOMOGENATES OF PHYTOPHTHORA INFESTANS ON VIABILITY OF POTATO PROTOPLAST. U. Roongruangsree, R. J. Young, and K. L. Deahl. Dept. of Plant Pathology, West Virginia University, Morgantown, WV 26506-6057, USDA-ARS, Bldg. 04, BARC-West, Beltsville, MD 20705.

Reaction of leaf protoplasts (PP) to culture filtrates and mycelial homogenates (MH) was studied using PP from Solanum tuberosum and fungal components of Phytophthora infestans (Pin). Comparisons were made between 2 r-gene cultivars (cvs.), cvs. with R-genes, and a non-host, S. carolinense. Culture filtrates and MH Pin were prepared from lima bean broth and agar plates. Viable PP were assessed by fluorescein diacetate at 0, 10, and 30 minutes. Culture filtrates had no effect on reducing PP viability. MH reduced viability of PP from all cvs. The mean number of viable PP after 10 and 30 minutes reaction time for R-gene cvs. was 68% and 42%, respectively, whereas viability in r-gene cvs. was 58% and 34% for the same reaction period. R-gene cvs. appeared to be less sensitive to MH treatment than r-gene cvs.

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THE EFFECT OF COMPOSTED MUNICIPAL SEWAGE SLUDGE (CMSS) ON PHYTOPHTHORA ROOT ROT AND SCLEROTINIA CROWN AND STEM ROT OF ALFALFA. R. P. Woodward and R. B. Carroll, Department of Plant Science, University of Delaware, Newark, DE 19717-1303.

Saranac alfalfa was planted into 15.2 cm pots containing autoclaved silt loam soil amended with CMSS obtained from three municipalities. Experimental design was a randomized complete block with five replications. Treatments were loading rates of 11.2, 22.4, 44.8, 112.0, and 224.0 Mg/ha of each CMSS. Phytophthora megasperma f. sp. medicaginis (Pmm) and Sclerotinia trifoliorum (St) inocula were applied separately via mixing into each amended soil prior to planting. After nine weeks, disease development was rated. No significant differences (P=0.05) were noted with respect to CMSS origin. Significant Pmm disease suppression occurred at 44.8 and 112.0 Mg/ha. Suppression of disease caused by St occurred at all loading rates. Isolations from infected root and crown tissues indicated less Pmm was recovered as the CMSS rates increased but St remained constant.

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EFFECT OF INSECT DEFOLIATION ON SEVERITY OF FUSARIUM CROWN-ROT OF ALFALFA. P. D. Colyer,[1] J. W. Lee,[2] and S. S. Quisenberry.[2] Louisiana State University Agricultural Center, Louisiana Agricultural Experiment Station, Red River Research Station,[1] Bossier City, LA 71113 and Department of Entomology,[2] Baton Rouge, LA 70803.

Alfalfa, Medicago sativa L. variety 'Florida 77', was inoculated with three different isolates of Fusarium and defoliated to varying levels with yellowstriped armyworms, Spodoptera ornithogalli (Guenee), to determine the effect of insect defoliation on the development of crown-rot under greenhouse conditions. There were no significant interactions between short-term insect defoliation and Fusarium crown-rot on forage quality, yield, or root carbohydrate reserves. Although insect defoliation alone did reduce plant height, yield, and maturity (18,33, and 30%, respectively) at the first harvest, no significant effects were observed at two subsequent harvests. Fusarium oxysporum Schlecht was the most virulent of the three isolates tested.

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CORRELATION BETWEEN SAMPLES FOR ESTIMATION OF INOCULUM DENSITY OF CYLINDROCLADIUM CROTALARIAE IN SOIL. A. K. Culbreath, M. K. Beute, and B. B. Shew, Depts. of Plant Pathology, Coastal Plain Expt. Stn., Tifton, GA 31793 and North Carolina St. Univ., Raleigh, NC 27695.

Four quadrants, each consisting of 63 (13.7 m^2) contiguous plots were established in a field naturally infested with Cylindrocladium crotalariae in Martin County, NC, in 1988. Half of the field was planted to corn the previous year and an adjacent area was planted to peanuts. Two quadrants (designated peanut or corn) were situated in each area. Two independent samples, each consisting of 12 (2.5 cm diam. x 16.5 cm) soil cores, were taken from all plots. Inoculum density (ID) of C. crotalariae was estimated using an elutriation-selective medium technique. Both samples gave similar estimates of mean ID in each quadrant; ID was greatest in peanut quadrants. Estimates of ID from the two samples were highly correlated ($P < 0.01$) in peanut quadrants but were not significantly correlated in corn quadrants.

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ROOT UPTAKE OF RUBIDIUM-86 BY CITRUS ROOTS AS AFFECTED BY ROOT PATHOGENS, SEASON AND IRRIGATION. J. A. Menge, E. L. V. Johnson, E. Pond and H. Liu. Dept. of Plant Pathology, University of California, Riverside, CA 92521.

Uptake of Rb^{86} by 1-cm pieces of excised citrus root tips was measured. In a greenhouse experiment, roots from Troyer citrange inoculated with *Phytophthora parasitica* absorbed 36% less Rb^{86} than roots not inoculated with *P. parasitica*. In the field, trees treated with nematicides and fungicides (oxamyl and metalaxyl) consistently exhibited a 27-57% greater uptake of Rb^{86} than did non-treated trees. Most of this increase in uptake was observed July-October when *P. parasitica* and nematode populations were high. Uptake of Rb^{86} was greater in roots of citrus receiving irrigation which was 80% and 120% of the evapotranspiration demand (ETD) than by those receiving 100% of the ETD. Root uptake of Rb^{86} fluctuated considerably on a seasonal basis and was greatest during the summer months and when root numbers were lowest. This indicates that citrus roots may be able to compensate for root damage or reduced numbers with increased efficiency of root uptake.

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SOILS SUPPRESSIVE TO BLACK ROOT ROT OF BURLEY TOBACCO IN WESTERN NORTH Carolina. Julie R. Meyer and H. D. Shew. Dept. of Plant Pathology, North Carolina State University, Raleigh, NC 27695-7616.

Soils suppressive to black root rot were identified by the absence of disease in fields planted in cultivars with low resistance to black root rot and containing 2-500 cfu/g soil of Thielaviopsis basicola. Suppressiveness was confirmed under controlled environmental conditions with several pathogen isolates and host cultivars. Suppressiveness was not the result of reduced survival of the chlamydospores of T. basicola. The suppressive factor appears to be abiotic and associated with soil acidity. Soil amendments were used to separate the effects of different components of acidity (soil pH, base saturation, exchangeable Al) on disease. The results suggest that soil Al may be the mechanism of suppressiveness in these soils.

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COLONIZATION OF SOLANUM TUBEROSUM L. BY COLLETOTRICHUM COCCODES (WALLR.) HUGHES A.W. Barkdoll and J.R. Davis, Univ. of Idaho R&E Center, Aberdeen, ID 83210

Colletotrichum coccodes can be found in roots, tubers, stem bases and apices of potato in the field. Tubers from foliar inoculated potatoes contained significant differences (P=0.01) in colony forming units (cfu's) of C. coccodes of 2000 and 300 in stem and bud ends respectively. Surface disinfestation of tubers with 10% clorox did not reduce cfu's. In view of the high cfu's of C. coccodes in tubers and stems an experiment was conducted to evaluate fungal colonization of potato originating from infested seed tubers. Russet Burbank mini-tubers were inoculated with a conidial suspension (50 ul of 9.2×10^6 conidia/ml) of C. coccodes and were planted in the greenhouse. The resulting plants were destructively sampled three times during the season. Stem apices and bases, roots and soil were sampled for C. coccodes at each period. At no sampling period was C. coccodes detected in stem bases or apices. In contrast, C. coccodes in soil increased from zero to above 0.2 cfu/g soil. Root infection at the last sampling increased from 0 to 15%.

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COLONIZATION AND PATHOGENICITY OF FUSARIUM OXYSPORUM AND F. SOLANI ON ESSEX SOYBEAN. G. M. Farias and G. J. Griffin. Department of Plant Pathology, Physiology and Weed Science. Virginia Polytechnic Institute and State University. Blacksburg, VA 24061.

Colonization of Essex soybean cotyledons by Fusarium oxysporum occurred 1 day after planting in naturally infested soil and the fungus was present at high frequency (25%) after 4 days. F. oxysporum and F. solani colonized the lower hypocotyl and emerging roots 2 and 3 days after planting, respectively. After 4 days both fungi were found colonizing the elongating, upper portion of the hypocotyl. The hypocotyl-root transition zone had the highest frequency (20-28% of 2-mm tissue segments) of colonization by each species at 4 days. F. solani had higher colonization rates per unit of inoculum than F. oxysporum (0.042 and 0.023 colonizations/m root/propagule/g soil after 4 days, respectively). In soil-temperature tank tests at 20 C and -0.01 MPa water potential, all of the four F. oxysporum and four F. solani isolates tested delayed seedling emergence and caused significant reductions in stem length and plant fresh weight.

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THE EFFECT OF PLANTING DATE ON FUSARIUM WILT OF MUSKMELON IN CALIFORNIA. D. J. Jacobson and T. R. Gordon, Dept. of Plant Pathology, University of California, Berkeley 94720.

A randomized complete block design was employed with planting dates as blocks and susceptible and resistant cultivars as treatments; the experiment was conducted for two years. Cortical root colonization by Fusarium oxysporum f. sp. melonis was measured on seedlings and at full fruit load. Rate of disease progress and incidence at harvest were both higher during hotter portions of the growing season. Root colonization by the pathogen was not affected by planting date; cultivar differences were apparent only at full fruit load and may represent xylem colonization of the susceptible host. Inoculum varied sig-

nificantly among plots but there was no correlation ($r^2 = 0.20$) between inoculum level and root colonization. In addition, root colonization by the pathogen was not correlated to disease. Thus, the increase in disease with temperature cannot be attributed to initial infection; however, temperature may influence subsequent systemic spread or symptom development.

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COMPARISON OF MEDIA FOR ISOLATING *RHIZOCTONIA SOLANI* FROM SOIL. P. C. Vincelli and C. M-S. Beaupre, Dept. of Plant, Soil & Insect Sciences, University of Wyoming, Laramie, WY 82071.

Radial growth of *R. solani* AG-1 through AG-5 at 27 C was fastest on water agar (WA; 14.9 mm/day), equal or slightly slower on Ko & Hora's medium containing 5 ul/l prochloraz 40EC (KH_p; 11.3 mm/day), and greatly reduced on ethanol-potassium nitrate medium containing 2% ethanol (EPN_2; 3.6 mm/day). KH_p and EPN_2 were both highly effective in inhibiting indigenous fungi and bacteria from three Wyoming agricultural soils. At least 97.9% and 98.8% of soilborne fungi in three soils were inhibited on KH_p and EPN_2 respectively, after 7 days at 19-22 C; and 99.9% of soilborne bacteria were inhibited on both media. WA inhibited at least 70.0% of bacteria after the same period but did not significantly inhibit fungi. No difference was observed between media in estimates of populations of *Rhizoctonia*-like fungi (RLF; characterization of isolates in progress) from three soils. Although efficiency of recovery of RLF from soil was equal among media, it was easier to locate and identify colonies of RLF on the two selective media than on WA. It was concluded that the high cost of materials for EPN_2 (approx. $22/l) as compared to KH_p (approx. $1/1) is not offset by any marked advantage.

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EFFECTS OF SOIL BULK DENSITY ON WILT/ROOT ROT OF CHICKPEAS. M. A. Bhatti and J. M. Kraft, Washington State University and USDA-Agricultural Research Service, Irrigated Agriculture Research and Extension Center, P.O. Box 30, Prosser, WA 99350.

Bulk densities of 1.2 g/cm^3 (loose) and 1.5 g/cm^3 (compacted) were produced to determine the effects of compacted soil on root disease severity and root growth of chickpeas (*Cicer arietinum*). The susceptible cultivar JG-62 was grown in soil infested with *Fusarium oxysporum* f. sp. *ciceri*, *F. solani* f. sp. *pisi*, *Pythium ultimum*, and/or *Thielaviopsis basicola* separately and in various combinations under controlled growth chamber conditions. There was more severe root disease and less root growth in the compacted soil than in loose soil when JG-62 was grown in soil infested with these pathogens individually or in combination. The effects of these root pathogens on severity of wilt and root rot of chickpea were additive when roots were exposed to various combinations. Compaction had no effect on wilt severity caused by *F. oxysporum* f. sp. *ciceri*. Root growth was inversely related to soil compaction.

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INFLUENCE OF SOIL MOISTURE ON WILT/ROOT ROT OF CHICKPEA. M. A. Bhatti and J. M. Kraft, Washington State University and USDA-Agricultural Research Service, Irrigated Agriculture Research and Extension Center, P. O. Box 30, Prosser, WA 99350.

The susceptible chickpea (*Cicer arietinum*) cultivar JG-62 was grown in soil infested with *Fusarium oxysporum* f. sp. *ciceri*, *F. solani* f. sp. *pisi*, *Pythium ultimum*, and/or *Thielaviopsis basicola*. These tests were conducted in greenhouse pot culture at 12, 18, and 25% soil moisture. Root rot or wilt increased with soil moisture as did rhizosphere populations of each pathogen. Soil infested with equal proportions of each pathogen together had as much or more disease when planted with JG-62 as in soil infested with an individual pathogen. The exception was a combination *F. oxysporum* f. sp. *ciceri* and *P. ultimum* where wilt severity decreased. Rhizosphere populations of *F. oxysporum* f. sp. *ciceri* were much higher than populations of other pathogens at the duration of each test.

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DNA RESTRICTION POLYMORPHISMS BETWEEN INTRA-SPECIFIC GROUPS OF AG 4 FROM *RHIZOCTONIA SOLANI*. R. Vilgalys[1], D. Gonzalez[1], T. B. Brenneman[2], D. R. Sumner[2], and A. S. Csinos[2]. [1]Department of Botany, Duke University, Durham, NC 27706, and [2]Coastal Plain Exp. Station, Dept. of Plant Pathology, University of Georgia, Tifton, GA 31793.

Plasmid probes containing cloned DNA fragments from an isolate of *R. solani* anastomosis group (AG) 4 were used to screen Southern blots of genomic DNA from 50 field isolates for restriction fragment polymorphisms. Most of the plasmid probes hybridized preferentially with a subset of the total isolates, with little or no cross-hybridization to the remaining isolates. Sub-group specificity of random-cloned DNA probes is concordant with previous observations of low genomic DNA/DNA reassociation (30-40%) between the two AG 4 subgroups. Restriction polymorphisms within each subgroup were also evident. Further studies using the plasmid library should permit us to determine if additional population substructure is evident in AG 4.

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COMPARATIVE GERMINATION OF CULTURE-PRODUCED AND PLANT-PRODUCED SPORANGIA OF *PYTHIUM ULTIMUM* IN RESPONSE TO SOLUBLE SEED EXUDATES AND EXUDATE COMPONENTS. Eric B. Nelson and Cheryl M. Craft, Department of Plant Pathology, Cornell University, Ithaca, 14853.

Sporangia of *Pythium ultimum* were produced on conventional culture media and on media amended with germinating seeds and excised radicles of several plant species. When produced on culture media commonly used for the cultivation of *Pythium* species, sporangia germinated in response to certain sugars and amino acids as well as cotton seed exudate. When produced in association with plant tissue or on media amended with α-phosphatidyl choline, however, sporangia failed to germinate in response to any sugar or amino acid tested, despite their ability to germinate in response to cotton seed exudate. It is believed that sporangia produced on plant-tissue amended media more closely reflect the behavior of those produced on diseased plant tissue in soil than do sporangia produced on conventional culture media. Therefore, molecules other than sugars and amino acids are probably responsible for activating sporangia of *P. ultimum* and establishing host-pathogen interactions under natural conditions.

99

EFFECT OF IRRIGATION OF SEEDLINGS ON SEVERITY OF PHYTOPHTHORA ROOT ROT OF SOYBEANS. A. F. Schmitthenner, Dept. of Plant Pathology, Ohio State Univ., Wooster, OH 44691.

Over a 3-yr period in soil infested with *P. megasperma* f.sp. *glycinea*, irrigation with 100 mm water 3 days after planting was compared with no irrigation on three cultivars, with and without metalaxyl applied in the seed furrow at planting time at rates of 560 g/ha. In nonirrigated plots, stand and seed yield was significantly increased by metalaxyl in low-tolerant cv. Sloan but not in high-tolerant cv. Asgrow 3127 or resistant (Rps 1-k) Century 84. Stands and seed yields of Asgrow 3127 were significantly lower in the irrigated treatments without metalaxyl. Sloan was severely damaged in the irrigated treatments and metalaxyl provided only partial control. Irrigation had no effect on stand or yield of Century 84. It was concluded that severe Phytophthora rot can be consistently induced by early irrigation and controlled by resistant cultivars or by metalaxyl soil treatment of high-tolerance cultivars.

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SUSCEPTIBILITY TO HELMINTHOSPORIUM CARBONUM RACE 1 in IOWA STIFF STALK SYNTHETIC MAIZE. K. M. Tubajika*, C. A. Martinson*, A. R. Hallauer°, and K. R. Lamkey°, *Dept. of Plant Pathology, °USDA/ARS Dept. of Agronomy, Iowa State University, Ames, Iowa 50011.

Iowa stiff stalk synthetic (BSSS) populations are important sources of commercial maize germplasm. Race 1 of *H. carbonum* (=*Bipolaris zeicola*), which is specific for the hm allele, severely attacked progeny in 59% of S2 lines of BS13(S)C5 in 1987. Differential inbreds Pr and Prl were susceptible and resistant, respectively. Three of 16 inbred progenitors of BSSS were susceptible. BSSS populations have been improved through recurrent selection (RS) since 1939. The intermated populations from C0 through C7 cycles of BSSS(HT) (half-sib RS), C0 through C5 cycles of BS13(S) (S1 RS) and C4, C8, and C11 cycles of BSSS(R) (reciprocal RS) were assayed for frequency of susceptible plants; 4 to 54% of plants in each cycle were susceptible. Modifying alleles and genes were common. Maize breeders need to assess their BSSS germplasm for susceptibility to this race of *H. carbonum*.

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EFFECT OF RELATIVE HUMIDITY ON GERM TUBE ELONGATION AND APPRESSORIAL FORMATION OF CERCOSPORA ZEAE-MAYDIS. P. R. Thorson and C. A. Martinson, Dept. of Plant Pathology, Iowa State University, Ames, Ia 50011.

Cercospora zeae-maydis, the cause of gray leaf spot of maize, has been reported to be favored by prolonged high RH and leaf wetness. Germ tube elongation and appressorial formation of *C.*

zeae-maydis were examined after exposure to different RH regimes. Conidia were atomized onto polysulfone membrane filter discs and allowed to germinate for 6 hr. Discs were then suspended above glycerol solutions maintained at 25.0 C to obtain 39-100% RH. Germ tube elongation and appressorial formation were observed microscopically after staining the discs with acid fuchsin in lactophenol. Appressoria formed in 2 to 3 days when germinated conidia were maintained at 95% RH. Appressoria did not form at 90% RH even after 15 days. However, when these conidia were then transferred to 95% RH, appressoria formed in 2 to 3 days. Conidia held at 39-80% RH for 15 days did not continue development when transferred to 95% RH.

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THE EFFECTS OF NITROGEN ON CHARCOAL ROT DEVELOPMENT IN SORGHUM BICOLOR. G.L. Cloud and J.C. Rupe, Dept. of Plant Pathology, University of Arkansas, Fayetteville, AR 72701.

Colonization of sorghum by Macrophomina phaseolina was determined at nitrogen fertilization rates of 0, 50, 100, and 150 lbs/acre in a randomized complete block design with five replications. Plant tissue and roots were sampled monthly to determine total nitrogen and percent root segments colonized by natural infection. Sorghum stalks also were inoculated with toothpicks infested with M. phaseolina, and four plants were rated monthly for lesion length. There were no significant differences between the level of soil nitrogen and incidence of root colonization. There was a positive correlation between the length of stalk lesions from the point of inoculation and plant tissue nitrogen levels at 119 and 139, but not 79 days after planting.

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AMBIGUITY OF THE DESIGNATION RACE 4 FOR ISOLATES OF CERCOSPORA SOJINA. D. V. Phillips and H. R. Boerma, Departments of Plant Pathology and Agronomy, Georgia Station, University of Georgia, Griffin, GA 30223.

Race 3 and Race 4 of Cercospora sojina were described in 1968 to designate isolates from North Carolina. In recent years, C. sojina isolates from the southern U.S. include Race 5 and several of Race 4. Since Race 4 was originally tested on only 16 cultivars, additional cultivars were tested for reaction to isolates of this race. Several cultivars reacted differently to isolates designated as Race 4, indicating that the 16 cultivars used to describe Race 4 are not adequate to distinguish Race 4 from other apparently new races. The reaction of additional cultivars to Race 4 cannot be determined because an authentic culture is not available. Since at least 3 isolates designated as Race 4 can be separated from each other and from Races 1, 2, 3, and 5 by cultivar reaction, the designation Race 4 is ambiguous and should not be used.

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STABILITY OF PLASMA MEMBRANE OF WHITE BEAN ASSOCIATED WITH WHITE MOULD RESISTANCE. J. C. Tu, Agriculture Canada, Research Station, Harrow, Ontario NOR 1GO.

Stability of plasma membrane of white bean to oxalic acid secreted by Sclerotinia sclerotiorum was associated with disease resistance. Leaf tissues of a susceptible (Fleetwood) and a resistant (ExRico 23) cultivar were treated with different concentrations of oxalic acid for thin sectioning and freeze fracturing. In thin sections, at a given oxalic acid concentration, the plasma membrane and chloroplasts of Fleetwood were affected more and ruptured quicker than those of ExRico 23. In replicas of freeze-fractured plasma membrane, protrusions, wrinkles and breakages increased with increasing oxalic acid concentration. The degree of damage was distinctly more severe in the plasma membrane of Fleetwood than ExRico 23. Conductivity measurements of the bathing water of leaf discs showed that Fleetwood had a higher conductivity reading than that of ExRico 23, indicating that the plasma membrane of Fleetwood was less stable than that of ExRico 23.

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INTERACTION OF DINITROANILNE HERBICIDES AND RHIZOCTONIA DISEASE ON SOYBEAN. E. M. Bauske and H. W. Kirby, 1102 S. Goodwin Ave. University of Illinois, Urbana, Illinois 61801

The effect of trifluralin, pendimethalin, and ethalfluralin and disease caused by Rhizoctonia solani on soybeans was determined with growth chamber and field studies. Steamed and nonsteamed soil was used in growth chamber studies. No significant interactions were found between herbicides and plant growth parameters in steamed soil. Significant interactions between herbicides and inoculum treatments affecting root and hypocotyl weight occurred in nonsteamed soil. Root and hypocotyl weights were significantly lower in inoculated soil than in noninoculated soil in the absence of herbicides. No similar significant differences were observed in treatments where herbicides were added to the soil. In field studies R. solani infested oats placed in furrow at planting significantly reduced plant growth parameters. No significant interactions were observed between herbicides and inoculum treatments. Effects of R. solani inoculum were significantly reduced with carboxin-pentachloronitrobenezene seed treatments.

106 Withdrawn

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CHARACTERIZATION OF *COLLETOTRICHUM GRAMINICOLA* ISOLATES WITH AMINOPEPTIDASE PROFILES AND POLYACRYLAMIDE GEL ELECTROPHORESIS OF SOLUBLE PROTEINS. Ali, M.E.K., H.L. Warren, D.M. Huber, F.E. Lytle, K. Hughes. USDA-ARS, Botany & Plant Pathology, and Chemistry Departments; Purdue University, W. Lafayette, IN 47907.

Filter aminopeptidase and gel electrophoresis methods were compared with a laser enhanced aminopeptidase technique to characterize isolates of *Colletotrichum graminicola* from three hosts. Filter and laser aminopeptidase profiles and gel electrophoretic patters of soluble protein indicate that isolates of *C. graminicola* from sorghum, corn and barnyard grass can be differentiated based on their host specificity. The laser aminopeptidase profiles further separated sorghum isolates to races, similar to their reactions on sorghum plants. Corn isolates showed less variability, which is consistent with results from pathogenicity tests. The percent hydrolysis of ß-napthylamides was highest in sorghum followed by corn and barnyard grass. The aminopeptidase assay is a powerful method which is sensitive enough to provide a rapid method of differentiating between isolates of *C. graminicola* from sorghum, corn and barnyard grass. Laser enhancement of the aminopeptidase system permits differentiation of races of *C. graminicola* pathogenic to sorghum.

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OCCURRENCE AND IMPACT OF VIRAL DISEASES OF WINTER WHEAT IN NEW YORK STATE. N. R. Miller, G. C. Bergstrom, and S. M. Gray. Dept. of Plant Pathology, Cornell University, Ithaca NY 14853.

The distribution and incidence of common virus diseases affecting soft white winter wheat in New York State (NYS) were determined in 1988 and 1989. Over 100 randomly selected fields were assessed for wheat spindle streak mosaic (WSSM), barley yellow dwarf (BYD), soilborne wheat mosaic, and wheat streak mosaic using ELISA techniques and symptom expression. At early stem extension, symptoms of WSSM were severe in fields planted to susceptible cultivars. BYDV was detected by ELISA in many fields at this growth stage, although no symptoms of the disease were observed. At spike emergence, BYDV was the only widespread wheat virus. In surveyed fields, as well as in field plot experiments, the cultivar Geneva consistently incurred a low incidence of WSSM. These findings, together with experiments examining the effect of WSSM on wheat yields, contribute to our understanding of the impact of virus diseases on wheat production in NYS.

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MANGANESE TOXICITY IN THE MARBLE CULTIVARS OF CHRYSANTHEMUM MORIFOLIUM. I. CYTOCHEMISTRY. R. H. Lawson and M. M. Dienelt. USDA-ARS, Florist & Nursery Crops Lab., Beltsville, MD.

Chrysanthemum morifolium (florists' chrysanthemum) cultivars of the Marble group are affected by necrotic spotting in lower leaves and some veinal necrosis while 'Vero' appears unaffected. Mn accumulation in necrotic lesions was determined by x-ray microanalysis and is similar to Mn toxicity in other species. Supplemental toxic levels of $MnSO_4$ increased the incidence of necrotic lesions in the Marbles and induced similar necrotic lesions on lower leaves as well as a top systemic chlorosis in 'Vero'. Aniline blue fluorescence in cell walls adjacent to necrosis indicated deposition of callose. There was no increase in callose in phloem from leaves or petioles of necrotic Marble plants over symptomless 'Vero' that would suggest the presence of an MLO. The DAPI stain for DNA did not reveal the presence of a fastidious prokaryote in Marble phloem. Leaf necrosis in the Marble group is associated with Mn toxicity.

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MANGANESE TOXICITY IN THE MARBLE CULTIVARS OF CHRYSANTHEMUM MORIFOLIUM. II. ELECTRON MICROSCOPY. M. M. Dienelt and R. H. Lawson, USDA-ARS, Florist & Nursery Crops Lab., Beltsville, MD.

Cell wall abnormalities associated with manganese-induced necrotic lesions in Chrysanthemum morifolium 'Pink Marble' (PM), 'Florida Marble' (FM), and 'Vero' were similar to those associated with necrotic lesions in untreated FM and PM. Although no pathogenic agents were observed, vesicles and filaments resembling MLO's occurred in new growth of all treated and untreated plants. These structures occurred in vacuoles of companion cells, gum duct epithelial cells and salt glands but not sieve elements. Transfer-cell-like wall ingrowths and papillae composed of membranes, phenolic substances (identified by the nitroso reaction) and a faintly stained amorphous matrix occurred in necrotic and adjacent tissue. Papillae often developed at plasmodesmata openings. Large papillae, corresponding to aniline blue fluoresence noted earlier, bordered necrotic cells. Papillae, which often occur in response to pathogenic invasion, may also reflect nutritional stress.

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DISEASE INCIDENCE AND BOTRYTIS CINEREA CONIDIAL CONCENTRATION AMONG GERANIUM STOCK PLANTS. M. K. Hausbeck and S. P. Pennypacker, Department of Plant Pathology, The Pennsylvania State University, University Park, PA 16802

Hourly Botrytis cinerea conidial concentrations among geranium (Pelargonium x hortorum) stock plants within a commercial greenhouse were estimated for selected time periods of the 1986 and 1987 growing seasons using a Burkard recording spore trap. Disease incidence occurring during 1987 was also documented. Maximum concentrations of conidia typically occurred at midday or in association with grower activity including watering, pesticide application, and harvesting of cuttings. The number of conidia trapped per day and the amount of disease increased during the season. The maximum number of conidia trapped in May was 2,000 conidia/m^3 air/hour and an average of 24% of the stems and 5% of the necrotic leaves were infected. Conidial concentrations and disease incidence continued to increase through July to a maximum of 14,000 conidia/m^3 air/hour and an average of 71% infected stems and 88% infected necrotic leaves.

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GERMINATION, INFECTION, AND SPORULATION OF ROSE POWDERY MILDEW ON LEAVES AT DIFFERENT WETNESS LEVELS UNDER LABORATORY CONDITIONS. R. E. De Long and C. C. Powell, Department of Plant Pathology, Ohio State University, Columbus, OH 43210.

Materials and methods were developed to observe conidial germination, formation of secondary hyphae (infection), and production of conidia (sporulation) of Sphaerotheca pannosa var. rosae on excised leaflets at five leaf wetness sensor readings (LWSR) over time in the laboratory (see Phytopathology 78:1520). Conidial germination varied in direct response to LWSR 24 hours after inoculation. Germination was 40, 42, 52, 62, and 64% at LWSR of 0, 8, 35, 65, and 100, respectively. Secondary hyphal development was 12% on dry leaves (0 LWSR) and 52% on wet leaves (100 LWSR) when observed 48 hours after inoculation. The production of conidia was observed 96 hours after inoculation and occurred at essentially all sites that developed secondary hyphae in both the dry and wet environments. Experiments observing secondary hyphal development were conducted in the laboratory to duplicate the diurnal LWSR fluctuations observed in a cut-rose greenhouse.

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DECLINE OF ASH TREES IN ARIZONA. J.C. Stutz, T.P. Lukacsko and J.L. Engle, Department of Botany, Arizona State University, Tempe, AZ 85287-1601.

Symptoms of the progressive decline of Arizona ash (Fraxinus velutina L.) in Arizona include chlorosis, leaf scorching, progressive dieback of twigs and branches, and proliferation of shoots along the main trunk. The incidence and severity of decline were observed in ash trees located at a study site in Tempe AZ during 1985 and 1988. In 1985, about 30% of the trees had dieback in more than one half of the crown. By 1988, this percentage had increased to 55% including 16% of the trees which had died in the interim. Mortality progressed at a rate of 4.6% per year. Investigations into the etiology of this problem are continuing.

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EPIDEMIOLOGY OF DOGWOOD ANTHRACNOSE IN CHEROKEE NATIONAL FOREST OF SOUTHEASTERN TENNESSEE. M. T. Windham. Department of Entomology and Plant Pathology, University of Tennessee, P.O. Box 1071, Knoxville, TN 37901-1071.

Dogwood anthracnose was first observed in the Applachian Mountains of southeastern Tennessee in 1988. Twenty-one plots in 1988 (elevation 274- 915m) and 35 plots in 1989 (elevation 250 - 915m) were monitored for incidence of dogwood anthracnose and disease severity. When plots were located more than 100m from water, disease severity values for trees within plots increased as plot elevation increased. In plots located within 50m of water, the disease was severe regardless of plot elevation. An average of 55% of the trees with trunk diameters > 5cm displayed symptoms of the disease and < 25% of the foliage was affected in infected trees. Disease incidence averaged 11% in trees with trunk diameters of less than 1.3cm; 40% of the foliage was affected in infected trees.

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ALTERNARIA LEAF SPOT OF GOMPHRENA GLOBOSA. Karen K. Rane and Robert L. Wick, University of Massachusetts, Suburban Experiment Station, 240 Beaver St., Waltham, MA 02154.

A severe leaf spot disease of Gomphrena globosa was found in a commercial cut-flower field in Massachusetts. Virtually 100% of the crop was affected and became unsalable as fresh cut flowers. Lesions consisted of dry, necrotic areas surrounded by red or purple margins, and ranged from 0.3 cm to 1.0 cm in diameter. Alternaria gomphrenae Togashi was recovered from lesions, and Koch's postulates were completed by foliar spray inoculation. A. gomphrenae was also recovered from two of four commercial gomphrena seed samples examined. This is the first report of this pathogen in the United States.

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SUSCEPTIBILITY OF FLORIST'S CHRYSANTHEMUM TO TOMATO SPOTTED WILT VIRUS AND WESTERN FLOWER THRIPS. J. A. Matteoni, W. R. Allen and A. B. Broadbent. Agriculture Canada, Vineland Station, Ontario, Canada L0R 2E0

A wide range in susceptibility to tomato spotted wilt virus (TSWV) and host preference of western flower thrips (Frankliniella occidentalis) was found among over 30 cultivars of florist's chrysanthemum (Chrysanthemum X morifolium). Significantly more plants became infected ($P<.001$) and developed symptoms ($P<.001$) when grown at 18/16 C (day/night) compared to 24/18 C. Economic losses resulted when tolerant cultivars were grown under cool conditions. There was no significant difference in percentage infection or in symptom development between flowering versus vegetative plants. Field resistance to TSWV was attributed to low thrips preference. Leaves of yellow-flowered cultivars were preferred by thrips to leaves of white-flowered cultivars.

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Cymbidium Mosaic Virus and Odontoglossum Ringspot Virus in Commercial Orchids in Singapore. S.-M. Wong, F. Soo, C. G. Chng, and G. Lim. Department of Botany, National University of Singapore, Kent Ridge, Singapore 0511.

A survey was conducted in commercial orchid farms in Singapore to assess the frequency of occurrence of cymbidium mosaic virus (CyMV) and odontoglossum ringspot virus (ORSV). The twelve surveyed orchid genera were: *Aranda, Ascocenda, Arachnis, Brassolaelio-cattleya, Cattleya, Dendrobium, Mokara, Oncidium, Paphiopedilum, Spathoglottis, Vanda, and Vandaneopsis.* The total number of orchid plants in the survey was 1146. Among the 12 major cultivated orchid genera 54.6% were infected with CyMV, 4.0% were infected with ORSV, and 14.2%

were mixed-infected with both CyMV and ORSV; 27.2% were not infected. The indirect ELISA method was used for virus detection.

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PHENOL OXIDASES OF FIVE ARMILLARIA BIOSPECIES. S.F. Marsh and P.M. Wargo, U.S.D.A. Forest Service, Hamden, CT 06514

Three isolates each of Armillaria biospecies groups I,III,V,VI and VII were assayed for constitutive phenol oxidases after 11, 18,25,32 and 39 days growth in liquid culture. Laccase was measured in the mycelium, rhizomorphs and extracellular culture medium. Tyrosinase was only found intracellularly. Averaged across isolates, intracellular laccase activity was highest at 11 days while tyrosinase and extracellular laccase were highest at 18 days. Activities of laccase and tyrosinase were not different among biospecies groups because there were large differences between isolates within a biospecies group. Among rhizomorph-producing isolates (12), isolates that produced more rhizomorphs had higher laccase activity. Laccase activity was also present in isolates that produced no rhizomorphs. Preliminary results indicate that peroxidase activity may be present in some isolates. No relation between the enzyme activities and reported pathogenicity, pH of culture medium, phenol production or mycelial pigmentation has been found.

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ASSOCIATION OF PEROXIDASE WITH SYSTEMIC RESISTANCE TO CLADOSPORIUM CUCUMERINUM INDUCED BY COLLETOTRICHUM LAGENARIUM IN CUCUMBER CULTIVARS RESISTANT OR SUSCEPTIBLE TO C. CUCUMERINUM. R. Reuveni and J. Kuć. Department of Plant Pathology, University of Kentucky, Lexington, KY 40546-0091.

Cucumber cultivars susceptible or resistant to C. cucumerinum were systemically protected by infection of leaf 1 with C. lagenarium. The protection of leaf 2 was positively correlated with the concentration of C. lagenarium inoculum, negatively correlated with the concentration of challenge inoculum and was not overcome by 10^6 conidia/ml of C. cucumerinum. Peroxidase activity increased in protected leaf 2. After challenge, peroxidase increased further in protected plants and was detected as early as 24 hr after challenge in resistant protected plants. The increase in peroxidase activity and the appearance and intensification of peroxidase isozymes occurred in the absence of visible damage. Peroxidase after challenge may be a useful marker for both induced and non-induced resistance.

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BIOSYNTHESIS OF SESQUITERPENOID PHYTOALEXINS IN BACTERIALLY INOCULATED COTTON LEAVES AND COTYLEDONS. M. Essenberg, H. Hamada, and G. D. Davis. Dept. of Biochemistry, Oklahoma State University, Oklahoma Agricultural Experiment Station, Stillwater, OK 74078-0454.

Chemical degradation of 2,7-dihydroxycadalene (DHC) and 2-hydroxy-7-methoxycadalene (HMC) produced in cotton cotyledons from [2-^{14}C,5-^{3}H]mevalonolactone following inoculation with an incompatible race of *Xanthomonas campestris* pv. *malvacearum* revealed that biosynthesis of DHC and HMC involves a hydrogen transfer to the isopropyl side chain, which probably occurs as a 1,3-hydride shift during cyclization of the farnesyl precursor. Gas chromatographic/mass spectrometric analysis of cotyledonary extracts showed the presence of inoculation-induced substances of molecular weights 204, 206, 216, 218, and 232, whose mass spectra suggest that they are intermediates on the pathway to DHC and HMC. High-resolution mass spectrometry and ^{1}H-nmr of the MW 218 compound indicate that it is $C_{15}H_{22}O$, 7-hydroxycalamenene.

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RELATIONSHIP OF β-1,3-GLUCANASE AND TOTAL SOLUBLE CARBOHYDRATE TO THE IMMUNIZATION OF TOBACCO AGAINST BLUE MOLD CAUSED BY PERONOSPORA TABACINA. S. Q. Pan, X. S. Ye and J. Kuc, Department of Plant Pathology, University of Kentucky, Lexington, Ky 40546-0091.

Stem injection of Ky14 tobacco with sporangiospores of P. tabacina or leaf inoculation with tobacco mosaic virus (TMV) systemically immunized plants against disease caused by both pathogens and systemically increased β-1,3-glucanase activity. After challenge with P. tabacina, β-1,3-glucanase also increased earlier and more rapidly in immunized plants and their tissue culture regenerants than in controls. One dominant intercellular isozyme of β-1,3-glucanase was associated with immunization before and after challenge and another with symptom expression after challenge only in controls. β-1,3-glucanase was not associated with immunization in Xanthi-nc tobacco. Total soluble carbohydrate increased in Ky14 and Xanthi-nc immunized with P. tabacina but not in regenerants of immunized Ky14 or Ky14 immunized by TMV.

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ASSOCIATION OF PATHOGENESIS-RELATED PROTEINS(PRs), PEROXIDASE (PER), β-1,3-GLUCANASE(GL) AND CHITINASE(CH) WITH INDUCED RESISTANCE OF TOBACCO TO PERONOSPORA TABACINA BUT NOT TO SYSTEMIC TOBACCO MOSAIC VIRUS. X. S. Ye, S. Q. Pan and J. Kuc. Dept. of Plant Pathology, University of Kentucky, Lexington, KY 40546-0091.

Tobacco Ky 14 inoculated with TMV and held at 23C for 3-12 days had localized necrosis on the inoculated leaves and was systemically protected against blue mold and TMV. Above 28C, systemic mosaic was apparent. Accumulation of PRs and activities of PER, GL and CH increased in induced plants. When TMV-inoculated leaves were removed 12 days after inoculation and plants were challenged with TMV or P. tabacina and transfered from 23C to 28C one day after challenge, induced resistance to P. tabacina but not TMV was apparent. Accumulation of PRs and activities of PER, GL and CH were further increased after challenge in induced plants at 23C and 28C. Transfer of plants from 23C to 28C for 7 days after induction and removal of inducer leaves did not affect induced resistance to P. tabacina and TMV when challenged and held at 23C for 7 days.

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ENHANCED PEROXIDASE AND ITS PERSISTENCE IN CUCUMBER PLANTS IMMUNIZED AGAINST ANTHRACNOSE BY FOLIAR INOCULATION WITH COLLETOTRICHUM LAGENARIUM. R. F. Dalisay and J. Kuc, Dept. of Plant Pathology, University of Kentucky, Lexington, KY 40546-0091.

Inoculation of the first true leaf of cucumber plants with conidia of C. lagenarium induces systemic resistance against the same fungus for 4-6 weeks. When the inoculated (inducer) leaf was detached one week after inoculation, resistance was reduced but persisted for the same time period. Peroxidase activity was 3-10 x greater in induced plants than in plants treated with water on leaf 1 and was consistently higher in plants with the inducer leaf attached. Leaves 4-12 in the bud after leaf 1 was induced followed the same pattern for protection and peroxidase activity as leaf 2. Results suggest that a signal produced during the first 7 days after inoculation of leaf 1 affects protection and peroxidase activity of expanded leaves and leaves in the bud. Once triggered, the response persists in the absence of the signal.

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PMG WALL GLUCAN IS AN EFFICIENT ELICITOR OF ISOFLAVONES BUT IS NOT AN EFFICIENT ELICITOR OF GLYCEOLLIN IN SOYBEANS. T. L. Graham and M. Y. Graham, Dept. of Plant Pathology, The Ohio State University, Columbus, OH 43210.

The cell wall glucans of Phytophthora megasperma f. sp. glycinea (PMG) elicit glyceollin at nearly hormonal (ng/ml) levels. However, they are non-race specific elicitors of glyceollin. Because we have demonstrated that preformed pools of conjugates of the glyceollin precursor, daidzein, are used in race specific accumulation of glyceollin in PMG infected tissues, we were interested to re-examine the specific role of the glucan elicitors in relation to these events. We discovered that PMG glucan is a highly efficient elicitor of daidzein, but is a very inefficient elicitor of glyceollin. Only 3-20% of the daidzein in PMG glucan treated tissues is converted to glyceollin, depending on factors such as light intensity, wounding and tissue age. The results complement earlier studies in suggesting that effective glyceollin elicitation may require either a second elicitor or a specific physiological state of the tissue perceiving the PMG wall glucan.

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BACTERIAL AND PLANT GENE EXPRESSION IN POTATO SOFT ROT. Z. Yang, C.L. Cramer, and G.H. Lacy, Laboratory for Molecular Biology of Plant Stress, VPI&SU, Blacksburg, VA 24061-0330.

A membrane-separated system involving potato tuber slices and *Erwinia carotovora* subsp. *carotovora* (Ecc) was used to study simultaneous *in planta* regulation of bacterial pathogenicity-related and defense-related genes in soft-rot interaction. Northern hybridization showed increases in pathogenicity-related genes including endo- and exo-pectate lyases (PLs) and endo-polygalacturonase (PG) within 3 hr reaching maxima beteen 6 and 12 hr. Host defense responses were monitored by phenylal-

anine ammonia lyase (PAL) and hydroxymethyl glutaryl CoA reductase (HMGR) activity and mRNA levels. Ecc induces rapid accumulation of PAL mRNA and enzyme activity superimposed on wound response. However, Ecc activates a specific HMGR isogene that is not induced by wounding, which rapidly elevated expression of a second isogene. Therefore, HMGR represents a novel defense-related gene useful in the study of molecular mechanisms of host defense responses.

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ELECTROPHORETIC CHARACTERIZATION OF PEANUT ENZYMES AFTER INFECTION WITH *ASPERGILLUS* SPP. J. B. Szerszen, R. E. Pettit, J. S. Neck and R. A. Taber. Department of Plant Pathology and Microbiology, Agricultural Experiment Station, Texas A&M University, College Station, Texas 77843-2132.

Isozyme patterns of buffer-extractable cotyledonary proteins from 10 peanut genotypes before and after infection with *Aspergillus flavus* or *A. parasiticus* were assayed electrophoretically using microprocessor-controlled native-PAGE (gradient 8-25%) and IEF-PAGE (pH 3-9). *Aspergillus*-inoculated and water control cotyledons from viable kernels were incubated (dark, 32 C, 95% RH) and sampled every 6 hrs for 3 days after inoculation. Non-inoculated cotyledons showed few inter-genotypic enzyme polymorphisms. Both fungi caused a rapid decrease of activity of alcohol dehydrogenase and acid phosphatase, 12 and 24 hrs after inoculation, respectively. After 24 hrs the fungi induced activity of alkaline phosphatase (not detected in water controls) and caused a rapid loss of activity of 6-P-gluconate dehydrogenase. Variations in banding patterns and activities of malate dehydrogenase and glucose-6-P-dehydrogenase as well as increased activity of esterase were recorded 30-72 hrs after inoculation with both fungi. Activities of catalase and β-glucosidase were not changed. Minor inter-genotypic isozyme variations were detected in infected cotyledonary tissue.

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DIVERSITY OF CUTINASES FROM FUNGAL PLANT PATHOGENS. Frances Trail and Wolfram Köller, Department of Plant Pathology, Cornell University, N. Y. State Agricultural Experiment Station, Geneva, NY 14456.

Our study on the enzymatic diversity of cutinases from various pathogens has revealed a relationship between cutinase characteristics and organ specificity. Cutin hydrolysis by *Cochliobolus heterostrophus*, a leaf pathogen, is optimal at pH 6.5, whereas cutin hydrolysis by *Rhizoctonia solani*, a stem-base pathogen, shows a pH-optimum of 9.5. pH-optima reported for cutinases isolated from leaf-infecting fungi *Botrytis cinerea* and *Venturia inaequalis*, and from stem-base pathogen *Fusarium solani* are in accordance with this relationship. *Alternaria brassicicola* and *Colletotricum lindemuthianum*, two pathogens that attack both stems and leaves, have cutinase pH optima at both acidic and alkaline values. The cutinase activity of *Alternaria brassicicola* has been resolved by chromatofocusing and represents two isozymes, one with each pH optimum.

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THE USE OF A STEROL INHIBITOR TO INVESTIGATE SUBSTRATE PARTITIONING IN THE ACETATE-MEVALONATE PATHWAY IN POTATO AFTER ELICITATION OF PHYTOALEXIN ACCUMULATION BY ARACHIDONIC ACID. M.N. Zook and J.A. Kuc, Department of Plant Pathology, University of Kentucky, KY 40546.

The levels of 2,3-oxidosqualene increased ten-fold in potato tuber tissue treated with ten micromolar U18666A, an inhibitor of 2,3-oxidosqualene cyclase. The same concentration of U18666A inhibited steroid glycoalkaloid accumulation by 90% but had no effect on the accumulation of rishitin and lubimin elicited by arachidonic acid (AA). In potato tuber tissue treated with U18666A, 2,3-oxidosqualene accumulated to lower levels in AA-elicited potato tuber tissue as compared to non-elicited tissue. These results indicate that AA alters the flow of substrate towards phytoalexin synthesis and away from sterol synthesis.

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MECHANISM OF REDUCTION OF PHOTOSYNTHESIS BY VERTICILLIUM DAHLIAE IN POTATO. R. L. Bowden[1], D. I. Rouse[1] and T. D. Sharkey[2], Dept. of Plant Pathology[1], Dept. of Botany[2], University of Wisconsin, Madison, WI 53706.

The decrease in leaf net photosynthesis (A) of potato 'Russet Burbank' infected with Verticillium dahliae was correlated with decreased stomatal conductance (G). The response of A to intercellular CO_2 concentration (Ci) at saturating light showed that partial stomatal closure was responsible for reduced A. Errors in the Ci calculation caused by uneven distribution of A across the leaf were tested for by $^{14}CO_2$ autoradiography but not found. G and A of diseased leaves were increased by increased relative humidity. In Verticillium-infected plants and drought-stressed plants, low G was correlated with low leaf water potential. However, leaves from Verticillium-infected plants had higher G at equal leaf water potentials. Pressure-volume curves showed that this was not due to osmotic adjustment.

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SODIUM BISULFITE ENHANCES ELECTROLYTE LEAKAGE, PEROXIDASE ACTIVITY AND SPORULATION OF BIPOLARIS MAYDIS RACE T (BMT) IN MAIZE. M. Akhtar and M. O. Garraway, Department of Plant Path., OARDC and The Ohio State University, Columbus, OH 43210.

Pretreatment (24 h at 28 C in the dark) of detached leaves of two isolines of the maize inbred W64A with an aqueous solution (500 μg/ml) of sodium bisulfite ($NaHSO_3$) increased electrolyte leakage and peroxidase activity compared to controls. Also, the Tms cytoplasm (susceptible) isoline of W64A treated with $NaHSO_3$ had significantly higher electrolyte leakage and peroxidase activity than the comparably treated N cytoplasm (resistant) isoline. Moreover, after $NaHSO_3$-treated leaves were inoculated with BMT the $NaHSO_3$-induced increase in sporulation was significantly more on the susceptible than on the resistant isoline. Also, trends in sporulation of BMT on amended agar media suggested a positive relationship between the $NaHSO_3$-induced increases in electrolyte leakage and peroxidase activity in maize leaves and the $NaHSO_3$-enhanced sporulation.

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EFFECTS OF XYLOSE ON PARTIALLY PURIFIED POLYPHENOLOXIDASE (PPO) FROM BIPOLARIS MAYDIS. R. C. Evans and M. O. Garraway. Biology Dept., Rutgers Univ., Camden, NJ 08102 and Dept. of Plant Path., OARDC, The Ohio State University, Columbus, OH 43210

When Bipolaris maydis race T was incubated on a basal agar medium supplemented with xylose (GX medium), sporulation on resulting mycelia increased but PPO activity decreased compared to mycelia incubated on a control medium lacking xylose (G-medium). PPO was partially purified using fractional precipitations with ethanol-chloroform and $(NH_4)_2SO_4$. A comparison of the $(NH_4)_2SO_4$ fractions from G- and GX-grown mycelia indicated that the two sources of PPO were similar in pH and buffer concentration optima, substrate specifity, response to inhibitors and electrophoretic mobility. Lineweaver-Burke plots indicated that the PPO from all fractions of GX mycelia was non-competitively inhibited with respect to PPO from all fractions of G mycelia. Thus, the difference in PPO activity between G and GX mycelia may involve a xylose-mediated inactivation of PPO.

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PLANT PARASITIC NEMATODES CONTAIN MULTIPLE ACETYL CHOLINESTERASE CLASSES. C. H. Opperman and S. Chang, Department of Plant Pathology, North Carolina State University, Raleigh, NC 27695.

Multiple molecular forms of acetyl cholinesterase (AChE) are common in many organisms. These forms often fall into discrete classes with similar biochemical parameters and responses to inhibitors. These classes, however, often differ markedly in these parameters. Experiments with Caenorhabditis elegans, Heterodera glycines, Meloidogyne arenaria, and M. incognita have demonstrated that these nematodes all possess at least two classes of AChE. There is a difference in distribution of classes between the Meloidogyne species and H. glycines which results in significant differences in in vitro enzyme inhibition. Heterodera glycines AChE is less sensitive to carbamate nematicides than that of Meloidogyne. The different kinetic parameters of these classes may help explain the responses to inhibitors.

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ACQUISITION OF GENETICALLY ENGINEERED PSEUDOMONAS STRAINS BY BEES DURING FORAGING ON STRAWBERRY BLOSSOMS. T. V. Suslow. DNA Plant Technology, 6701 San Pablo Avenue, Oakland, CA 94608.

The potential for pollinator bees to acquire and vector applied biological control bacteria under field conditions was quantitated for strawberry blossoms. Ice-minus deletion mutation strains RGP36R2, Pseudomonas syringae, and GJP17BR2, P. fluorescens, were applied to strawberries and established populations on leaves and blossoms. Up to 160 individual bees were assayed on a given sampling date. On one date the relative

abundance of pollen attached to a bee was recorded. Pollen concentration was not correlated to acquired Ice-minus population densities. Bees acquired Ice-minus strains, non-nucleating bacteria, and Ice-plus bacteria from strawberry blossoms. The prevalence of strain GJP17BR2 on bees as compared to RGP36R2 was inversely related to their relative population densities on blossoms from the same sampling period. Among the interpretations of this observation is the possibility that the two strains may occupy spatially distinct habitats on strawberry blossoms that are reflected in the foraging habits of bees.

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INDUCED SYSTEMIC RESISTANCE TO PERONOSPORA TABACINA IN TENNESSEE 86 TOBACCO AND TISSUE CULTURE REGENERANTS OF INDUCED PLANTS. E. M. Nuckles and J. Kuc. Department of Plant Pathology, University of Kentucky, Lexington, KY 40546.

The tobacco cultivar Tennessee 86 is resistant to tobacco etch virus and tobacco vein mottling virus but is highly susceptible to tobacco mosaic virus and to blue mold caused by P. tabacina. Stem injection with sporangiospores of P. tabacina induced systemic resistance to blue mold in Tn 86. Resistance was expressed as a reduction in number, size, and sporulation of lesions. Tissue culture regenerants of Tn 86 plants stem-injected with P. tabacina were protected against blue mold compared to regenerants from plants stem-injected with water. Induction of systemic resistance may provide a technology for rapidly introducing resistance to plants resistant to one or more pathogens.

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FIELD PERFORMANCE AND GREENHOUSE ASSAY OF FUNGI FOR BIOCONTROL OF RESIDUE-BORNE PYRENOPHORA TRITICI-REPENTIS. W. F. Pfender, W. Zhang, and A. Nus. Dept. of Plant Pathology, Kansas State University, Manhattan, KS 66506.

Candidate biocontrol fungi (grown in bran/millet seed culture) were applied to field plots containing Pyrenophora-infested winter wheat straw, with the goal of reducing ascocarp (primary inoculum) production by the pathogen in the residue. Limonomyces reduced ascocarp production by 86% and by 60-80%, respectively, in two years of field tests. Among fungi tested in one year only, an unidentified fungus significantly reduced ascocarp production, Laetisaria gave inconsistent results, and several fungi were ineffective. To screen candidate biocontrol fungi under controlled conditions, a method was developed in which Pyrenophora-infested straws are inoculated with test fungi and placed on a greenhouse bench with intermittent wetting cycles. Test conditions (straw and inoculum types, wetting periods) have been adjusted to give results consistent with those in field tests with selected fungi.

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COMPATIBILITY OF SOME COMMONLY USED SOIL DRENCH FUNGICIDES AND INSECTICIDES WITH THE BIOCONTROL AGENT GLIOCLADIUM VIRENS. J. C. Locke and R. D. Lumsden, Florist and Nursery Crops Lab. and Biocontrol of Plant Diseases Lab., Plant Sciences Institute, USDA-ARS, Beltsville, MD 20705.

The interaction of the biocontrol agent Gliocladium virens with fungicides and insecticides, which can be used as soil drenches in bedding plant production systems, was investigated. The pesticides tested included: Aliette 80W, Banrot 40WP, Benlate 50W, Subdue 2E, Terraclor 75W, Truban 25EC, Diazinon AG4E, and Vydate L. The pesticides were evaluated for their effect on both proliferation of G. virens and the degree of damping-off control achieved against Pythium ultimum and Rhizoctonia solani on zinnia seedlings. None of the pesticides evaluated, except Benlate applied prior to introduction of the biocontrol agent, altered proliferation of the biocontrol agent in a soilless growing medium. Similarly, none of the pesticides decreased nor increased efficacy against either Pythium or Rhizoctonia. These results demonstrate the compatibility of this biocontrol agent with these pesticides at their labelled application rate.

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INTERACTIONS BETWEEN TRICHODERMA HAMATUM AND THERMOPHILIC FUNGI IN BARK COMPOST IN SUPPRESSION OF RHIZOCTONIA DAMPING-OFF. Y. R. Chung and H. A. J. Hoitink, Dept. of Plant Pathology, Ohio State Univ., Wooster, OH 44691.

A white zone of microbial growth typically is present in compost piles where process temperatures range from 40-50 C. Humicola spp. were the predominant fungal taxa isolated from this zone. The ability of Trichoderma hamatum 382 to induce suppression to Rhizoctonia damping-off in media prepared with 40-50 C compost was significantly reduced as compared to that in media prepared with higher or lower temperature composts. Humicola isolates, unable to grow on PDA at 25 C, specifically reduced efficacy of the biocontrol agent in paired biocontrol radish bioassays. Population development of T. hamatum 382 or of Rhizoctonia solani in media prepared with composts from various temperatures did not differ. Results suggest that compost process temperature impacts performance of Trichoderma-fortified composts.

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INCREASE OF SCLEROTINIA SCLEROTIORUM AND VERTICILLIUM DAHLIAE FOLLOWING CERTAIN FOLIAGE FUNGICIDE SPRAYS ON POTATO. Gene D. Easton, Washington State University, Irrigated Agriculture Research and Extension Center, Prosser, WA 99350.

Fungicides were sprayed on potato foliage in plots infested with Colletotricum coccodes (Cc), Sclerotinia sclerotiorum (Ss), and Verticillium dahliae (Vd). Visual ratings of symptoms and laboratory propagule counts after culture from stems showed: 1) Sclerotinia stem rot was not different from the control in plots sprayed with vinclozolin and thiophanate methyl in 1986-1988, 2) Sclerotinia stem rot was 5- to 10-fold greater in 1987 plots sprayed with chlorothalonil or Bravo C/M and their combination and in 1988 plots sprayed with chlorothalonil or fentin hydroxide and their combination than the control, 3) significantly more visible microsclerotia and stem propagules of Vd were present in 1988 plots treated with chlorothalonil or fentin hydroxide than in control plots, and 4) Cc was not reduced or enhanced by any spray treatment. Application of fungicides did not alter tuber yield.

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PROTECTION OF POTATO FROM RHIZOCTONIA-CANKER WITH BINUCLEATE RHIZOCTONIA-LIKE FUNGI. A. Escande and E. Echandi, Department of Plant Pathology, North Carolina State University, Raleigh, 27695-7616.

Fourteen isolates of binucleate Rhizoctonia-like fungi (BNR) were studied as potential biocontrol agents for protection of potato from Rhizoctonia-canker in greenhouse and potato fields naturally infested with Rhizoctonia solani (AG-3). Eight of the BNR reduced incidence and severity of Rhizoctonia-canker by an average of 78 and 85%, respectively, in greenhouse experiments. In the field, six of the eight BNR reduced incidence and severity of Rhizoctonia-canker by an average of 43 and 41%, respectively. In a field heavily infested with R. solani, selected BNR and the fungicide Tops 2.5D (thiophanate methyl) were equally protective of potato from Rhizoctonia-canker. Cultivars Atlantic, Irish Cobbler, Kennebec, Norchip, Russet Burbank, and Superior were equally protected from Rhizoctonia-canker by selected BNR under field conditions. Isolates of BNR have potential as biocontrol agents for protection of potato from Rhizoctonia-canker.

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EFFECTS OF DEW TEMPERATURE, DEW PERIOD, AND REPEATED INOCULATIONS WITH PUCCINIA JACEAE ON YELLOW STARTHISTLE. A. R. BENNETT and W. L. BRUCKART, USDA-ARS, Ft. Detrick, Bldg. 1301, Frederick, MD 21701.

Puccinia jaceae was evaluated for biological control of yellow starthistle (YST, Centaurea solstitialis) in greenhouse

studies. YST plants were uniformly inoculated with urediniospores 4 wk after planting and placed in dew chambers for 4, 8, 12, or 16 hr at temperatures ranging from 10 to 30 C. Disease severity (number of pustules) was evaluated 2 wk after inoculation, and the most pustules ($1.6/cm^2$ leaf area) developed after incubation at 20 C for 12 or 16 hr. No infection occurred at 10 or 30 C. Inoculation of YST plants up to four times on a weekly basis beginning 4 wk after planting resulted in mean shoot biomass values of 0.88, 0.60, 0.52, 0.46, and 0.41 g for 0, 1, 2, 3, and 4 inoculations, respectively. Under suitable conditions, significant reduction of YST biomass can occur from infection by P. jaceae.

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TRICHODERMA HARZIANUM USED IN INTEGRATED CONTROL OF BOTRYTIS CINEREA ROT ON APPLE. Arne Tronsmo. Department of Microbiology. Agricultural University of Norway. N-1432 Aas-NLH. Norway.

By the use of the fungal antagonist Trichoderma harzianum we have been able to reduce Dry Eye Rot (Botrytis cinerea) on apple. However, we are not able to perform biological control of all other diseases and pests in commercial fruit growing. Integrated control with fungicide resistant antagonists is therefore of interest. By testing several Trichoderma spp. isolates for fungicide resistance, we were able to select isolates with much higher tolerance to Carboximid and other fungicides than the parent strain. One isolate, Trichoderma harzianum 220 were able to control Dry Eye Rot in a field with commercial spray program against other pest and diseases, both alone and together with reduced dosage of Vinclozolin. Even if integrated control with T. harzianum and Vinclozolin was not significantly more effective than biological control on its own, the combination of two different control methods will probably give safer control, and less danger of resistance development in the pathogen.

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BIOLOGICAL AND CULTURAL MANAGEMENT OF ROOT INFECTING FUNGI. Brad Melvin, Joe Vargas, Jr., Lee Berndt and Ron Detweiler. Dept. of Botany and Plant Pathology, Michigan State University, East Lansing, Michigan. 48823.

Management of necrotic ring spot, a disease caused by an ectotrophic root infecting fungus (Leptosphaeria korrae), was obtained on Poa pratensis receiving daily irrigation and/or bio-organic fertilizer treatments. Highly significant reduction of necrotic ring spot was achieved with a 2.5mm per day irrigation treatment compared to an 80% evapo-pan irrigation treatment or no supplemental irrigation treatment. Significantly less disease incidence occurred with the bio-organic fertilizers, Turf Restore and Sustane in the 80% evapo-pan irrigation treatment compared to the untreated control. A correlation between disease reduction and an increase in total thatch bacterial populations in daily irrigated plots during May and June was observed. The early season period of turf growth is the optimum time of year for infection of P. pratensis by L. korrae. Total bacterial populations were higher throughout spring and early summer in the Turf Restore and Sustane plots compared to the untreated control. Many of the bacteria in these studies are antagonistic to L. korrae in vitro.

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A QUANTITATIVE ASSAY OF NEMATODE BIOCONTROL AGENT ACTIVITY AND ITS USE IN A CLANDOSAN AMENDED SOIL. J. C. Doney, Jr. and J. B. Kotcon. Div. of Plant and Soil Sciences, West Virginia Univ., P. O. Box 6057, Morgantown, WV 26506-6057.

Nematode biocontrol agent (NBA) activity, defined as mortality resulting from biotic activity, was assayed by comparing survival of Pratylenchus penetrans (PP) in raw (R) and sterile (S) soils. PP were added to 100-g samples of orchard soil (cherty silt loam) and incubated at 25 C. Percent recovery of viable PP was lower (P=0.05) in R than in S soils after 11 days of incubation. This difference in percent recovery was greater at 20 than at either 14 or 30% soil moisture, and increased as initial inoculum was increased beyond 200 PP. This assay was used to evaluate NBA stimulation by the nematicide Clandosan. Mean percent recovery was significantly lower in R amended with 22-g of Clandosan per L (0%) than in unamended R (17%), and, in Clandosan-amended S (16%) than in unamended S (33%). The results of this assay are inconsistent with an enhanced NBA mode of action for Clandosan.

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PREVENTION OF AFLATOXIN CONTAMINATION WITH STRAINS OF ASPERGILLUS FLAVUS. P. J. Cotty, USDA, ARS, Southern Regional Research Center, P.O. Box 19687, New Orleans, LA 70179.

Cottonseed can be infected by strains of Aspergillus flavus Link that do not produce aflatoxins. Furthermore, virulence of the fungus is not correlated with aflatoxin production. These observations suggest that strains of A. flavus which do not produce aflatoxins may exclude other strains from crops and prevent aflatoxin contamination. Greenhouse studies confirmed that non-toxigenic strains of A. flavus can prevent aflatoxin contamination of cottonseed by toxigenic strains. When simulated exit holes of the pink bollworm were inoculated with a strain which did not contaminate cottonseed with aflatoxins, subsequent contamination of developing cottonseed by two toxigenic strains was prevented. Inoculation with toxigenic and non-toxigenic strains simultaneously resulted in at least 100 fold reductions in toxin contamination. Non-toxigenic strains may be useful as protective agents in all susceptible crops.

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POSTHARVEST BIOCONTROL OF GRAY MOLD OF PEAR BY PSEUDOMONAS GLADIOLI. G. H. Mao and R. A. Cappellini, Department of Plant Pathology, Rutgers University, New Brunswick, NJ 08903.

Pseudomonas gladioli and its cell-free filtrate (CFF) inhibited growth of Botrytis cinerea in vitro. The bacterium was tested in vivo for biocontrol of gray mold of Anjou pears. Bacterial suspensions were applied to wounded pears. The pears were then inoculated with B. cinerea ($5x10^3$-$5x10^5$/ml) 1 hr later and incubated for 7-9 days in a moist chamber at 23-25 C. P. gladioli at concentrations of $1x10^8$) colony-forming units/ml (CFU/ml) retarded disease development on pears challenged with B. cinerea spore suspensions. When the antagonist was applied at $1x10^9$ CFU/ml, the pathogen did not produce lesions on pears. Control of gray mold was not obtained on wounded pears applied with CFF at concentrations of 20-80 units/ml at the same pathogen inoculum levels.

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

Widely differing levels of benomyl-resistance in Venturia

inaequalis has been attributed to allelic mutations in the beta-tubulin gene. To study this phenomenon at the molecular level, genomic DNA was isolated from 6-wk-old broth cultures of a benomyl-sensitive (WC-S) and a benomyl-resistant (KV3C) field isolate of V. inaequalis and partially digested with the restriction enzyme Sau3A. Sucrose gradient fractionated DNA (16-20 kb) and BamHI/EcoRI digested lambda EMBL3 DNA were ligated with T4 DNA ligase and packaged to prepare a library. The library was screened for clones with a heterologous Erisyphe graminis beta-tubulin probe. DNA sequence analysis of the clones showed extensive sequence similarities with the probe thereby confirming that the beta-tubulin gene had been cloned.

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OVERWINTER SURVIVAL IN THE FIELD OF COLLETOTRICHUM ACUTATUM ON STRAWBERRY FRUIT IN OHIO. L. L. Wilson, M. A. Ellis, and L. V. Madden, Dept. of Plant Pathology, Ohio State Univ., Wooster, OH 44691.

Survival of Colletotrichum acutatum on infected strawberry fruit was evaluated in the field. Infected fruit were sealed in nylon mesh bags (5 fruit/bag) and placed on and 5-8 cm beneath the soil surface in Nov 1988. Both treatments were covered with 8 cm of straw mulch. At 1-mo intervals, starting in Dec 1988, one bag from each of three replications was removed from on and beneath the soil surface and fruit were assayed for viable C. acutatum. From Nov to Mar 1989, ambient air temperatures (above straw mulch) ranged from -18 to 20 C, and soil temperatures ranged from -3 to 13.2 C. C. acutatum was recovered from 100% and >95% of fruit from on and within soil, respectively, for the first 3 mo. After 4 mo the fungus was recovered from 80 and 67% of the fruit from on and within soil, respectively. Sampling will continue through May 1989. Results will be discussed.

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CONTROL OF CYTOSPORA CANKER AND BACTERIAL CANKER IN A YOUNG SWEET CHERRY ORCHARD IN OREGON. R.A. Spotts, T.J. Facteau, and L.A. Cervantes. Oreg. St. Univ., Mid-Columbia Agric Research and Extension Center, Hood River, OR 97031.

A field study to evaluate control of Cytospora canker was initiated in 1981 by planting sweet cherry trees cv. Bing. Treatments included white trunk paint, 3 levels of nitrogen, application of benomyl (1.35g/l) after dormant pruning or at popcorn, petal fall, and shuck split. Trees were evaluated annually from 1982 to 1986 for active trunk cankers, and isolations made from margins of cankers. Between 1 and 7% of the trees were infected with C. cincta each year, and 26% were infected by 1986. Bacterial canker, caused by Pseudomonas syringae, occurred in 13% of the trees in 1982 and 25% by 1986. Death of trees infected with C. cincta and P. syringae was 14 and 26%, respectively. Nitrogen or benomyl did not reduce incidence of cankers. White trunk paint reduced the incidence of both Cytospora and bacterial trunk cankers. Disease incidence was highest in trees close to an old cherry orchard.

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PATHOGENICITY OF PHIALOPHORA SP. AND RHIZOCTONIA-LIKE FUNGI ON CRANBERRY (VACCINIUM MACROCARPON). L. P. Chang, E. H. Varney, and J. L. Peterson. Plant Pathology, Rutgers University, New Brunswick, NJ 08903.

Cranberry cuttings inoculated with a high level of Phialophora developed severe symptoms, including leaf yellowing, defoliation, desiccation, and root necrosis. Symptoms were significantly milder at low inoculum levels. Controls were symptomless. There was a significant difference in fresh weight and root length between Phialophora-inoculated and control cuttings. Rhizoctonia-like fungi isolated from cranberry roots and shoots had binucleate hyphae. Three selected isolates (Rh-1, Rh-2, and Rh-3) had different modes of infection. Rh-1 penetrated and colonized the epidermal and cortical cells. Rh-2 penetrated and produced typical hyphae and monilioid cells in the epidermis. Rh-3 did not penetrate the epidermal cells, but colonized the root surface.

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PHOMOPSIS BUD AND TWIG BLIGHT OF PEACH. F. F. Hendrix, Jr., Department of Plant Pathology, University of Georgia, Athens, GA 30602.

Phomopsis twig blight of peach, caused by Phomopsis sp., has been a major problem in North Georgia for the last 5 years. The fungus infects through healthy buds after harvest. Isolations were made from buds of cv. Red Haven, Crest Haven, Correll and Sentinel bi-weekly from May 27 through September 26, 1988. Buds were surface sterilized in 0.05% sodium hypochlorite in 10% ethanol, and plated on acid potato dextrose agar. Elevated isolation frequencies from 4% to 32% were recorded immediately after harvest for each cultivar. Cankers develop on bearing wood under the buds from December to February. Twigs die immediately after bloom. Benomyl sprays (1 lb/A a.i.) on August 1 and September 1 reduced the incidence of twig cankers in 1989 from 48% to 8%.

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ISOLATION, PURIFICATION AND CHARACTERIZATION OF A PHYTOTOXIN FROM LIQUID CULTURES OF LEUCOSTOMA PERSOONII AND L. CINCTA. *A.M. Svircev, °A.R. Biggs, *N. Miles and *C. Chong. *Horticultural Research Institute of Ontario, Vineland Station, Ontario, Canada, LOR 2EO; °Agriculture Canada Research Station, Vineland Station, Ontario, Canada, LOR 2EO.

Liquid cultures of L. persoonii and L. cincta were grown in 2% malt extract medium on a rotary shaker. Fourteen-day-old cultures were filtered and the cell-free culture filtrate was separated by ultrafiltration into specific molecular size fractions. Excised peach, Prunus persica, shoot tips (ca. 4 cm in length) were used to test the crude fractions for toxin activity. Peach canker-like symptoms were induced only by the < 1,000 dalton fraction. Further purification of the phytotoxin by isoelectric focusing and granulated bed electrophoresis has identified the toxin as a small polypeptide. Further purification and identification of the phytotoxin is in progress. The toxin is being tested as selective agent in the tissue culture program to obtain peach plants with an increased resistance to Leucostoma spp.

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CYTOCHEMICAL PROCEDURES FOR ILLUSTRATING THE RESPONSE OF PECAN TO INFECTION BY CLADOSPORIUM CARYIGENUM. S. V. Diehl, C. H. Graves, and P. A. Hedin. Dept. of Plant Path. & Weed Sci. and Crop Sci. Res. Lab, USDA, Mississippi State, MS 39762.

Several fungitoxic phenolics affect resistance in pecan to infection by C. caryigenum. Transmission electron microscopy can be used to locate phenolics within leaf vacuoles. Distribution of phenolics within vacuoles varied depending upon the fixation procedure used. Localization and quantification of juglone, isoquercitrin and condensed tannins in fresh microscopic tissues can be accomplished with the use of the Hoepfner-Vorstatz stain and butanol-HCl and a microspectrophotometer. This technique showed that juglone was found consistently in higher concentrations in all greenhouse seedling leaf tissue than the other two compounds. Both infected and noninfected leaf tissue can be compared with scanning electron microscopy. Combined use of these procedures should give a composite illustration of host response to infection by C. caryigenum.

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DIFFERENTIATION OF COLLETOTRICHUM SPP. PATHOGENIC TO STRAWBERRY. P. S. Gunnell and W. D. Gubler, Department of Plant Pathology, University of California, Davis, CA 95616.

The morphology of both conidia and setae produced on strawberry leaf piece agar were found to be reliable criteria to distinguish Colletotrichum spp. pathogenic to strawberry. von Arx proposed that C. fragariae Brooks was synonomous with C. gloeosporioides Penz., however, conidia of C. fragariae isolates, including specimens collected by Brooks, were predominantly clavate whereas conidia of C. gloeosporioides were predominantly cylindrical with rounded ends. Conidia of C. fragariae were also longer and narrower than those of C. gloeosporioides. Setae of C. fragariae were brown, several septate, somewhat sinuous, not tapered, and usually produced conidia when mature. Setae of C. gloeosporioides although also brown and septate, were strongly tapered, did not produce conidia, and were finely warted toward the top. C. acutatum Simmonds produced fusiform spores and comparatively short, dark brown, thick-walled setae which were usually aseptate.

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RELATIONSHIP OF TEMPERATURE TO THE FUNGI INVOLVED IN CRANBERRY FRUIT ROT. F.L. Caruso, Cranberry Experiment Station, University of Massachusetts, East Wareham, MA 02538.

More than ten different fungi are capable of causing field or storage rot in cranberry fruit. Cranberries

(cultivars 'Early Black' and 'Crowley') with diverse rot symptoms were sampled in July, August, and September (field collection), and in October and January (while in storage). Individual berries were cut into thirds, surface-sterilized, plated on three ACMA plates. Plates were incubated at 15, 22, and 30 C for three weeks. Seven additional cultivars were sampled in February. Proper diagnosis of the primary causal agent is dependent on incubation temperature. Godronia, Sporonema, and Apostrasseria preferred cooler temperatures whereas Phyllosticta, Physalospora and Phomopsis preferred warmer temperatures. There were diverse differences in fungi isolated at different sampling times among different cultivars.

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POWDERY MILDEW OF PECAN: VARIETAL SUSCEPTIBILITY AND EFFECTS ON KERNEL DEVELOPMENT. T. B. Brenneman and P. F. Bertrand, Coastal Plain Experiment Station and Rural Development Center, respectively, Dept. of Plant Pathology, University of Georgia, Tifton, GA 31793.

Studies were conducted on powdery mildew (Microsphaera penicillata) of pecan to monitor disease progress and determine its effects on kernel development. A total of 716 nuts (cv. Woodard) were rated six times during the season between July 10 and September 17, 1987. Severe powdery mildew developed, and disease levels at the third through last evaluations were significantly negatively correlated with kernel weight. The percent kernel weight was not correlated with disease severity. Within a cluster, the further a nut was from the terminus, the less powdery mildew it had. An additional study monitored disease progress on 14 pecan cultivars. Woodard was extremely susceptible but the other cultivars had moderate to good resistance.

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EFFECT OF FLOOD DURATION ON SEVERITY OF PHYTOPHTHORA ROOT AND CROWN ROT OF KIWIFRUIT IN CALIFORNIA. K. E. Conn and W. D. Gubler. Department of Plant Pathology, University of California Davis, CA 95616.

Six month old kiwifruit seedlings grown for 3 months in soil artificially infested with Phytophthora citrophthora, P. cryptogea, P. megasperma, or one of two unidentified Phytophthora spp., were subjected to biweekly flooding periods of 0, 6, 12, 24, or 48 hours. Root rot (RR) and crown rot (CR) caused by P. cryptogea and an unidentified Phytophthora sp. increased with the length of the flooding period. With 48 hours flooding, consistently severe RR (83-96%) was observed. In contrast, P. citrophthora, P. megasperma, and another unidentified Phytophthora sp. caused variable RR (13-80%) with 0 to 48 hours flooding while CR developed only sporadically on seedlings flooded for 48 hours. These results indicate that soil-water management that avoids prolonged and repeated saturation may minimize losses due to Phytophthora, depending on the species present.

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VARIATION IN PREDICTION OF FRUITLET CORE ROT WITHIN AND BETWEEN PINEAPPLE FIELDS. J.E. Yuen and G.Y. Taniguchi, Department of Plant Pathology, 3190 Maile Way, Honolulu, HI 96822.

Routine application of acaricides for control of fruitlet core rot (FCR) and other fruit diseases (interfruitlet corking, eye inhibition, and leathery pocket) caused by *Penicillium funiculosum* is not economically feasible, possibly because FCR can also be caused by *Fusarium moniliforme* f. sp. *subglutinans*. Application of acaricides only when triggered by suitable 'predictors' has been proposed to reduce chemical application. The accuracy of prediction for any area will be limited by the natural variation of disease and the 'predictor' within this area. A survey was made of 10 fields to examine the correlation of pre- and post-force mite and fungal populations (*P. funiculosum* and *F. moniliforme* f. sp *subglutinans*) to FCR incidence at harvest. The use of entire fields as the statistical unit showed a correlation (r) between pre-force mite populations and FCR of 0.82. Due to variation within these fields, however, the correlation dropped to 0.42 when the 60 subplots (6 within each field) were analyzed. Fungal isolations pre- and post-force showed high levels of *F. moniliforme* f. sp. *subglutinans* but this was not statistically significant as a disease predictor.

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ROOT ROT OF KIWIFRUIT VINES CAUSED BY PYTHIUM SPP. A. J. Latham, W. A. Dozier, Jr., J. M. Mullen, and H. L. Campbell. Ala. Agric. Exp. Stn., Auburn University, AL 36849.

Plantings of kiwifruit (Actinidia chinensis) have been made in several southeastern states. A planting on land previously in long-time peanut production sustained over 50% loss to root rot following an extended wet period during June. Pythium spp. were consistently obtained from rotted roots. One isolate cultured on corn meal agar at 25 C developed oogonia 23.4 um diam typical of Pythium ultimum var. ultimum. Eight isolates cultured in the dark on a blue grass-water medium at 22 C produced oogonia ranging from 21.7 to 24.0 um typical of Pythium ultimum var. sporangiiferum. Pure cultures were increased on corn meal-sand mixtures and used to inoculate potted kiwi plants. Within six weeks, both species caused root decay similar to that observed in the field.

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REGULATION OF PECTIN LYASE (PNL) PRODUCTION IN ERWINIA CAROTOVORA SUBSP. CAROTOVORA (Ecc) STRAIN 71 BY DNA DAMAGING AGENTS: ISOLATION OF A NONINDUCIBLE MUTANT USING A pnlA-lacZ TRANSCRIPTIONAL FUSION. James L. McEvoy, Russell O. Nordeen, and Arun K. Chatterjee. Department of Plant Pathology, University of Missouri-Columbia, Columbia, MO 65211, U. S. A.

To analyze the regulation of PNL production we cloned the structural gene, pnlA, and constructed pnlA-lacZ fusions using the mini-Mu element, POI1734. In six of eleven insertions, lacZ expression was controlled by the pnlA promoter. The induction of ß-galactosidase (ß-gal) by mitomycin C (MC) occurred in RecA$^+$ but not in RecA$^-$ Ecc strains. A typical fusion was placed by marker exchange into the chromosome of a Lac$^-$ derivative of Ecc71. In this construct (AC5022), ß-gal was induced 60-fold with MC. By EMS mutagenesis of AC5022 followed by screening for ß-gal production, we obtained a mutant (AC5023) that was noninducible by MC. AC5023 was not defective in RecA nor was it complemented by a PnlA$^+$ plasmid. These data suggest that in AC5023 there is a defect in pnlR, a gene locus that positively regulates PNL production.

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CLONING OF eep GENES THAT DETERMINE EXTRACELLULAR ENZYME PRODUCTION IN ERWINIA CHRYSANTHEMI (EC16) AND E. CAROTOVORA SUBSP. CAROTOVORA (ECC71). H. Murata, W. Chun, and A. K. Chatterjee. Dept. of Plant Pathology, Univ. of Missouri, Columbia, MO 65211, U.S.A.

During the isolation of pectinase-deficient mutants, we obtained Eep$^-$ mutants of EC16 and Ecc71 that produced very low levels of pectate lyase, polygalacturonase, cellulase and protease activities. The EC16 mutant also was mucoid and produced lower levels of phospholipase C. The Eep$^-$ mutants did not macerate potato tuber tissue. By mobilizing EC16 and Ecc71 gene libraries, we isolated clones that restored production of these enzymes and tissue-macerating ability in the cognate mutants. The Eep$^+$ plasmids did not restore extracellular enzyme production in export deficient (Out$^-$) mutants of EC16 or Ecc71. Our data reveal the presence of a gene(s), other than out, that pleiotropically affects the production of extracellular proteins in these bacteria.

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EVIDENCE FOR CATECHOL SIDEROPHORE PRODUCTION BY ERWINIA CAROTOVORA SUBSP. CAROTOVORA. C.T. Bull[1], C.A. Ishimaru[2], and J.E. Loper[2]. [1]Department of Botany and Plant Pathology, Oregon State University, [2]USDA-ARS, HCRL, Corvallis, OR 97330.

Erwinia carotovora subsp. carotovora strain W3C105, which

causes soft rot disease of potatoes, was investigated for production of a catechol siderophore. Catechol production was detected colorimetrically, by bioassay, and by homology to genes determining biosynthesis of enterochelin, the catechol siderophore of Escherichia coli. Results suggested that strain W3C105 produces a catechol siderophore with functional similarity to enterochelin. Genes involved in catechol production were identified from a genomic library of strain W3C105 in E. coli strain AN192, which is deficient in enterochelin production. Three clones producing a catechol were detected on a universal siderophore-detection medium. These clones provided iron to enterochelin-deficient indicator strains. The role of catechol siderophore production in the pathogenicity and ecology of E. carotovora will be evaluated.

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CHARACTERIZATION OF AN rcsA-LIKE GENE OF ERWINIA AMYLOVORA THAT STIMULATES EXTRACELLULAR POLYSACCHARIDE (EPS) PRODUCTION IN ERWINIA SPP. AND OTHER ENTEROBACTERIA. W. Chun, A. Chatterjee, R. N. Goodman and A. K. Chatterjee, Department of Plant Pathology, University of Missouri-Columbia, Columbia, MO 65211.

EPS production by E. amylovora is required in the elicitation of the fire-blight disease in apples and pears. To examine the regulation of EPS biosynthesis, we obtained from an E. amylovora cosmid library, several E. coli (HB101) clones that were mucoid on agar plates. The complementation of an rcsA mutation in E. coli and the stimulation of EPS production in various enterobacteria (E. amylovora, E. stewartii, E. coli, and Salmonella typhimurium) associate the mucoid phenotype conferred by the cloned DNA to an E. amylovora regulator gene. This rcsA-like gene along with its own promoter has been localized on a 2.2 kb DNA segment. Nucleotide sequence homology determined by Southern hybridizations and functional complementation of the mucoid phenotype by the cloned DNA indicate that the genes for the regulation of EPS biosynthesis have been conserved in these enterobacteria.

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CLONING OF AN AVIRULENCE GENE FROM *PSEUDOMONAS SOLANACEARUM* STRAIN AW1 AND ITS INVOLVEMENT IN HOST RANGE. B. F. Carney and T. P. Denny, Dept. of Plant Pathology, UGA, Athens, GA 30602.

A locus responsible for the hypersensitive response (HR) in *P. solanacearum* strain AW1 (avirulent on tobacco, but pathogenic on tomato) was cloned by complementation in *P. solanacearum* strain K601 (pathogenic on tobacco and tomato). *Pseudomonas solanacearum* K601 transconjugants [K601(pBC73) and K601(pBC62)] were nonpathogenic on tobacco, suggesting that cloned locus had the potential to restrict the host range of K601. DNA analysis of the clones pBC73 and pBC62 indicated that a common 4.2 Kb *Eco*RI/*Bam*HI fragment was responsible for the induction of HR in K601 transconjugants. Transposon mutagenesis of the wild type locus in strain AW1 resulted in the loss of HR on tobacco but retention of pathogenicity on tomato, indicating that this locus contains an avirulence gene. Pathogenicity on tobacco was not acquired upon inactivation of this avirulence gene in AW1, suggesting that AW1 lacks positive acting host range genes that are required for pathogenicity on tobacco.

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CLONING OF TWO GENES FOR PRODUCTION OF EXTRACELLULAR POLYSACCHARIDE FROM *PSEUDOMONAS SOLANACEARUM* AND THEIR CONTRIBUTION TO VIRULENCE. S.-R. Baek and T. P. Denny. Dept. of Plant Pathology, University of Georgia, Athens, GA 30602.

Previous research had marked two loci of *P. solanacearum* strain AW1 that are involved in the production of extracellular polysaccharide (EPS) with Tn*5* insertions. These Tn*5* insertions and flanking DNA of *P. solanacearum* were cloned and used to locate eight cosmid clones that contained homologous sequences in a genomic library of *P. solanacearum* AW1. The cosmids were restriction mapped and found to contain unique and overlapping regions that spanned a total of 55 kilobases; the Tn*5* insertions in the two loci were centrally located and 12.5 kb apart. The Tn*5* inactivated genes in strains AW1-1 and AW1-41 were designated *epsA* and *epsB*, respectively. Seven of the eight cosmids completely restored EPS production to strain AW1-41. Three cosmids partially restored EPS production to strain AW1-1 and these transconjugants were more virulent on tomato than was strain AW1-1. These results support the idea that EPS has a major role in *P. solanacearum* causing wilt symptoms on tomato.

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THE HYPERSENSITIVE RESPONSE IS ELICITED BY ESCHERICHIA COLI CONTAINING A CLUSTER OF PATHOGENICITY GENES FROM ERWINIA AMYLOVORA. S. V. BEER; C. H. ZUMOFF; D. W. BAUER; B. J. SNEATH; and R. J. LABY. Department of Plant Pathology, Cornell University, Ithaca, NY 14853 U.S.A.

Hrp$^-$ mutants of E. amylovora are deficient in both pathogenicity to pear fruit and the ability to elicit the hypersensitive response in tobacco. A cosmid, pCPP430, containing wild-type DNA of E. amylovora, was identified that restores pathogenicity and HR-eliciting ability to 18 transposon-induced Hrp$^-$ and two naturally occurring Hrp$^-$ mutants. pCPP430 contains a cluster of hrp genes, dispersed throughout a 45 kb region of chromosomal DNA. When Escherichia coli, strain DH5, containing pCPP430, was infiltrated into tobacco leaf tissue, strong collapse occurred extremely rapidly. In addition, the cosmid conferred HR-eliciting ability (in tobacco), to several other species of Erwinia. These results clearly indicate that pCPP430 contains all the genes needed for elicitation of the HR, and that they are expressed in E. coli and in other Erwinia species.

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CHARACTERIZATION OF PROMOTER-ACTIVE FRAGMENTS FROM XANTHOMONAS USING A NEW BROAD HOST RANGE PROMOTER SELECTION VECTOR. S. Swarup, R. DeFeyter and D.W. Gabriel. Plant Pathology Dept., University of Florida, Gainesville, FL 32611.

A broad host-range (Inc Q) promoter selection vector, pUFC600, was constructed that enables a direct selection of promoter-active fragments. pUFC600 is 9.4 kb in size, Kn^r, has a promoterless Sm^r gene, multiple cloning sites and tandem transcriptional terminators upstream of the cloning sites. In the absence of any promoter-active fragment, E. coli strain DH5α and Xanthomonas strains containing the plasmid were sensitive to streptomycin at 15 μg/ml in minimal and 25 μg/ml in complete media. A genomic library (~4 kb insert size) of X. citri strain 3213 was constructed in pUFC600. Clones were introduced into X.c. pv. citrumelo strain 3048 Sp^r at an average frequency of 10^{-4} per recipient. Promoter activity was selected or screened on both minimal and complete media with streptomycin and various classes of promoter active fragments were identified. The relationship of pathogenicity genes to promoter-active fragments which were induced on minimal media will be discussed.

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Tn5-induced mutations of *Xanthomonas campestris* pv. *citrumelo* affecting pathogenicity and host-species specificity. M.T. Kingsley and D.W. Gabriel. University of Florida, Gainesville, Florida 32611

Tn5-induced mutations affecting pathogenicity (PATH$^-$) and host species specificity (HSS$^-$) were recovered in *X. campestris* pv. *citrumelo*. All 3048::Tn5 exconjugants (including auxotrophs) were screened on both bean and citrus. Auxotrophic mutations had significant effects *in planta*. For example, uracil auxotrophy resulted in a path$^-$ phenotype, while isoleucine-valine auxotrophs were hss$^-$ in bean but were relatively unaffected in citrus. None of the Tn5-inserts affecting pathogenicity or host range appeared to be clustered by hybridization analyses, nor did the affected DNA regions hybridize with the *P. solanacearum hrp* cluster (i.e. pVir2). A high level of polymorphism was observed between, but not within 12 species or pathovars of *Xanthomonas* probed with HSS-related DNA fragments, which may indicate a lack of conservation of the gene(s) present at the affected loci.

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MOLECULAR CHARACTERIZATION OF A LOCUS REGULATING PRODUCTION OF EXTRACELLULAR POLYSACCHARIDE SLIME AND VIRULENCE IN *PSEUDOMONAS SOLANACEARUM*. S. M. Brumbley and T. P. Denny, The University of Georgia, Athens, GA 30602.

A well known phenomenon of *P. solanacearum* is the spontaneous mutation from a mucoid to a nonmucoid form. A variety of other traits, including virulence, are affected. A previously isolated Tn*5* mutant (AW1-80) is identical to the naturally occurring spontaneous mutant AW1-A in every way tested. An *Eco*RI fragment containing the Tn*5* plus flanking DNA from AW1-80 was cloned and used as a hybridization probe to identify two cosmids from a genomic library of the wild type *P. solanacearum* strain (AW1). These cosmids restored wild type traits when conjugated into AW1-80, AW1-A and several other spontaneous avirulent mutants strains of *P. solanacearum*. These results suggest that this locus (designated *rpc*) contains a regulatory element(s) with global functions. This system does not appear to be the same as that regulating the production of alginate in *Pseudomonas aeruginosa*.

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THE EFFECT OF EXPRESSION OF AGROBACTERIUM RHIZOGENES ROLB AND C GENES ON ROOT INDUCTION AND GROWTH. F. Shaheen and F. F. White, Dept. of Plant Pathology, Kansas State University, Manhattan 66506.

The Ri plasmid of A. rhizogenes contains a set of unique T-DNA genes (rolA, B, and C) which, upon expression in plant cells, induce hairy root syndrome. Each gene induces independent effects in transgenic plants, while their combined effects direct the hairy root syndrome. RolB gene causes root induction and flower hyperstyly. RolC gene expression results in fast root growth, reduced plant height, small flowers, hyperstyly, poor fertility, and wrinkled leaves. Deletion of the DNA sequences that separate the rolB and C promoters decreases the rooting response on kalanchoe leaves. A 1.2 kb DNA fragment containing wild-type regulatory sequences was fused to the reporter gene, β-glucuronidase gene (GUS), in both orientations to study the effect of rolB and C genes expression.

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THE COAT PROTEIN OF TOBACCO MOSAIC VIRUS: AN ELICITOR MOLECULE OF THE HYPERSENSITIVE REACTION. J. N. Culver and W. O. Dawson. Dept. of Plant Pathology, Univ. of California, Riverside, CA 92521.

Recently, specific nucleotide changes in the coat protein gene of tobacco mosaic virus (TMV) have been shown to be responsible for the induction of hypersensitivity (HR) on *Nicotiana sylvestris*. These nucleotide changes resulted in amino acid substitutions in the coat protein. To determine if the altered RNA or altered protein was responsible for the induction of HR, the coat protein translational start was removed from a full-length cDNA clone of mutant TMV 25, which normally induces HR on *N. sylvestris*. Infectious transcripts of the altered genome failed to induce HR on inoculated leaves of *N. sylvestris*. However, infectious viral RNA was recovered 10 days post-inoculation and Western blot analyses revealed the presence of the TMV encoded 126 KD protein and the absence of coat protein. This study demonstrates the coat protein of TMV to be the elicitor molecule responsible for the induction HR on *N. sylvestris*.

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PHYSIOCHEMICAL ANALYSIS OF A SEROLOGICALLY DISTINCT TOMATO SPOTTED WILT VIRUS STRAIN. M. D. Law and J. W. Moyer, North Carolina State University, Box 7616, Raleigh, NC 27695-7616.

Tomato spotted wilt virus (TSWV) is the type member of a monotypic group of plant viruses (tomato spotted wilt group). We have isolated a TSWV variant from impatiens (TSWV-I). The symptoms produced by TSWV-I were typical for TSWV but the host range was limited to some ornamentals and Nicotiana sp. Electron micrographs of TSWV-I infected tissue revealed cytopathic effects distinct from TSWV, consisting primarily of filaments in a lattice arrangement. TSWV-I is composed of three distinct RNA species of approximately 8.3 Kb, 5.2 Kb and 3.4 Kb, which comigrate with TSWV RNA. Purified TSWV-I virions are composed of three proteins, approximately 78 KD (G1), 52 KD (G2), and 28 KD (NC) which comigrate with TSWV proteins. The TSWV and TSWV-I nucleocapsid proteins were not serologically related by Western blot analysis with polyclonal antibodies. In contrast, TSWV and TSWV-I G1 and G2 proteins were found to be related in Western blot analysis.

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SEROLOGICAL RELATIONSHIPS OF THE CAPSID PROTEINS OF THE TYPE ISOLATE OF MAIZE CHLOROTIC DWARF VIRUS (MCDV-T). C. M. Maroon, D. T. Gordon and R. E. Gingery (USDA/ARS). Dept. of Plant Pathology, Ohio State Univ., Wooster 44691.

MCDV-T consistently yielded three capsid proteins, designated CP1 (MW=33.8 kD), CP2 (MW=25.8 kD) and CP3 (MW=23.6 kD), and occasionally a fourth, CP4 (MW=19.6 kD), in SDS-PAGE. CPs 1, 2 and 3 were separated by SDS-PAGE, eluted, concentrated and used to raise polyclonal antisera (PcAs) in rabbits. The dilution end points of these antisera, tested against 150 ng of the homologous CP in Western blots (WB), were: CP1-PcAs=1:1,200,000; CP2-PcAs=1:38,400; and CP3-PcSa=1:9600. In WB, CP1-PcAs did not react with CP2 or CP3, whereas CP2-PcAs and CP3-PcAs cross-reacted with CP2 and CP3, but did not react with CP1. We conclude that CP1 is distinct from CP2 and CP3 and hypothesize that CP1 is expressed by a distinct viral gene. While appearing serologically related, we refrain from a conclusion on the relationship of CP2 and CP3; pending further study of cross-contamination of CP2 and CP3 preparations.

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IDENTIFICATION AND NUCLEOTIDE SEQUENCE OF THE COAT PROTEIN GENE OF POTATO LEAF ROLL VIRUS. O. P. Smith, USDA-ARS, Frederick, MD 21701 and K. F. Harris, Dept. of Entomology, Texas A&M University, College Station, TX 77843

The open reading frame (ORF) for the coat protein of potato leaf roll virus (PLRV) has been sequenced and identified using cloned PLRV cDNA. Verification was obtained by the expression of a portion of this ORF as an Escherichia coli B-galactosidase fusion protein followed by dot-blot ELISA analysis employing polyclonal antisera to purified PLRV. The predicted molecular weight of the protein is 23 K (207 amino acids). Analysis of the protein revealed an amino acid sequence homology of 38% with the 22 K coat protein of barley yellow dwarf virus (BYDV). As reported for BYDV (Virology 165:306) the PLRV coat protein gene contains the complete overlap (+1 frame) of a second ORF. This ORF encodes a potential protein of 17 K (155 amino acids) and shares ca. 27% amino acid sequence homology with the corresponding 17 K ORF of BYDV.

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THE ROLE OF PHENYLPROPANOID PATHWAY ENZYMES IN THE RESISTANCE RESPONSE OF SOYBEAN TO SOYBEAN MOSAIC VIRUS. C.W. Choi, C. L. Cramer, D.C. Bays and S.A. Tolin, Department of Plant Pathology, Physiology and Weed Science, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061.

In the soybean cultivar York, which has single gene resistance to the type or G1 strain of soybean mosaic virus (SMV), the G4 strain induces local and systemic necrosis and the G5 strain induces systemic mosaic. Total mRNA isolated from leaves through 72 hr after inoculation with SMV-G4 or -G5, separated on agarose gels, and probed with cDNA specific for each of two enzymes in the phenylpropanoid pathway, phenylalanine ammonia-lyase (PAL) and chalcone synthase (CHS), was positive only for CHS by 48 hr with SMV-G4. Enzymatic activity of PAL, assayed in mock or virus-inoculated leaves or hypocotyls, increased with both SMV-G4 and -G5, but the time course differed. The induction of defense responses in this host-virus system will be compared to other pathogen strain-specific, induced host defense responses.

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CHARACTERIZATION OF MAIZE CHLOROTIC DWARF VIRUS (MCDV) RNA. X. Ge, D. T. Gordon and R. E. Gingery (USDA), Dept. of Plant Pathology, and M. D. McMullen (USDA), Dept of Agronomy, Ohio State Univ., Wooster, OH 44691.

MCDV RNA isolated from virions was fractionated on oligo(dT) cellulose into polyA+- and polyA-RNAs. The polyA+RNA contained predominantly 10-kb RNA, considered to be the full-length genomic RNA, while the polyA-RNA contained a range of small to large (10-kb) RNAs with no discrete bands. The polyA-RNA hybridized to cDNA synthesized from the polyA+RNA. The two RNA fractions directed synthesis of similar sets of proteins in cell-free translation systems. Northern hybridization analysis using cDNA synthesized from the 10-kb MCDV-virion RNA detected only full-length MCDV RNA in total RNA, polyA+RNA, and RNA released from polyribosomes by either EDTA or puromycin from MCDV-infected tissue. No subgenomic RNAs were detected. These results support the hypothesis that MCDV-virion RNA is a monopartite, positive sense, polyadenylated RNA which is expressed as a polyprotein.

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MAPPING OF POTYVIRUS-SPECIFIC AND GROUP-COMMON ANTIGENIC DETERMINANTS WITH MONOCLONAL ANTIBODIES BY WESTERN-BLOT ANALYSIS AND COAT PROTEIN AMINO ACID SEQUENCE COMPARISONS. Ramon Jordan. USDA-ARS, Florist and Nursery Crops Laboratory, Beltsville, MD

The reactivities of bean yellow mosaic virus (BYMV)-specific, BYMV subgroup-specific, and potyvirus cross-reactive monoclonal antibodies (McAbs) were tested in immunoblot analysis with SDS-PAGE separated coat protein subunit and enzymatically or chemically generated peptide fragments. BYMV-specific antigenic determinants were located in the 40-amino acid (aa) residue trypsin-cleaved N-terminal peptide. BYMV subgroup-specific sites were located in the 18-aa C-terminal peptide and in less-conserved regions of the 218-aa trypsin-resistant-core (TRC) protein. Cross-reactive McAbs reacted with conserved regions located in the TRC peptide. A highly conserved determinant, recognized by a broad-spectrum McAb (which has reacted with more than 95 potyvirus isolates so far tested), was mapped to a 14-aa residue peptide in the TRC. Progress in the ELISA analysis of overlapping synthetic octa-peptides, to further identify and delineate McAb-binding domains, will also be presented.

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PRODUCTION OF TRANSGENIC TOBACCO CONTAINING COWPEA MOSAIC VIRUS (CPMV) COAT PROTEIN GENES. D. L. NIDA and S. A. Ghabrial, Dept. of Plant Pathology, University of Kentucky, Lexington, KY 40546-0091

Preliminary data in our laboratory indicate that tobacco may serve as a model to study the potential of cross-protection against CPMV. ELISA results show that tobacco supports virus multiplication and cell-to-cell movement. Because CPMV coat proteins VP37 and VP23 are derived from a 60k polyprotein precursor by proteolytic processing, it was of interest to determine whether constitutive expression of the 60k polypeptide in transgenic plants would interfere with CPMV infection. Therefore, plant expression vectors were constructed by inserting cDNA representing the CPMV 60k precursor into the binary Ti vector pMON530. Constructs containing CPMV coat protein genes in sense and antisense orientations were generated. Transgenic plants are being produced via Agrobacterium cocultivation and selection on kanamycin-containing medium.

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CELL-FREE STUDIES OF TEV 49KDA PROTEINASE PROCESSING: DIFFERENTIAL CLEAVAGE RATES AT DIFFERENT POLYPROTEIN JUNCTIONS. W. G. Dougherty and T. D. Parks, Dept. of Microbiology, Oregon State Univ., Corvallis, OR 97331-3804.

Tobacco etch virus (TEV) 49kDa proteinase recognizes a specific heptapeptide sequence at five locations on the TEV polyprotein. This consensus cleavage sequence contains both conserved and nonconserved positions. In cell free studies, cleavage at the 50kDa/71kDa protein junction proceeded at a slow rate relative to processing at the 58kDa/30kDa cleavage site. Site directed mutagenesis of TEV cDNA sequences was performed, such that the nonconserved positions of the 50kDa/71kDa site were converted into those amino acids found at the 58kDa/30kDa cleavage site. Reciprocal mutations of the 58kDa/30kDa site were also tested. In each case, processing of the 58kDa/30kDa sequence proceeded at a faster rate. These data suggest that post translational regulation of gene expression in TEV may be possible via differential proteolytic processing at various gene product junctions.

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DETECTION OF PLANT RNA VIRUS-SPECIFIC RIBONUCLEOPROTEIN COMPLEXES IN INFECTED CELL EXTRACTS. R. French. USDA, ARS, Department of Plant Pathology, University of Nebraska, Lincoln, NE 68583.

Within cells, both cellular and viral RNAs probably exist as complex ribonucleoproteins. In order to detect RNA virus-specific ribonucleoproteins in a general way, antiserum to double-stranded RNA (dsRNA) was used to immunoprecipitate complexes from barley stripe mosaic virus (BSMV), brome mosaic virus, or tobacco mosaic virus-infected barley protoplasts. Radiolabeled protein components of the complexes were then detected by SDS-PAGE and fluorography. Immunoprecipitates from mock-inoculated protoplasts contained little protein while those from infected cells revealed several proteins unique for each virus, including coat protein. Extracts from BSMV-infected cells contained polypeptides of ca. 140, 60, 25 (coat), 20 and 18 kD. UV irradiation of cells prior to extraction increased the yield of protein suggesting that the immunoprecipitated proteins are intimately associated with RNA in vivo. Several types of complexes may be precipitated because synthetic dsRNA and BSMV virion RNA were both partially effective in competition assays.

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COMPARISON OF SYMPTOMS ASSOCIATED WITH EXPRESSION OF CaMV GENE VI WITH TWO PROMOTERS IN TRANSGENIC PLANTS. K-B. Goldberg, J.M. Kiernan, and R.J. Shepherd. Dept. of Plant Pathology, Univ. of Kentucky, Lexington, KY 40546.

Datura innoxia and Nicotiana edwardsonii were transformed with gene VI of cauliflower mosaic virus (CaMV) strains CM1841 and D4 with their homologous 19S promoters or a chimeric 35S promoter-gene VI of CM1841 using the Ti-plasmid vectors pGA472 or pKYLX-7, respectively. These plants are not hosts for CM1841. Expression of P62, the gene VI protein, in D. innoxia was associated with stunting accompanied by chlorosis or necrosis. The maximum levels of P62 in these plants with either a 19S or 35S promoter were similar, as determined by western blot analysis using antiserum to P62. The transgenic N. edwardsonii were mildly chlorotic when expressing gene VI of CM1841 but overall the levels of P62 were much lower than observed with D. innoxia. The accumulation of P62 in N. edwardsonii was at least ten times greater using gene VI in combination with a 35S promoter compared to the 19S promoters.

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THE EFFECT OF IN PLANTA PRODUCED HUMAN INTERFERON ON TYMV INFECTION.

G. A. de Zoeten, J. R. Penswick and T. Hohn. Friederich Miescher-Institut, P. O. Box 2543, CH-4002, Basel, Switzerland

Cauliflower mosaic virus (CaMV) DNA was engineered to carry the human IFN αD gene to the infected plant. Inoculation of turnip (Brassica rapa cv "Just Right") with CaMV strains carrying the IFN αD gene resulted in the production of biologically active IFN αD in infected plants (ca. 2μg/g Fwt.) In planta produced IFN αD did not hamper superinfection with a single stranded (+) RNA plant virus, turnip yellow mosaic virus (TYMV).

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GENOMIC CHARACTERIZATION OF BEET CURLY TOP VIRUS ISOLATES. D. C. Stenger and J. E. Duffus, USDA-ARS, Salinas, CA, 93905.

Full-length, infectious DNA clones have been constructed for three distinct isolates of beet curly top virus (BCTV). Progeny virus derived from cloned genomes of the Logan (severe on Beta vulgaris, wide host range), Worland (mild on B. vulgaris, wide host range), and Horseradish (narrow host range) isolates were transmitted by Circulifer tenellus, and displayed the same phenotypes as the original isolates. A fourth BCTV genome (severe, wide host range) was inadvertently cloned as a contaminant of the Horseradish isolate. Southern hybridization assays indicated that each cloned genome shared sequence relatedness with a full-length, infectious BCTV DNA clone previously characterized by Stanley et al (EMBO J 5:1761). Endonuclease restriction maps developed for the cloned BCTV genomes were distinct from one another. Infectivity assays determined that plasmids containing tandem repeats of BCTV genomes were generally more infectious than excised linear BCTV DNA inserts.

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MUTAGENESIS OF GENE VI of CAULIMOVIRUSES RESULTS IN CHANGES IN THE DISEASE PHENOTYPE. E. P. Broglio and R. J. Shepherd. University of Kentucky, Lexington, KY 40506.

A CaMV hybrid genome was constructed which contained unique restriction sites bordering gene VI that allowed its sequences to be subcloned, mutagenized and then ligated into the remainder of the genome. Changes in the primary structure of gene VI were made by linker-insertion mutagenesis. Of the several mutants constructed, three were found to be infectious and were examined for disease phenotype on Brassica campestris and several solanaceous hosts. The infectious mutants and the parent virus displayed a similar phenotype on B. campestris and two Nicotiana species, N. edwardsonii and N. bigelovii. In Datura stramonium one of the mutants induced a distinctly different phenotype. This result supports previous reports that gene VI is largely responsible for disease induction.

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MOLECULAR CHARACTERIZATION OF NON-ENVELOPED BACILLIFORM VIRUSES. N. E. Olszewski, S. L. Medberry and B. E. L. Lockhart, University of Minnesota, St. Paul, MN 55108.

Genomes of several non-enveloped bacilliform viruses have been

shown to be double-stranded circular DNA. We have constructed genomic clones of two non-enveloped bacilliform viruses, kalanchoe top-spot virus (KTSV) and Commelina yellow mottle virus (CoYMV), and are using these clones to characterize these genomes and the transcripts they encode. The CoYMV genome is 7.5 kb in size and each strand contains a single-stranded region (discontinuity). The CoYMV genome encodes a 7.6-7.7 kb polyadenylated transcript. DNA sequencing has identified a region of the genome with homology to the 3'-end of the initiator $tRNA^{met}$ of wheat and bean. The $tRNA^{met}$ homology and one discontinuity map to the same region. These features of CoYMV suggest that this virus replicates by a mechanism similar to that of caulimoviruses. The host for CoYMV, Commelina diffusa, contains a 1 kb transcript which shares homology with CoYMV DNA. This transcript occurs in both healthy and infected leaves but its significance is unknown.

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PROXIMITY OF PSEUDOMONAS FLUORESCENS STRAIN PRA25rif TO PEA TAPROOTS. J. L. Parke and C. M. Liddell, Dept. of Plant Pathology, University of Wisconsin, Madison 53706.

Pea seeds coated with a spontaneous mutant strain of P. fluorescens, PRA25rif, resistant to rifampicin, were sown in soil held at -6 kPa matric potential. Seven days later, PRA25rif populations associated with the pea taproot segment 0.5-1.5 cm below the seed were assessed from dilution plate counts. Root segments with various amounts of adhering soil (0-1200 mg cm^{-1}) were sampled to determine if soil mass affects quantification of root-colonizing bacteria. The amount of soil adhering to roots did not significantly affect the number of bacteria recovered, and the density of PRA25rif was greatest in the inner rhizosphere. After sonication of the samples, 99.8% of the bacteria recovered were isolated from the soil suspension, and only 0.2% were recovered from macerated roots.

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EFFECTS OF SOIL PH ON SUPPRESSION OF TAKE-ALL BY PSEUDOMONAS FLUORESCENS 2-79. B.H. Ownley, D.M. Weller, and L.S. Thomashow. USDA-ARS, Pullman, WA 99164-6430

Pseudomonas fluorescens 2-79 suppresses Gaeumannomyces graminis var. tritici (Ggt) which causes take-all of wheat. The major mechanism of suppression is phenazine-1-carboxylate; fluorescent siderophore and a second iron regulated factor have only minor roles. Mycelial growth of Ggt was inhibited by 2-79 on Kanner's agar (used for phenazine production) adjusted to pH 4.5, 5.5, 6.5, 7.2, 7.6, or 8.5. To determine the effect of soil pH on take-all suppression, seeds were treated with 2-79 or mutants deficient in production of phenazine (Phz^-), siderophore (Sid^-), antifungal factor (Aff^-), or a combination and sown in a steamed Ritzville silt loam (pH 7.6, 29.6% sand, 64.0% silt, 6.4% clay) with bulk soil pH adjusted to 4.9, 5.7, 6.2, 7.3, 7.6 or 8.0. Strain 2-79 and a $Phz^-Sid^-Aff^+$ mutant significantly suppressed take-all at all soil pH values. Phz^- mutants were less suppressive and generally failed to suppress take-all at low (<6.2) and at high (>7.6) soil pH.

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BIOLOGICAL CONTROL OF RHIZOCTONIA SEEDLING DISEASES IN BEDDING PLANT NURSERIES WITH THOUGHTS TOWARDS COMMERCIAL DEVELOPMENT. D.A. Schisler[1], M.H. Ryder[1], R.G. Rowden[2], [1]CSIRO-Division of Soils, Glen Osmond, South Australia and [2]Incitec Ltd., Gibson Island, Brisbane, Queensland, Australia.

A cooperative research project between CSIRO and Incitec Ltd. was initiated to investigate the feasibility of using antagonistic microorganisms to control Rhizoctonia diseases of seedlings in bedding plant nurseries. Fifty soil samples from 30 nurseries in South Australia were assayed for suppressiveness to *Rhizoctonia solani*. From these assays, 13 highly suppressive soils were selected and fungi, bacteria and actinomycetes were isolated from bulk and rhizosphere soils. Ninety-five prokaryotic antagonists were assayed against *R. solani* (anastomosis group 8) on *Capsicum annuum* cv "Green Giant" and *Celosia argentea* using commercial potting, seeding and watering equipment. More than one-third of the organisms significantly decreased disease ($p<0.01$) on *Capsicum*, and 3 decreased disease on *Celosia*. Nonpathogenic, binucleate Rhizoctonias isolated from nursery soils also significantly decreased Rhizoctonia disease, occasionally to levels equal to noninoculated controls. Commercial biological control of Rhizoctonia diseases of seedlings has considerable potential and formulations of superior performers are currently being developed.

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PENICILLIUM FUNICULOSUM AS A BIOCONTROL AGENT FOR PHYTOPHTHORA ROOT ROT OF AZALEA. J. G. Fang and P. H. Tsao. Dept. of Plant Pathology, Univ. of California, Riverside, CA 92521.

Penicillium funiculosum, which showed antagonism to several *Phytophthora* spp. in vitro, suppressed azalea root rot caused by *Ph. cinnamomi* or *Ph. parasitica* in some, but not all, greenhouse experiments. The antagonist was grown in a bran/peat medium and mixed into a planting medium [peat/perlite (3:1)]; this was followed by inoculation with *Phytophthora* at 5-7 days. Results obtained over a 2-month period showed that suppression of *Ph. parasitica* was in general greater than *Ph. cinnamomi*. Introduction of the biocontrol agent by dipping the roots in a spore suspension was not effective. *P. funiculosum*, however, improved the growth of azalea plants even in experiments where *Phytophthora* was not suppressed. Plants in the antagonist-containing treatment grew better (70-220%) and had greener foliage than those in the control without the antagonist, possibly due to suppression of minor root pathogens present in the planting medium.

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INCREASE IN RHIZOSPHERE COMPETENCE OF TRICHODERMA HARZIANUM FOLLOWING PROTOPLAST FUSION. A. Sivan and G.E. Harman, Dept. of Horticultural Sciences, Cornell Univ., Geneva, NY, 14456.

Rhizosphere competence of strains of Trichoderma harzianum was tested by treating seeds with conidial suspensions and assessing colonization of roots of plants grown from inoculated seeds. A strain (1295-22) derived by protoplast fusion exhibited a greater degree of rhizosphere competence than the parental strains (T12 and T95). T12 colonized mainly the upper third of the roots, while T95 was found primarily on the upper and the lower parts of the roots. However, 1295-22 showed a greater and more uniform colonization of the whole rhizosphere than did T12 or T95. Colony forming units of T95 and T12 along the lower half of corn roots were primarily confined to the rhizosphere. Conversely, 1295-22 colonized both the rhizosphere and rhizoplane equally. Although population densities of the tested strains were, in general, lower in cotton rhizosphere than in corn, 1295-22 colonized more cotton root segments than the parental types.

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INHIBITION BY PSEUDOMONAS CEPACIA, A POTENTIAL BIOCONTROL AGENT, OF SELECTED SOILBORNE PATHOGENS. K. E. Conway, C. J. Foor, D. Malvick, and C. Bender, Department of Plant Pathology, Oklahoma State University, Stillwater, OK 74078-9947.

A bacterium isolated from soil at the Oklahoma Forest Regeneration Center in Washington, OK, exhibited strong antibiotic activity in culture against Macrophomina phaseolina. The bacterium was identified as Pseudomonas cepacia (Pc) by fatty acid profile analysis. In dual culture on PDA, Pc strongly inhibited M. phaseolina, Rhizoctonia solani AG 1, AG 2 type 1, AG 3 and AG 4, Fusarium oxysporum, Pythium irregulare, and Laetisaria arvalis, but not Sclerotium rolfsii or Trichoderma harzianum. However, Pc strongly inhibited S. rolfsii in pairings on KMB. This indicated possible siderophore production in addition to antibiotic production by Pc. Potential of the bacterium as a biocontrol agent is being assessed in soil systems.

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INFLUENCE OF WHEAT ROOT PATHOGENS ON MAINTENANCE OF POPULATIONS OF PSEUDOMONAS FLUORESCENS Q72a-80 AND 2-79 IN THE WHEAT RHIZOSPHERE. M. Mazzola and R.J. Cook, Dept. of Plant Pathology and USDA-ARS, Washington State University, Pullman 99164

P. fluorescens Q72a-80 and 2-79 are suppressive, respectively, to Pythium spp. and Gaeumannomyces graminis var. tritici (Ggt) on wheat. A 10-fold increase in the population of 2-79 has been observed in the presence of Ggt, but the influence of other wheat root pathogens on biocontrol pseudomonads has not been investigated. Therefore, the effects of root infections by Ggt, Rhizoctonia solani AG8 and Pythium irregulare on the maintenance of populations of Q72a-80 and 2-79 were monitored in Thatuna silt loam. Initial bacterial populations of 10^6 cfu/cm root were established on seminal roots R, -2A and -2B. Populations of Q72a-80 and 2-79 in the rhizosphere of roots infected by Ggt and R. solani remained at or above populations on healthy (check) roots but the population of both bacterial strains was significantly lower (1-2 log cfu/cm root) after 30 days in the rhizosphere of roots infected by P. irregulare.

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SEED TREATMENT WITH THE BIOPESTICIDE TRICHODERMA HARZIANUM STRAIN KRL-AG2: 1988 FIELD TRIALS. A. G. Taylor, T. E. Stasz[1], and G. E. Harman. NYSAES, Cornell Univ., Geneva, NY, and [1]Eastman Kodak Co., Rochester, NY.

A biopesticide seed treatment using a strain (KRL-AG2) of T. harzianum produced by protoplast fusion is being developed for control of plant diseases. In 1988, field trials were conducted in five states, i.e. Florida, Idaho, Mississippi, New York and Texas. Crops included cabbage, canola, cotton, cucumber, snap bean, soybean, sweet corn and tomato. In general, seed treatment with KRL-AG2 and solid matrix priming provided reliable and effective control of seedrot diseases. Plant stands from seeds with biological treatments were equal or superior to those from seeds treated with chemical fungicides. Biological seed treatment increased yield in sweet corn and slightly increased yield in cotton relative to seed treatment with highly effective mixtures of fungicides. Expanded trials, including additional crops and states, are planned for 1989.

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EFFICACY OF BINUCLEATE RHIZOCTONIA AGENTS (BN) IN BIOCONTROL OF RHIZOCTONIA ROOT ROT OF SUGAR BEET IN THE FIELD. L. J. Herr, Dept. of Plant Pathology, Ohio State Univ., Wooster, OH 44691.

Similar field tests of three BN (as biocontrol agents) were run on a silty clay loam (SC) and a sandy loam (SL) soil during 1988. BN agents (1, 3 and 4) plus no agent controls, were banded over rows at two rates (11.2 and 67.4 kg dried barley inoculum/ha) in early June. On 15 June, beets were side-dressed with R. solani AG-2,T2 barley inoculum (45 kg/ha). Harvest (10 Oct) data taken were % plant loss (% PL); disease rating (DR), on a scale of 0=healthy to 5=80-100% rotted; and yield. On SC, BN-1 and 3 agent treatments (not diff.) had lower % PL, DR, and a >50% increase in yield over the BN-4 agent and no agent (not diff.) treatments. The 67.4 kg/ha rate was significantly better than the 11.2 kg/ha rate, however the rate x agent interaction was non-significant. Whereas on SL, the rate x agent interaction was significant and BN-3 at 67.4 kg rate ranked best in all categories, including a 383% increase in yield over the no agent treatment.

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A NEW BIOCONTROL FORMULATION FOR TRICHODERMA AND GLIOCLADIUM. J.A. Lewis, G.C. Papavizas, and R.D. Lumsden, USDA, ARS, Beltsville, MD 20705.

Biocontrol formulations were prepared with vermiculite, wet fermentor biomass of isolates of Trichoderma spp. and Gliocladium virens, and dilute HCl. The mixture was air-dried and activated with wheat bran and additional dilute acid to develop germlings (actively-growing hyphae) of the fungi. Aseptic conditions did not have to be maintained during formulation and activation. Dry preparations survived in storage at 5 and 25 C for at least 12 wk. Fungi in activated preparations proliferated in soil up to $2x10^6$ cfu/g of soil within 1 wk of amendment. Several preparations significantly reduced survival (>60%) of Rhizoctonia solani in pathogen-infested beet seed in soil and prevented saprophytic growth in soil. Preparations containing various isolates significantly prevented damping-off of cotton (>60%) in R. solani-infested soil, damping-off and blight of bean (>90%) in Sclerotium rolfsii-infested soil, and damping-off of zinnia in soilless mix infested with R. solani.

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SELECTING FOR ALKALINE TOLERANCE IN *TRICHODERMA HARZIANUM*. E. G. Ruppel, USDA-ARS Crops Research Lab, 1701 Center Avenue, Fort Collins, CO 80525.

Trichoderma spp. are favored by moist, acid soils but do persist in western calcareous soils at low population densities, which may indicate genetic diversity for alkaline tolerance. Selections from over 200 random isolates of *Trichoderma* spp. were made in vitro at increasing medium pH. One isolate of *T. harzianum* (TpH) grew and sporulated on pH 11 medium. At soil pH 6.7 with six consecutive plantings, TpH and a known biocontrol isolate of the fungus (THW) at 10^6 cfu/g significantly suppressed sugar beet damping-off in the first two plantings. By the sixth planting, treatment effects were nonsignificant. In soil at pH 8.2 with three plantings, no significant damping-off suppression occurred at planting 1, but both isolates suppressed disease at plantings 2 and 3. At pH 6.7, TpH was not as effective as THW in increasing seedling survival, and was no more effective at pH 8.2.

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PARASITISM OF SCLEROTIA OF SCLEROTIUM ROLFSII BY TALAROMYCES FLAVUS. Lea Madi and Y. Henis, The Hebrew University of Jerusalem, Faculty of Agriculture, Rehovot 76100, Israel.

Twelve isolates of Talaromyces flavus were examined for their capacity to parasitize pre-dried sclerotia of Sclerotium rolfsii on water agar (2% Bacto agar in tap water) and in natural sandy loam soil adjusted to 70% MHC. The dried sclerotia were dipped in a conidial suspension of T. flavus containing 10^7 spores/ml and incubated at 30 C for 3-4 days. All the tested isolates parasitized the sclerotia on water agar, but only 9 were capable of parasitizing the sclerotia in soil. Dipping the sclerotia in a heat-activated T. flavus ascospore suspension (10^7 spores/ml), resulted in up to 5% mycoparasitism of the sclerotia in either water agar or soil, as compared to 5-100%, depending on the isolate and on the medium, obtained with T. flavus conidia. The differences in mycoparasitic capacity observed among the tested T. flavus isolates were found to be correlated with their growth rates as well as with their glucose oxidase, β-1,3-glucanase and chitinase activities.

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DIRECTED ENHANCEMENT OF BIOCONTROL IN *PSUEDOMONAS* BY CONSTITUTIVE ANTIBIOTIC BIOSYNTHESIS. W. Howie, D.Matsubara, N. Gutterson, and T. Suslow. DNA Plant Technology Corportation, Oakland, CA 94608.

Constitutive biosynthesis of oomycin A in *Pseudomonas fluorescens* strain Hv37aR2 was achieved by placing a strong promoter sequence upstream of an introduced copy of the *afuE* locus. *In situ* oomycin A biosynthesis was monitored by bioluminescene from a reporter *afuE-lux* fusion in either a constitutive (WH157) or control (WH161) strain. Biosynthesis of oomycin A *in situ* was determined over a 7-day period in both strains. Biosynthesis in strain WH157 was consistently 50 to 100-fold higher than in strain WH161 on seeds, roots, hypocotyls, and cotyledons of cotton, Acala SJ2. There were no significant differences in populations of the two strains on any of the plant parts assayed. Seed treatment with WH157 resulted in an average of 15 to 20% increase in emergence and decrease in root infection by *Pythium ultimum* as compared to strain WH161. Clearly, constitutive antibiotic biosynthesis by a rhizobacterial strain can mitigate deficiencies in efficacy.

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EFFECT OF SUBLETHAL HEATING ON WEAKENING, AND ON HEAT SHOCK PROTEIN SYNTHESIS, IN PROPAGULES OF FUSARIUM. S. Freeman, C. Ginzburg* and J. Katan, Dept. Plant Pathology & Microbiology, Faculty of Agric., Rehovot 76100; and *Dept. of Ornamental Horticulture, The Volcani Center, Bet Dagan 50250, Israel.

The weakening of propagules of F. oxysporum niveum (FON) by sublethal heating may be expressed in a delay in germination, and in reduced survival and disease incidence. Germlings of FON respond to heating by synthesizing a set of heat shock proteins (HSPs) corresponding to 95, 83, 80, 74, 70, 35 and 18 kD. Vital fluorescent staining in germlings decreases in intensity which corresponds with appearance of HSPs. HSPs are observed 10 min after heating. Recovery from a shock of 40 C is more rapid at 25 C than at 36 C. Thermotolerance to a lethal temperature of 44 C is acquired by preheating germlings at sublethal levels, as determined by vital staining and survival. Solarization at sublethal temperatures may cause either a weakening or an acquired thermotolerance, but the direction is probably dependent on the environment into which the propagules are introduced.

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IDENTIFICATION OF HORTICULTURAL TRAITS FOR PREDICTING BROCCOLI CULTIVAR SUSCEPTIBILITY TO BACTERIAL SOFT ROT. C. H. Canaday, Dept. of Entomology and Plant Pathology, University of Tennessee, West Tennessee Experiment Station, Jackson, TN 38301.

Regression analyses of disease incidence versus horticultural traits identified three traits useful for predicting the susceptibility of broccoli cultivars to bacterial soft rot caused by Pseudomonas marginalis pv. marginalis and Erwinia carotovora. Data on eight traits thought to influence cultivar susceptibility were collected in a field evaluation of 25 cultivars and lines maturing simultaneously under disease-conducive conditions. The percentage of harvested heads with soft rot (12.2-85.7%) was regressed on the horticultural traits of 13 cultivars. The best model was head tightness, doming, and looseness versus disease incidence (R^2=0.9352; $P<0.05$ for traits). This model predicted 85% of the variability in disease incidence when applied to the remaining 12 entries. Further validation and refinement of the model and quantification of traits are in progress.

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RESISTANCE OF CUCUMBER PLANT INTRODUCTIONS TO POWDERY MILDEW C. C. Block and R. L. Clark, North Central Regional Plant Introduction Station, Iowa State University, Ames, IA 50011

Over 750 cucumber plant introduction (PI) lines were evaluated

in the greenhouse for resistance to powdery mildew caused by Sphaerotheca fuligenea. Seedlings were inoculated by spraying the cotyledons and the first true leaf with conidia suspended in a solution of distilled water and Tween 20 (2 drops/l). A suspension containing 5 x 10^5 conidia/ml gave a very uniform infection. Twenty-nine PI's were resistant and 46 additional PI's had intermediate resistance. Over 50% of the resistant PI's were from China. Eight resistant accessions were selected to study disease progression. Disease progression, measured by a weekly analysis of spore populations on leaves, was significantly slower on the resistant PI's than on the cultivars 'Marketer' (susceptible) and 'Ashley' (intermediate). Distinct differences were also detected in disease progression among some of the resistant accessions. Powdery mildew developed most slowly on the PI's 197088, 288238, 321006, and 390258.

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DYNAMICS OF A WHITE MOLD (WHETZELINIA SCLEROTIORUM) EPIDEMIC IN FLORIDA CABBAGE. D. P. Weingartner, AREC, Hastings,FL 32045.

Temporal and spatial development of white mold was studied on a 140 ha commercial cabbage farm during the 1987-88 and 1988-89 cabbage seasons. Apothecia were observed Dec to April, however, were most numerous 10-14 days following periods of rain or irrigation. Successive "crops" of apothecia were produced in individual fields, many from sclerotia buried 5-8 cm in soil. Abundant apothecia were present during infection periods. New infection, however, did not always occur when apothecia were present. Two apothecia were observed on current season's sclerotia during 1988. During 1989 stipes were produced within 30 and 43 d, respectively, at 12 and 8 C, by sclerotia formed on current season's infections. Degree of aggregation was assessed by mapping distribution of infection in nine different fields. Data suggest that most infection is due to within field inoculum and that frequent cultivation may be an effective control measure. Significantly more 'Savoy' plants were infected than 'Bravo'.

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EFFECT OF OIL AND ENDOSULFAN ON SPREAD OF POTYVIRUSES IN FALL-PLANTED WATERMELON. S. E. Webb and P. Groves, University of Florida-IFAS, CFREC, 5336 University Ave., Leesburg, FL 32748

We evaluated oil and endosulfan (to control colonizing aphids) alone and in combination in large plots (0.13 ha) of fall-planted (18 August) watermelon in Central Florida to determine if virus spread could be delayed. Plants showing mosaic symptoms were mapped every three days and were periodically tested for watermelon mosaic virus 2 (WMV-2), papaya ringspot virus type-W (PRSV-W), and zucchini yellow mosaic virus (ZYMV) using ELISA. In mid-October, plots treated with oil or oil and endosulfan averaged 50% fewer virus-infected plants than those treated with endosulfan alone, but by the end of October after a large aphid flight all plots were almost 100% infected. In late September, 91% of plants tested (n=150) were infected with WMV-2 and 15% with PRSV-W. In mid-October percentages were equal for WMV-2 and PRSV-W. By early November, 84% (n=860) were infected with WMV-2 and 97% with PRSV-W. Fewer than 0.1% were infected with ZYMV.

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ETIOLOGY OF TOMATO DECLINE. J. S. Gerik[1], A. F. Van Maren[2], D. C. Stenger[1], and J. E. Duffus[1]. [1]USDA-ARS, Salinas, CA 93905 & [2]Univ. Calif. Coop. Ext. Serv., El Centro, CA 92243.

Tomato decline (TD) of fresh market tomatoes in the desert areas of California has been observed since 1977. The disease occurs only in fields with a history of previous tomato crops. Although TD is known to be soil borne, the cause of the disease, until now, has not been determined. Tomatoes grown for 3 weeks in a growth chamber at 14 C in soil collected from a field with a history of TD became infected with a Tombusvirus serologically indistinguishable from the BS-3 strain of tomato bushy stunt virus (TBSV). Field grown, symptomatic plants collected during 1987 and 1988 were consistently found to be infected with TBSV, as determined by ELISA and bioassay. Eight tomato cultivars, which in field observations were considered to be very susceptible or resistant to TD, were mechanically inoculated with TBSV. The symptoms were most severe on the TD susceptible plants, but were very mild on TD resistant plants. These experiments implicate TBSV as the etiological agent of TD.

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TOWARDS MANAGEMENT OF FUSARIUM YELLOWS OF CELERY. K. F. Ireland and M. L. Lacy. Department of Botany and Plant Pathology, Michigan State University, E. Lansing, MI 48824.

Moderately resistant (MR) and susceptible (S) celery had higher Fusarium yellows disease ratings when grown in the greenhouse in muck soil from a field monocropped to celery than in soil from an area in the same field rotated into leeks for one summer. Rotating another celery field into onions for 1 yr reduced the pre-planting population (popn) of *Fusarium oxysporum* f. sp. *apii* race 2 from 80 propagules/ gram of soil (ppg) to 26 ppg, but growing celery the following yr increased post-harvest popn to 160 ppg. Race 2 popn in an infested celery field rotated into other vegetables for 5 yr was too low to measure, and remained low after 1 yr back into celery. An apparently suppressive soil (SS) was identified in a field with a low Fusarium yellows incidence on a farm where all other fields were highly infested. MR and S celery had lower disease ratings when grown in the greenhouse in the SS than in soil from a conducive field on the same farm. Race 2 popn was 120 ppg in the conducive soil, but too low to measure in the SS.

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ETIOLOGY OF CARROT CAVITY SPOT IN CALIFORNIA. E. Vivoda, R. M. Davis. Department of Plant Pathology, University of California, Davis, CA 95616.

Pythium violae and P. ultimum were isolated from cavity spot lesions on carrots from the San Joaquin Valley. Healthy, mature carrots were inoculated with the fungi and incubated in moist chambers. Symptoms developed after 7 days. Either P. violae or P. ultimum were reisolated from inoculated carrots exhibiting cavity spot lesions. Fourteen cultivars of carrots were susceptible to P. violae but only ten to P. ultimum. In growth chamber studies 3 month old carrots were transplanted into soil infested with either Pythium spp. (200 cfu/g soil) at 15, 20 and 25°C. Symptoms developed after 2 weeks. P. violae caused the greatest number of lesions per carrot at 15°C while the optimum temperature for disease development for P. ultimum was 20°C. Overall, more carrots were infested by P. violae than P. ultimum. The role of inoculum density, soil moisture and carrot age was also investigated.

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COLONIZATION OF CROP RESIDUE BY FUSARIUM OXYSPORUM F. SP. MELONIS AND NONPATHOGENIC STRAINS OF F. OXYSPORUM. T.R. GORDON, Department of Plant Pathology, University of California, Berkeley, CA 94720.

Colonization of crop residue, by Fusarium oxysporum f. sp. melonis (FOM) and nonpathogenic strains of F. oxysporum (FON), was studied in an agricultural field soil. Inoculum densities of the two fungi in this soil were approximately equal. Shoot tissue incubated for five days in moist soil was heavily colonized by both pathogenic and nonpathogenic strains. Tomato shoots supported significantly higher levels of FOM than FON, while the reverse was true for wheat. Residue colonization averaged over seven crop species showed significantly higher levels of the pathogen relative to the nonpathogenic strains. Five days after shoot removal, FOM was present at significantly higher levels than FON on roots of both muskmelon and tomato. However, the proportion of total colonization (FOM + FON + F. equiseti + F. solani) represented by FOM and FON was the same on living roots as on roots five days after shoot removal.

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FUSARIUM WILT OF TOMATO IN FLORIDA BEFORE AND AFTER AN OVERSEASONING PERIOD. John Paul Jones, J. W. Scott, and J. P. Crill, Gulf Coast Res. and Edu. Center, 5007 60th St. E., Bradenton, FL 34203

A 2.5 month midwinter overseasoning period between crops greatly reduced the incidence of Fusarium wilt or race 1-tolerant Rutgers (caused by race 1, 2, or 3), race 1-resistant Manapal (caused by race 2 or 3), race 1 and 2-resistant Walter (caused by race 3). Disease incidence caused by race 1 (but not race 2 or 3) was slightly decreased on susceptible Bonny Best. Yield losses were sharply reduced by the overseasoning period on Rutgers (caused by race 1, 2, or 3), Manapal (caused by race 2 or 3), Walter (caused by race 3), and Bonny Best (caused by race 1 or 2, but not race 3). Based on yields and disease incidence, race 2 survived just as well as race 1, and race 3 survived just as well as race 1 or 2. Manapal was less susceptible to race 2 or 3 than Bonny Best and no more susceptible than Rutgers. Walter was less susceptible to race 3 than Bonny Best and no more susceptible than Rutgers or Manapal.

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SQUASH SILVERLEAF AND ITS ASSOCIATION WITH THE SWEETPOTATO WHITEFLY. R. K. Yokomi[1], L. S. Osborne[2], and K. A. Hoelmer[1].

USDA, ARS, 2120 Camden Road, Orlando, FL 32803[1], Univ. of Florida, IFAS, CFREC, Apopka, FL 32703[2]

In 1988, a new disorder called squash silverleaf (SSL) appeared in squash and other cucurbits across south and central Florida. SSL symptoms first appear as a veinal chlorosis followed by a whitening of veins which can extend throughout a mature leaf. Sectored discoloration of fruit was also observed. Extracts from affected tissue did not react with an array of cucurbit and whitefly-transmitted virus antisera. SSL was not graft or mechanically transmitted. SSL symptoms appeared within 10 days of plant colonization from all local populations of the sweetpotato whitefly (SPWF) tested. Ten nymphs per plant induced symptoms, but severity increased with more SPWF. New growth following elimination of the whitefly by insecticides was normal. These data suggest that an insect toxin may be involved in SSL symptom expression.

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EFFECT OF DOWNY MILDEW (PSEUDOPERONOSPORA CUBENSIS) ON SUGAR CONTENT OF WATERMELON. C. L. Patterson, Wes Watkins Agri. Res. and Ext. Center, Oklahoma State Univ., Lane, OK 74555.

Sugar content of watermelon fruit decreased as the incidence of downy mildew (Pseudoperonospora cubensis) increased to 25% or greater. Bravo 720, Ridomil/Bravo 81W, and Ridomil/MZ58 reduced the rate of the downy mildew epidemic. The Ridomil formulations delayed the epidemic 2-wk and resulted in excellent disease control; sugar content of fruit in those treatments was 13.1% and 12.8%, respectively. Bravo 720 delayed the epidemic 1-wk, resulted in moderate disease control, and sugar content was reduced to 8.5%. Leaves in the non-treated control plots were 100% infected, severe defoliation occurred, and sugar content was reduced to 6.4%. Thus, infection and defoliation of watermelon leaves by P. cubensis decreased fruit quality and subsequent yield as determined by sugar content.

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SOURCES OF INOCULUM AND CONTROL OF FUSARIUM CROWN AND ROOT ROT OF TOMATOES IN GREENHOUSE ROCKWOOL SYSTEMS. L. Mihuta-Grimm and R. C. Rowe, Dept of Plant Pathology, Ohio State Univ., Wooster, OH 44691.

Fusarium oxysporum f.sp. radicis-lycosersici readily colonized rockwool with or without plant growth nutrients, but disease rarely occurred away from the inoculum site. Introduction of the pathogen on infected seedlings, contaminated soil, or irrigation water was investigated. Use of infected transplants resulted in severely diseased mature plants and reduced yields. The addition of infested soil resulted in significant disease incidence but no corresponding yield loss. Use of contaminated irrigation water produced little or no disease. Benomyl drenches (0.023 to 0.112 g a.i./L) were applied once to 2-wk-old transplants grown in rockwool starter cubes, and at 10- and 20-day intervals to plants growing to maturity on rockwool slabs. Although initial phytotoxicity and stunting were observed on treated transplants, there was no effect on yield. Benomyl applications to 2-wk-old transplants and plants on rockwool slabs were effective in control of this disease.

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INCIDENCE AND TEMPORAL DISTRIBUTION OF MUSKMELON VIRUSES AND APHIDS IN SOUTH TEXAS. M. E. Miller, J. V. Edelson, and E. L. Cox. Texas A&M University, Weslaco, TX 78596.

Distribution of 6 muskmelon viruses in South Texas was determined by randomly sampling 345 leaves exhibiting virus-like symptoms during weekly surveys. Samples were assayed with an enzyme-linked immunosorbent assay (ELISA). Tobacco ringspot virus was detected first on 7 April and had the highest incidence, 23.2% of samples. Squash mosaic virus was detected on 20 April. Papaya ringspot virus, Watermelon mosaic virus, and Zucchini yellow mosaic virus were not detected until 27 May. Two or more viruses were found in many samples. ELISA readings were negative for 80 samples. Aphid movement was monitored by placing yellow-green pan traps around melon fields and surveying plants weekly at 4 locations. Aphid abundance on plants did not increase above a mean of 1/vine. Transitory winged aphids were trapped throughout the growing season and peak abundance occurred in April and early May.

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VIRUS TITER AS AN INDEX OF RESISTANCE IN CUCUMIS MELO. V. L. Hunt, M. E. Miller, and E. L. Cox, Texas A&M University, Weslaco, TX 78596.

Resistance levels of 20 muskmelon cultivars to squash mosaic virus (SqMV) were determined by rating the number of days from inoculation to first symptom (DTS), disease severity, and number of plants infected. Significant differences ($p=0.05$) occurred among cultivars in DTS and disease severity ratings. 'Perlita', 'Sunshine', and 'Hiline' had 50, 42, and 33%, respectively, symptomless plants. Symptomless plants were reinoculated and those remaining symptomless were self-pollinated. The proportion of symptomless plants in the selfed progenies was greater than in the original populations. A double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) was developed to provide a more precise method for comparing plant responses to SqMV. Titer cut-off values were determined from titration curves of clarified viral concentrates. Symptomless plants were associated with reduced virus titer.

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EPIDEMIC WAVES OF RUST ON ALFALFA. R. D. Berger and D. A. Roberts, University of Florida, Gainesville, FL 32611.

Waves of disease have been assumed in the early stages of epidemics caused by foliar pathogens, but such waves have never been detected and characterized in natural epidemics. Discrete waves of rust (Uromyces striatus Schroet.) on alfalfa (Medicago sativa L.) were observed when disease intensity was estimated thrice weekly with a logistic disease-assessment scale. Pictorial keys were prepared for the mid-point of each of the ten classes of severity in the range $0.00035<y<0.5$, and two leaves on each of 20 tagged plants were assessed. Even though the epidemic was well advanced on the lower leaves, two distinct waves of initial disease, on the logit scale, were observed in four crops on each of the two assessed leaf layers. The delay for waves on the upper leaves was the same as the time differential between the emergence of leaves of the two layers. The latent period (p) varied from 11 days in the spring to 8 days in the summer when p was estimated from the waves with a modification of Van der Plank's basic infection equation.

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DYNAMICS OF PSEUDOMONAS SYRINGAE STRAINS COEXISTING ON LEAF SURFACES. L. L. Kinkel and S. E. Lindow, Department of Plant Pathology, University of California, Berkeley, CA 94720.

The extent and nature of interactions among bacteria coexisting on leaves are not well understood. We compared the population dynamics of pairs of Pseudomonas syringae strains inoculated onto bean or potato leaves separately, co-inoculated, or sequentially inoculated (24 h period between inoculation of strains). Plants were maintained in growth chambers under alternating wet and dry conditions. Combined population size of strains when co-inoculated was less than the combined size when inoculated onto separate leaves, suggesting that strains do affect the growth and survival of one another. Order of arrival and environmental conditions both influenced the relative population sizes of co-existing strains. Consideration of the relationship through time between the population sizes of different strains when co-inoculated may identify successful co-existors and provide means for investigating the mechanisms of phylloplane interaction.

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PERIODIC SPATIAL PATTERNS OF BACTERIAL BROWN SPOT IN A 90 M SNAP BEAN ROW SEGMENT. **B. D. Hudelson, M. K. Clayton[1], and C. D. Upper[2]. Departments of Plant Pathology and Statistics[1], and USDA-ARS[2], University of Wisconsin-Madison, Madison, WI 53706.**

Snap bean (Phaseolus vulgaris L.) plants within a 90 m row segment (1550 plants) were evaluated for bacterial brown spot incidence using a systematic sampling plan in which 6 of every 31 plants were sampled. In this plan a minimum number of sampled plants was spaced in a way that allowed construction of a complete autocorrelation function. Autocorrelation analysis of these data suggested the presence of previously undetected periodic patterns of bacterial brown spot. A modified spectral analysis was used to further define the patterns. Thirty-two percent of the variability in the data set could be explained by patterns of disease that occurred at wavelengths of approximately 0.5, 1, 6.5, 13, 15, 20, 25 and 39 m.

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SPATIAL ANALYSIS OF CITRUS BACTERIAL SPOT EPIDEMICS IN FLORIDA CITRUS NURSERIES. T. R. Gottwald and J. H. Graham, U.S. Dept. of Agriculture, ARS, Orlando, Florida 32803.

Spatial patterns of disease were studied in four central Florida nurseries infected with citrus bacterial spot. Both indices of dispersion and ordinary runs analysis indicated aggregation in all four nurseries. Spatial lag correlation analysis indicated within-row autocorrelation corresponded to mechanical spread of disease down nursery rows. Some across-row autocorrelation corresponded to natural or mechanical spread. High disease incidence on rootstock plants was directly related to disease on new scion shoots in the same spatial area. Two nurseries had discrete areas of high disease incidence which were interpreted as potential foci of disease. Shallowest disease gradients corresponded to down-row mechanical spread from hedging in one nursery, and across-row natural spread to the southeast in a second. Natural vs. mechanical spread was directly related to citrus cultivars, bacterial strain aggressiveness, use of bactericides, and cultural management.

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EFFECT OF SELECTED CONSERVATION-TILLAGE PRACTICES ON WETNESS DURATION OF WHEAT STRAW RESIDUE AND ASCOCARP PRODUCTION BY PYRENOPHORA TRITICI-REPENTIS. W. Zhang, W. F. Pfender, E. Adee, and A. Nus. Dept. of Plant Pathology, Kansas State University, Manhattan, KS 66506.

Field plots of wheat straw infested with Pyrenophora tritici-repentis were subjected to no-till or two different levels of reduced tillage. Tillage type and microsite (location of straw relative to soil surface) were studied as factors influencing ascocarp production and wetness duration of the straw. Ascocarps were counted on straws sampled in mid-winter. Straw wetness was monitored by electrodes connected to a micrologger. Ascocarps/ha were greatest in no-till plots. Ascocarps/g were not affected by tillage type, but were reduced in straws near the soil surface and on the lower portion of standing stubble. Cumulative duration of straw wetness differed by tillage type and, across tillage types, was longer for straws near the soil surface. In two tillage types, number of ascocarps/g was negatively correlated with number of wetness periods of >10 hr.

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A STOCHASTIC MODEL FOR THE INITIAL OCCURRENCE OF FUNGICIDE RESISTANCE IN PATHOGEN POPULATIONS. M. G. Milgroom. Department of Plant Pathology, Cornell University, Ithaca, NY 14853.

The probability that resistance will occur in a population and increase to unacceptable levels is estimated as a function of inital pathogen population size (N_0), mutation rate (p), pathogen growth rates (r) and fungicide efficacy ($1-\alpha$) using a stochastic model. Model results support the intuitive prediction that there is a greater probability of resistance occurring when N_0, p, and r are large. In contrast, higher levels of fungicide efficacy reduce the risk of resistance occurring in some circumstances, depending on values of N_0, p, and r. When N_0 is small, high values of $1-\alpha$ may result in lower risks of resistance occurring. These model results support the intensive use of selective fungicides as a resistance management tactic under some conditions.

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SPATIAL DISTRIBUTION OF SOYBEAN RHIZOCTONIA FOLIAR BLIGHT AND EFFECT OF AGGREGATION OF EARLY INFECTION ON DISEASE DEVELOPMENT. X.B. Yang, J.P. Snow, and G.T. Berggren. Dept. of Plant Path. & and Crop Physi. La. Ag. Expt. Sta., LSU Ag. Center, Baton Rouge, LA 70803.

Soybean foliar blight caused by *Rhizoctonia solani* Kuhn is initiated by inoculum from soil and spreads by means of interplant mycelial growth. Six soybean fields with a history of the disease were subdivided into 0.75 by 0.75 m quadrats. Disease in each qudrat in each field was assessed early and late in the growing season. Spatial pattern analysis showed a clustered distribution of the disease. Disease incidence late in the season was predicted by the amount and the degree of aggregation of diseased leaves early in the season. Incorporation of an aggregation factor, AF, into the logistic disease growth model, dx/dt=rx(1-x)/AF, improved disease prediction ability.

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SPATIOTEMPORAL MODELS TO DESCRIBE EPIDEMICS OF LEPTOSPHAERULINA LEAFSPOTS ON WHITE CLOVER AND ALFALFA. O. Modesto Olanya and C. Lee Campbell, Department of Plant Pathology, North Carolina State University, Raleigh, NC 27695-7616.

Leafspot epidemics on white clover and alfalfa caused by Leptosphaerulina spp. were studied during spring and fall 1987 and spring 1988. Disease severity was assessed twice/wk on plants of alfalfa (A) or white clover (C) in radial arms around disease foci. The plant combinations AA, AC, CC and CA (first letter = focus host, second letter = recipient host) were arranged randomly with four replications in each season. Disease progress was described (R^2=0.85-0.96) for all treatment combinations in each season by the model $y=a[1-\exp(-bt)]/[1+\exp(cd)]$ where y is the proportion disease at distance d from the focus at time of assessment t, and a, b, and c are parameters. The appropriateness of this new model for all combinations and parameter estimates suggests that characteristics of Leptosphaerulina leafspot epidemics are similar. However, the rate of disease spread to and increase on white clover was slower than on alfalfa regardless of the disease focus host.

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SPATIAL PATTERN OF CERCOSPORA LEAFSPOT ON WHITE CLOVER IN TWO WHITE CLOVER/TALL FESCUE GRASS PASTURES. Scot C. Nelson and C. Lee Campbell. Department of Plant Pathology, North Carolina State University, Raleigh 27695-7616.

Severity of Cercospora leafspot of Ladino white clover was estimated on every leaf in each of four 0.09 m^2 samples per quadrat within four blocks of 64 2.4 x 2.4 m contiguous quadrats in two white clover/tall fescue grass pastures in September 1988. Disease incidence and severity were correlated significantly (r = 0.71-0.79) in all four blocks. Using estimates of Taylor's b, aggregation was indicated from disease severity data while spatial pattern based on incidence data ranged from regular to aggregated. Significant autocorrelations were found in all four blocks for disease incidence and in three of four blocks for disease severity, indicating that similar levels of disease tended to occur in quadrats of close proximity. From results of the Greig-Smith blocked quadrat variance procedure and estimates of sampling costs, optimum quadrat size among those tested was 5.76 m^2 with an optimum number of samples per quadrat of one or two.

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RAIN SPLASH DISPERSAL OF COLLETOTRICHUM ACUTATUM OF STRAWBERRY. X. Yang, L. L. Wilson, L. V. Madden, and M. A. Ellis, Dept. of Plant Pathology, Ohio State Univ., Wooster, OH 44691.

A rain simulator developed at OSU/OARDC was used to investigate the influence of rain intensity (15 and 30 mm/hr), duration (15-60 min), and types of ground cover on splash dispersal of conidia of C. acutatum on strawberry fruit. Potted strawberry plants were held in two concentric circles within a wood frame and inoculated fruits with sporulating lesions were placed in the center. Inner and outer circles, 15 and 30 cm from the inoculum source, were exposed to a uniform zone of generated rain. Studies were conducted with bare soil, soil covered with fresh straw (>6 cm deep) or plastic mulch. Disease incidence generally was less in the outer circle, but increased over time only with soil. With a plastic cover, 100% incidence was obtained at both distances and all times. Horizontal airflow also was tested and resulted in less disease up-wind compared to down-wind.

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DENSITY OF OVERWINTERING POPULATIONS OF *UNCINULA NECATOR* ON BARK OF GRAPEVINES. David M. Gadoury and Roger C. Pearson, Department of Plant Pathology, Cornell University, New York State Agricultural Experiment Station, Geneva 14456.

As they mature, cleistothecia of *U. necator* are washed by rain to bark of grapevines, where they overwinter. In 1988, we monitored the development of powdery mildew, then trapped cleistothecia in rainwater collected by funnels beneath vines, and finally determined the number of ascocarps on bark in 17 commercial vineyards. Delayed disease development was associated with the subsequent delayed dispersal of ascocarps. The density of ascocarp populations ranged from 291,905 cleistothecia/kg bark on unsprayed vines of *Vitis vinifera* 'Chardonnay' to 8,864 cleistothecia/kg bark on *V. labrusca* 'Concord'. Areas under disease progress curves were correlated with subsequent ascocarp numbers on bark. However, the most consistently accurate predictor of population density on bark was the number of cleistothecia trapped in funnels. Density of asococarp populations on bark is probably a function of both disease incidence and subsequent efficiency of dispersal. Trapping cleistothecia during dispersal provides more reliable assessments of inoculum dose than estimates derived from assessments of disease. Future studies will examine relationships between primary inoculum dose and development of epidemics.

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SEASONAL OCCURRENCE OF SPORES OF MONILINIA OXYCOCCI IN A

WISCONSIN CRANBERRY FIELD. P. G. Sanderson and S. N. Jeffers. Dept. of Plant Pathology, Univ. of Wisconsin, Madison 53706.

A Burkard 7-day recording volumetric spore trap was used to determine when spores of Monilinia oxycocci, the causal agent of cranberry cottonball, were present in a commercial cranberry field. In 1987 and 1988, ascospores were first detected on 5 May, ca. one week before bud break, and continued to be trapped until one week before bloom. They occurred for 31 consecutive days in 1987 and 28 consecutive days in 1988. Conidia were first detected ca. 10 days before bloom in 1986, at the start of bloom in 1987, and at 50% bloom in 1988. Conidia were present for 34, 30, and 26 consecutive days beginning 7, 6, and 17 June in 1986, 1987, and 1988, respectively. The two spore types never occurred concurrently. Both ascospores and conidia exhibited a diurnal periodicity. Ascospores were trapped predominantly between 1000-2100 hr and were most abundant between 1600-1800 hr. Most conidia were trapped during the daylight hours, particularly around midday.

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THE INFLUENCE OF TEMPERATURE AND FRUIT AGE ON INFECTION OF APPLE FRUIT BY *VENTURIA INAEQUALIS*. K. L. Reynolds and R. C. Seem. Department of Plant Pathology, Cornell University, New York State Agricultural Experiment Station, Geneva, NY 14456.

Several studies have reported ontogenic resistance to *V. inaequalis* in apple fruit, but none has observed the effects of this resistance on germination, appressorium formation, or colonization. Attached and detached fruit were inoculated with ascospores or conidia every 3 weeks beginning at petal fall. Attached fruit were kept moist at ambient orchard temperatures for 1, 2, 4, 8 or 16 days. Detached fruit were kept moist and incubated at 13, 22, or 31 C for 94 hr. Epidermal sections were stained in cotton blue/lactophenol and examined microscopically. The proportions of spores that had germinated, developed appressoria, and successfully penetrated and formed a subcuticular stroma were recorded. Temperature had a pronounced effect at all stages of infection. Fruit age did not affect germination or appressorium formation, but the proportion of successful penetrations decreased significantly as fruit matured. These results suggest that ontogenic resistance is not due to inability of the pathogen to germinate or form appressoria, but rather to inability of the fungus to penetrate the cuticle or an interaction with the host that prevents formation of a subcuticular stroma.

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SURVIVAL OF MUCOR PIRIFORMIS ON COLONIZED STONE FRUIT ENDOCARPS. T. J. Michailides, Univ. of Calif., Berkeley, Kearney Agric. Center, Parlier, 93648, and J. M. Ogawa, Dept. of Plant Pathology, Univ. of Calif., Davis, 95616.

Sporangiospores of Mucor piriformis attached to peach and nectarine endocarps and partially buried survived better in dry (-1,300 bars matric potential) soil than in wet (-0.3 bar matric potential) soil and longer at 0 and 10 C than at 27 and 33 C. Sporangiospores declined over time in a polynomial (quadratic) fashion in wet soil at all temperatures and in dry soil only at 33 C. The fungus grew and sporulated on endocarps incubated at 0, 10, and 21 C but not at 27 or 33 C. Washings from endocarps induced germination of sporangiospores of M. piriformis (up to 50%) and increased its growth and sporulation. The decline of M. piriformis propagules on endocarps buried in soil in a peach orchard was exponential after the initial 5 mo. Chlamydospore-like structures developed in mycelia and sporangiophores were found in decayed tissues. More than 75% of the colonies of M. piriformis recovered from propagules on endocarps originated from sporangiospores.

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INTERFERTILITY OF 'S' AND 'P' GROUPS OF HETEROBASIDION ANNOSUM IN NORTH AMERICA. T.E. Chase, W.J. Otrosina, and F.W. Cobb, Jr. USDA-Forest Service, Pacific Southwest Expt. Sta. P.O. Box 245, Berkeley,CA 94701 and Department of Plant Pathology, University of California, Berkeley, CA 94720.

H. annosum in North America consists of two biological species designated 'S' and 'P'. In experiments to be discussed interfertility (% dikaryons formed) between groups in the western U.S. was quantified as ca. 18%. Interfertility is not random but can be ascribed to presence of '+' intersterility alleles in both groups. The distribution of intersterility alleles, the nature of inter-group hybrids, their stability, and significance in the pathology and speciation of the fungus will be discussed. Isozymes provide additional markers for monitoring heterokaryon and dikaryon formation and stability.

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VIRULENCE OF SINGLE AECIOSPORE ISOLATES OF CRONARTIUM QUERCUUM F. SP. FUSIFORME. E.G. Kuhlman and F.R. Matthews, USDA, Forest Service, SEFES, Athens, GA, 30602.

Single gall isolate LHNC-2 produced galls on twice as many seedlings of rust resistant loblolly pine family 10-5 as did standard composite isolate 1-73. Thirty-two single aeciospore isolates from LHNC-2 were used to produce basidiospore inoculum for inoculating seedlings of family 10-5. Six weeks after inoculation symptoms were unusually frequent. Three months after inoculation with the single aeciospore isolates, the percentage of seedlings with galls varied from 33-85. In the same experiment isolate LHNC-2 and 1-73 produced galls on 60 and 44% of the seedlings, respectively. Single aeciospore isolates from virulent single gall isolates appear to have great potential for determining variations in virulence in the pathogen population and in resistance among pine families.

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COMPARATIVE VIRULENCE OF *HETEROBASIDION ANNOSUM* ISOLATES. F. Cobb, Jr., T. Chase, W. Otrosina, A. Ratcliff, and T. Popenuck. Dept. of Plant Pathology, Univ. of CA, Berkeley, 94720 and USDA-For. Serv. Pacific Southwest Forest Exp. Station, Berkeley

No differences in mortality of white fir or ponderosa pine seedlings inoculated with dikaryons and progeny homokaryons of the 'S' and 'P' groups, respectively, were apparent but results among homokaryons varied considerably. In another test, four dikaryon isolates each of 'P' and 'S' and one of *H. araucariae* from Australia were inoculated into seedlings of nine conifers. All 'P' isolates were highly virulent on ponderosa and sugar pines and less virulent on fir, Douglas-fir, Norway spruce, giant Sequoia and western hemlock. The 'S' isolates were most virulent on non-pine species and more variable than 'P' (one was avirulent). The Australian isolate was moderately virulent only on ponderosa pine, Sequoia, and Sitka spruce. Sitka spruce was highly susceptible to all isolates and incense-cedar was highly resistant to all isolates.

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EFFECTS OF WATER STRESS ON WHITE FIR WOUND RESPONSE TO TRICHOSPORIUM SYMBIOTICUM, FUNGAL SYMBIONT OF THE FIR ENGRAVER BEETLE. W. J. Otrosina and G. T. Ferrell, USDA Forest Service, Pacific Southwest Forest and Range Experiment Station, Berkeley, CA.

Effects of water stress on white fir reaction to stem inoculation with the fungal symbiont of the fir engraver beetle (Scolytus ventralis LeC.) were assessed in greenhouse and field tests. Inoculum was selected from surveys of fungal populations carried by individual beetles. Among the most prevalent fungi recovered from beetle washings were Trichosporium symbioticum, unidentified species of Cladosporium, Graphium, Paecilomyces, Penicillium, and yeasts. In both greenhouse and field inoculations with T. symbioticum, firs under high water stress (xylem pressure potentials under -2.0 mPa) produced lesions which were longer and less resinous than those of firs under lower stress. The differential host reactions to this fungus may be potentially useful in assessment of susceptibility of drought stressed white fir to attack by the bark beetle-fungus complex.

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NUCLEI IN MATURE AECIOSPORES FROM TWO ZYMODEMES OF PERIDERMIUM HARKNESSII IN CALIFORNIA. D. R. Vogler, F. W. Cobb, Jr., and L. Epstein, Department of Plant Pathology, University of California, Berkeley, CA 94720.

We used epifluorescent microscopy to count nuclei in aeciospores from two zymodemes (electrophoretic types) of Peridermium harknessii J. P. Moore. For each single-gall isolate, spores were fixed overnight at 4C in 3.7% formaldehyde, pelleted by centrifugation, resuspended in 0.1 ug/ml 4,6-diamidino-2-phenylindole (DAPI) for 6 min, pelleted and rinsed with water 6 times, and mounted in 75% glycerol in 25% phosphate buffer (pH 8). Nuclei were counted at 800X using filters recommended for DAPI by the microscope manufacturer (Zeiss). Spores from zymodeme I (collected from several pine hosts throughout California) were predominantly (>75%) binucleate, while spores from zymodeme II (collected from lodgepole pine in the central Sierra Nevada) were predominantly (>85%) uninucleate. Implications for the nuclear behavior of this endemic forest pathogen are discussed.

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INDUCTION OF RESISTANCE TO BLIGHT IN CHINESE CHESTNUT. Louis Shain and Joseph B. Miller. Dept. of Plant Pathology, University of Kentucky, Lexington, KY 40546.

The mechanism of resistance to blight, caused by the Asian pathogen Cryphonectria (Endothia) parasitica, by Asian species of chestnut is unclear. Previous workers focused on preformed compounds, i.e. those present prior to infection, to explain the resistance of Chinese chestnut (Castanea mollissima). Bark tannins, ether solubles, and proteins were implicated. Our hypothesis is that compounds induced after infection may play a definitive role in host resistance. Preformed inhibitors would be expected to protect the host during the growing as well as the dormant season. Our inoculation results indicate, however, that while cankers expanded more quickly on American than on Chinese chestnut during the growing season, canker expansion on dormant stem segments was comparable for both hosts. Preliminary bioassays of extracts from challenged and unchallenged bark from both species, furthermore, indicate that compound(s) inhibitory to C. parasitica were produced by bark of challenged Chinese chestnut only during the growing season.

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SCREENING YOUNG SEEDLINGS OF CHESTNUT FOR BLIGHT RESISTANCE. F. V. Hebard and L. Shain. University of Kentucky, Lexington, KY 40546.

Greenhouse-grown chestnut seedlings, 1 and 2 years old, were tested for blight resistance by direct inoculation with *Endothia parasitica* and by measuring ethylene production in stem segments exposed to mycelium for 2 days. Tests included highly blight-resistant Chinese chestnut and blight-susceptible American chestnut, their moderately blight-resistant first generation hybrids, and first backcrosses to American chestnut, as well as grafted scions of very highly blight-resistant Chinese chestnut, cv Nanking. The mean of blight resistance in a backcross population should be intermediate between the hybrids and American chestnut. In the direct inoculation test, there was little canker expansion on cv Nanking, confirming its very high resistance to blight. Rates of canker expansion were quite similar for the other populations. Seedling Chinese chestnut could be distinguished from the remaining populations due to superficiality of cankers and sparse fructification. Thus direct inoculation of young chestnut seedlings could distinguish very highly resistant plants from highly resistant plants, and these in turn from moderately resistant and susceptible plants, but it could not distinguish moderately resistant and susceptible plants. In contrast, the ethylene technique correctly ranked all classes of plants. However, we were unable to precisely distinguish resistance classes within the backcross population because of large variance associated with the technique. This problem may be solvable by increasing the number of replicates.

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CELLULAR CHANGES IN SLASH PINES CAUSED BY COMPATIBLE AND INCOMPATIBLE REACTIONS TO CRONARTIUM QUERCUUM F. SP. FUSIFORME. C. H. Walkinshaw. USDA, Forest Service, Southern Forest Experiment Station, Gulfport, MS 39505.

Fifty nine slash pine (Pinus elliottii Engelm. var. elliottii) families that vary greatly in rust resistance were inoculated with single-gall field isolates of Cronartium quercuum (Berk.) Miyabe ex Shirai f. sp. fusiforme. Tissues were fixed 9 to 30 days after inoculation, processed, sectioned, and stained for light microscopy. Cortical cells invaded by the fungus acted as carbohydrate sinks. In cells of susceptible plants this was evident by a high affinity for stains. Nuclei in these cells were normal in size and shape. In contrast, cells in resistant seedlings were swollen, had faded chromatin, and showed signs of hydrolytic enzyme activity. Deposition of ergastic substances in cells of resistant seedlings did not coincide with changes in cortical cells.

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SURVEY OF THE NATIONAL MALL FOR ELM LEAF SCORCH ASSOCIATED WITH XYLELLA FASTIDIOSA. J. L. Sherald and J. Lei, Center for Urban Ecology, National Park Service, Washington, DC, 20242 and Twyford International, Inc., Santa Paula, CA 93060.

Elm trees of the National Mall in Washington, D.C. were examined for leaf scorch symptoms characteristic of those associated with Xylella fastidiosa. Examinations were made of 580 trees, predominantly Ulmus americana, in Sept. of 1986, 1987, and 1988. Approximately 25% of the trees were affected each year. Trees were classified according to the percentage of the canopy affected: (0%) 75%, (trace - 5%) 13%, (6 - 25%) 4%, (26 - 50%) 3%, (51 - 75%) 1%, (76 - 99%), 3%, (100%) 1%. Leaves were collected in June and Sept., 1987 from 47 trees for detection of X. fastidiosa with an ELISA test kit. Samples were collected from all symptom classes. Of 18 leaf scorch-affected trees 12 were detected in June prior to symptom development and 17 in Sept. after symptom development. In Sept., 2 of the 29 symptomless trees produced weak positive ELISA reactions.

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ROOT DAMAGE IN WHITE ASH INFECTED WITH MYCOPLASMALIKE ORGANISMS. A. T. Dyer and W. A. Sinclair, Department of Plant Pathology, Cornell University, Ithaca, NY 14853.

Pathological changes were monitored in roots of white ash (*Fraxinus americana*) infected with the mycoplasmalike organisms (MLOs) associated with ash yellows. Seedlings in their 4th year were potted in soil or installed in hydroponic culture after grafting with MLO-infected shoots or (for controls) with strips of their own bark. The DAPI (4',6-diamidino-2-phenylindole•2HCl) fluorescence test indicated MLO infection of roots of all inoculated but no control seedlings 33 days after grafting. Dead and dying lateral roots were first noted at 34 days, and leaves on some plants began wilting at 68 days, after grafting. In secondary phloem of living, diseased lateral roots, starch accumulation was reduced, hyperplasia developed, many sieve tubes and companion cells collapsed, and sclerenchyma was commonly observed adjacent to the collapsed elements. Tap roots and stems remained alive. After vernalization, diseased plants grew feebly. Control plants showed none of the above symptoms. Root damage may be important in the decline of ash naturally infected with MLOs.

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PRODUCTION OF MONOCLONAL ANTIBODIES AGAINST SPECIES OF THE ARMILLARIA MELLEA COMPLEX.

I. A. Bérubé, and M. Dessureault. Centre de Recherche en Biologie Forestière, Faculté de Foresterie, Université Laval, Ste-Foy, Québec, Canada, G1K 7P4.

The root pathogen, *Armillaria mellea* in the broad sense is comprised of nine reproductively isolated groups or biological species in North America. Soluble proteins characteristic of species I and V were separated and purified by SDS-PAGE. These proteins were used to generate specific monoclonal antibodies against species I and V from immunized spleen cells of BALB/c strain and murine myolema cell line Sp 2/0. Immunoblotting analysis and ELISA confirmed specificity against species I and V. The usefulness of this technique to recognize haploid and diploid isolates of mycelia, rhizomorphs and fruiting bodies of all nine species from different geographical area will be discussed.

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INCIDENCE AND SILVICULTURAL SIGNIFICANCE OF ENDOCRONARTIUM HARKNESSII ON JACK PINE IN ARTIFICIALLY REGENERATED AREAS OF NORTHWESTERN ONTARIO. J. Juzwik and N. Chong, Pest Management Section, Ont. Min. Nat. Res., Sault Ste. Marie, Ontario, P6A 5N5.

Pine-pine gall rust (Endocronartium harknessii [J. P. Moore]) accounted for >99% of all rusts encountered during a 1987 survey of jack pine (Pinus banksiana Lamb.) in 71 artificially regenerated sites in the Northwestern Region of Ontario. The sites had been regenerated with planted stock (bareroot or container seedlings) or by direct seeding (ground or aerial). In the six districts of the Region, district averages for rust incidence ranged from 6.7 - 17.2%. Significant differences in mean disease incidence among regeneration strategies were not detected. Galls occurred on the main stem of 11.4% of the jack pine observed. These main stem infections were associated with no damage except for the actual gall on 67% of the trees, with crooks or multiple leaders on 29%, and with mortality of 3.4%.

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CONE HARVESTING PRACTICES AFFECT THE INCIDENCE OF BLACK SEED ROT OF SLASH PINE CAUSED BY LASIODIPLODIA THEOBROMAE. S. W. Fraedrich, T. Miller, USDA Forest Service, Olustee, FL 32072, F. J. Spirek, Nekoosa Packaging Co., Valdosta, GA 31603.

Cones were harvested from 4 ramets of 4 clones on 3 dates (8/31, 9/14, 9/26/88) and divided among 3 treatments: no ground contact/no storage (NGC/NS), no ground contact/storage (NGC/S), ground contact/storage (GC/S). Seeds from each cone were assayed for fungus damage and fungi. Specific gravity was determined for cones from each tree at each sample period. Fungus-damaged seed were absent in the NGC/NS treatment, and occurred at low frequency (0.25-2.25%) among cones in the NGC/S treatment. Incidence of fungus-damaged seeds was high (12%) among cones harvested early in the GC/S treatment; incidence decreased with later harvest dates. Analysis of data by clones provides additional evidence that degree of cone maturation at harvest may be critical for seed colonization and infection by pathogenic fungi. Lasiodiplodia theobromae was the predominant fungus isolated from diseased seeds.

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CORRELATION OF ARMILLARIA ROOT ROT WITH DAMAGE TO TREES DEFOLIATED BY THE JACK PINE BUDWORM. K.I. Mallett and W.J.A. Volney. Forestry Canada, Northern Forestry Centre, 5320 - 122 St. Edmonton, Alberta, Canada T6H 3S5

Pinus banksiana trees severely defoliated by the jack pine budworm (Choristoneura pinus) in 1985 and '86 were dissected to determine the extent of root infections by Armillaria sp. The severity of damage to the tree crowns was correlated with the incidence of root infection: 100 % of the dead trees were infected with Armillaria sp., 60% of the trees whose upper crowns had been killed were infected, but only 20% of the healthy trees were infected. The value of these observations in hazard-rating jack pine stands is discussed.

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VARIABILITY IN VIRULENCE OF OOSPORE INOCULUM OF PHYTOPHTHORA CAPSICI AND THE RELATIONSHIP OF THE DENSITY OF OOSPORES IN SOIL TO PLANT MORTALITY. J.H. Bowers and D.J. Mitchell. Dept. of Plant Pathology, Univ. of Florida, Gainesville, FL 32611.

Oospore progeny from 20 crosses of pathogenic isolates of Phytophthora capsici were found to vary in pathogenicity to pepper. Only oospore inoculum from specific crosses caused disease in pepper seedlings with an initial inoculum density of 25 oospores per gram of soil. The highest mortality (30 to 75%) resulted from oospore inoculum produced from crosses involving certain isolates of A2 compatibility type. Oospore inoculum from other crosses caused little or no disease (0 to 5%). Oospores produced from one cross resulted in 40 and 60% plant mortality with inoculum densities of 10 and 15 oospores per gram of soil, respectively. The ID50 was calculated to be 41 oospores per gram of soil. Inoculum efficiency was calculated using the multiple infection transformation as the estimated number of infections per propagule and was found to be 0.011.

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SURVIVAL OF *FUSARIUM OXYSPORUM* F. SP. *VASINFECTUM* IN SOIL AS AFFECTED BY FOUR CROPPING SYSTEMS. J. P. McEntee, R. D. Martyn, and J. L. Starr. Department of Plant Pathology and Microbiology, Texas A&M University, College Station, 77843.

Microplots infested with a sand:cornmeal inoculum of *Fusarium oxysporum* f. sp. *vasinfectum* (FOV) were planted with a FOV-susceptible cotton cultivar (Rowden), a resistant cultivar (Auburn 634), a nonhost (Sorghum), or left fallow. Populations of FOV were determined initially and again at mid- and end-of-season. At the end of the season, plants were examined for vascular browning (VB) and isolation frequency of FOV from root and stem tissue. Final FOV populations were highest in microplots planted to sorghum, while no difference was observed between FOV populations from Rowden and Auburn 634. The fallow treatment had the lowest FOV populations. Differences in plant numbers with VB and in the nature of the VB were observed between cotton cultivars. VB incidence and severity was greatest in Rowden; however, no significant difference in isolation frequency of FOV was observed between cultivars. Examination of the sand:cornmeal inoculum revealed that it was composed largely of microconidia, chlamydospores and mycelia. When dried under sterile conditions, the microconidial and mycelial populations decreased rapidly, leaving primarily chlamydospores.

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ECOLOGY OF FUSARIUM OXYSPORUM F.SP.NIVEUM IN FUSARIUM WILT-SUPPRESSIVE AND -CONDUCIVE SOILS. R. P. Larkin, D. L. Hopkins, and F. N. Martin, Dept. of Plant Pathology, University of Florida, Gainesville, FL 32611.

Population dynamics, root colonization, and chlamydospore germination of Fusarium oxysporum f.sp. niveum were monitored in relation to other microorganism populations and incidence of fusarium wilt of watermelon in four soils. An UV-derived orange-colored mutant of F.o.niveum which was comparable to the wild-type strain in growth, pathogenicity, and root colonization was used to differentiate the pathogen from indigenous F.oxysporum strains. Pathogen populations were relatively stable in a fusarium wilt-suppressive soil developed through prolonged monoculture to watermelon cv. 'Crimson Sweet' (Phytopath.77:607-611) and in a non-suppressive 'Florida Giant' monoculture soil, but increased over time in a wilt-conducive fallow soil and a microwave-treated suppressive soil. Planting of cv. 'Crimson Sweet' resulted in substantial changes in microbial populations which were related to suppressiveness.

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SUPPRESSION OF TAKE-ALL ON WHEAT UNDER WHEAT:SORGHUM DOUBLECROPPING SYSTEMS. C.S. ROTHROCK, Department of Plant Pathology, University of Arkansas, Fayetteville, AR 72701.

Factors were studied that limited take-all development on wheat following sorghum in doublecropping systems. Two field studies with plots exhibiting decreased take-all severity following sorghum had lower soil pH than plots where wheat followed soybean. Bioassays for microbial suppressiveness indicated wheat:sorghum doublecropped soils were suppressive to take-all at levels similar to soils with long histories of wheat monoculture. Growth chamber studies indicated no differences in disease development on wheat in artificially infested soils after amending with various summer crop residues, including sorghum, or planting with these summer crops, indicating no differences in pathogen survival.

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RHIZOSPHERE COMPETENCY OF FIVE Fusarium SPECIES ON MAIZE PLANTED IN THE FIELD. C. M. Ocamb, T. Kommedahl, and P. M. Burnes, Department of Plant Pathology, University of Minnesota, St. Paul, MN 55108.

In 1988, maize seeds were coated with one of five species of Fusarium and planted in the field. Virtually the entire root systems of the seedlings were colonized by each of three species. Of these, F. moniliforme constituted 90%; F. proliferatum, 48%; and F. oxysporum, 39% of the colonies isolated from roots. Seedling root systems were not extensively colonized by F. solani (9%) or F. equiseti (11%). The seedling root systems of the dry control, water control, and the captan treatment yielded primarily F. oxysporum and F. solani. Rhizosphere competence differed in laboratory, greenhouse, and field tests. Field studies offer evidence that Fusarium species present in the seed differ as potential inoculum sources for root infections.

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EFFECT OF INOCULUM DENSITY OF *CEPHALOSPORIUM GRAMINEUM* AND SOIL pH ON CEPHALOSPORIUM STRIPE OF WINTER WHEAT. L. P. Specht and T. D. Murray. Department of Plant Pathology, Washington State University, Pullman, WA 99164-6430.

Winter wheats (cvs. Stephens and Nugaines) were grown in soil adjusted to pH values of 4.5-7.6 and maintained at -0.01 MPa. Soil in pots was drenched with conidial suspensions of *Cephalosporium gramineum* when seedlings were in the four- to five-leaf stage. Inoculum density of *C. gramineum*, soil pH, and cultivar all had significant ($P<0.05$) influences on the percentage of tillers of mature plants with disease symptoms. Incidence of diseased tillers averaged 0, 4.8, 11.2, and 22.0% at 0, 2.5 x 10^4, 2.5 x 10^5, and 2.5 x 10^6 conidia/g soil, respectively. Incidence of diseased tillers averaged 17.5, 14.6, 10.6, and 8.0% at soil pH values of 4.5, 5.7, 6.7, and 7.6, respectively. Cvs. Stephens and Nugaines averaged 15.5 and 9.8% diseased tillers, respectively. There were no significant interactions among these factors.

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TOXIC, CYTOCHALASIN-LIKE COMPOUNDS FROM MACROPHOMINA PHASEOLINA. A. Karr, T.D. Wyllie, F. Beleid-El Moshaty, A. Novacky, Department of Plant Pathology, University of Missouri, Columbia, MO 65211.

We report the production of phytotoxin(s) by Macrophomina phaseolina, the cause of charcoal rot of soybean. Isolates of M. phaseolina were grown in the dark in Czapek-Dox liquid medium in stationary culture at 33 C for 2, 3 or 4 weeks. The culture filtrate was fractionated by solvent partitioning. The 70% [v/v] ethanol/methylene chloride soluble fraction was found to contain a component(s) which was toxic to E. coli, soybean root cap cells, soybean cotyledonary cells and soybean plants. When the sample was fractionated by elution from a C_{18}-RP semipreparative column, this toxic activity eluted coincident with a single symmetrical peak detected by A_{254}. The UV spectrum of this component was similar to that of known cytochalasins and its toxic activity in the bioassays could be reproduced with commercially available cytochalasins Band D. The toxin will be purified by HPLC and the structure confirmed by mass spectrometry. The characterization of this toxic metabolite will facilitate studies of the biochemistry of charcoal rot disease development in soybeans and may permit screening for identification of cultivars resistant to M. phaseolina.

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INFLUENCE OF *GAEUMANNOMYCES GRAMINIS* VAR. *TRITICI* ON MANGANESE OXIDIZING BACTERIA IN WHEAT RHIZOSPHERES. T.S. Roseman and D.M. Huber. Department of Botany & Plant Pathology; Purdue University, W. Lafayette, IN 47907.

Take-all root, crown and foot rot was reduced with Mn amendment and

by suppression of Mn oxidizers in the wheat rhizosphere. This study was initiated to determine if the pathogenicity of Ggt influenced rhizosphere populations of Mn oxidizing bacteria. Nine isolates of Ggt, differing in pathogenicity, grown on PDA and Bromfield's media were evaluated on wheat plants grown for 5 weeks in a low-N field soil. The population of rhizosphere Mn oxidizing organisms was determined by dilution plating rhizosphere soil on Gerretsen's medium. The Ggt isolates strongly influenced population levels of Mn oxidizers (600 CFU/g soil - 63,000 CFU/g soil); however, virulence of Ggt was independent of population of Mn oxidizers in the rhizosphere.

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SUGAR BEET STAND ESTABLISHMENT AND SOILBORNE PATHOGENS IN TARE SOIL. Carol E. Windels and Donna J. Nabben, Northwest Experiment Station, University of Minnesota, Crookston, 56716.

When sugar beets are piled for storage in the Red River Valley, soil removed from beets during piling (tare soil) is returned to fields. In 1986-88, tare soils from 45 fields were evaluated for pathogens by a seedling assay. Aphanomyces cochlioides was isolated from dying seedlings in four of six tare soils where it had been verified during the growing season; no emergence occurred in the other two soils. Of 39 tare soils collected at random, Rhizoctonia solani was isolated from dying seedlings in 22 sites; in 1987, 66% of the cultures were AG-4, 27% AG-2-2, and 7% were unidentified. Seedling emergence in paired tare and field soils was statistically less (55 vs 89%, respectively) for 22 of 44 locations. At four of these sites, emergence averaged 3% in tare soils, and was associated with high soil pH, organic matter, soluble salts, phosphorus, and potassium compared to most tare soils and all field soils. Since tare soils often become mixed, returning tare soil to beet fields can spread soilborne pathogens and affect beet emergence.

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INOCULUM DENSITY OF PYTHIUM MYRIOTYLUM AND ITS RELATION TO PEANUT PLANT VIGOR. R. Garcia-Espinosa, R. Rodriguez-Kabana, and P. A. Backman, Alabama Agricultural Experiment Station, Dept. of Plant Pathology, Auburn University, AL 36849-5409.

On a quantitative basis, the damage caused by P. myriotylum to the peanut host has been documented mainly for seedling disease and pod rot. The effect of increasing inoculum levels of P. myriotylum on peanut plants allowed to germinate free of, but immediately exposed to the pathogen was evaluated. Significant negative correlations were observed between retrieved populations of the pathogen and plant vigor parameters. Based on exponential correlation analysis, a 50% reduction in leaf index can be expected with 30 to 40 propagules/gram (ppg) of soil, and a 50% reduction in fresh weight can be expected with 50 ppg of soil. On average, populations >100 ppg of soil caused a profound proportional stunting of all the aerial parts of the plant. The importance of this pathogen to peanut production in the southeastern U.S.A. is discussed.

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SYMPTOM DEVELOPMENT OF SOYBEAN SUDDEN DEATH SYNDROME IN RELATION TO T-2 TOXIN PRODUCTION BY FUSARIUM SOLANI. B.S. Corwin, T.D. Wyllie, G.E. Rottinghaus, and F.P. Ross, Department of Plant Pathology and Vet. Med. Diag. Lab., University of Missouri, Columbia, MO 65211, and USDA/APHIS/NVSL, Ames, IA 50010.

We report the possible involvement of a toxin with soybean sudden death syndrome (SDS). Fusarium solani isolate MO1247 was obtained from field grown soybean plants with symptoms of SDS. Pathogenicity of this isolate was confirmed in the greenhouse. The isolate was grown in Czapek-Dox broth in stationary culture at 22 ± 2 C. Culture filtrates were extracted with ethyl acetate, concentrated, and fractionated by thin layer chromatography. One component of the fraction was identified as T-2 toxin and the identification was confirmed by tandem mass spectrometry. Soybean plants, cv. 'Lee 74', in growth stage V3 were root dipped in 200 µl of 100, 500, or 1000 ppm T-2 toxin in 20 ml of water for 45 min. Plants exposed to all concentrations of T-2 exhibited marginal necrosis and chlorosis of leaflets, as well as cupping within 2 days posttreatment. No symptoms developed on the controls. The similarities in foliar symptoms between plants inoculated with F. solani isolate MO1247 and plants treated with T-2 toxin suggest that the toxin may be involved in soybean SDS.

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PHYTOPHTHORA ROOT ROT OF WHITE PINE IN PENNSYLVANIA. S. H. Kim, T. N. Olson, and P. Keller. Pennsylvania Department of Agriculture, Harrisburg, 17110 and Messiah College, Grantham, Pennsylvania 17027.

Phytophthora root rot of Eastern white pine, Pinus strobus (Ps), occurred in field and container grown trees during August of 1988. Both Phytophthora citricola (Pci) and P. cryptogea A^2 (Pcr) were isolated from dead roots of 6-year-old trees in a Bucks County field where 44% and 8% out of 360, were dead and yellowed, respectively. The disease occurred more in clay soils and lower slopes than in upper shale areas. Pci was isolated from a shipment of 200 container grown 3-year-old Ps from western PA. Both Pcr and Pci caused sudden wilting, greying, tip-curling, and browning of needles as a result of root rot when 2-year-old Ps were transplanted into a soil mix containing the inoculum grown in V8 juice-vermiculite medium; Pcr caused a greater tree mortality and shoot stunting than Pci.

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SUSCEPTIBILITY OF SUGAR BEET CULTIVARS TO AG-2-2 CULTURES OF RHIZOCTONIA SOLANI ISOLATED FROM LEGUMES AND SUGAR BEET. Cheryl A. Engelkes and Carol E. Windels, Department of Plant Pathology, University of Minnesota, St. Paul, 55108 and Northwest Experiment Station, Crookston, 56716.

Crown and root rot of sugar beet (Beta vulgaris L.) caused by AG-2-2 of Rhizoctonia solani occurs in Minnesota and North Dakota. Cultures of AG-2-2 isolated from fababean, pinto bean, and soybean (crops rotated with beet) and from sugar beet were field-tested for pathogenicity on the roots of three sugar beet cultivars. Roots were rated on a 0-7 scale (0=healthy, 7=100% rotted). 'Maribo Ultramono' gave a higher root rot index (3.3) compared to 'ACH 184' (2.1) and 'Fc712' (1.8). Four cultures from pinto bean gave an average root rot index of 4.4; two cultures from soybean averaged 3.7; ten cultures from sugar beet averaged 1.8; and three cultures from fababean averaged 1.6. In fields where AG-2-2 is present, rotation of legume crops can provide inoculum that causes crown and root rot of sugar beet.

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SHOOT TIP PROPAGATION USED TO PRODUCE AGROBACTERIUM-FREE MUSCADINE PLANTS. D. E. Griffin, C. H. Graves, Jr., and K. L. Thies, Department of Plant Pathology and Weed Science, Miss. State, MS 39762.

Because of the incidence of crown gall caused by Agrobacterium tumefaciens (AT) in muscadine (Vitis rotundifolia) plantings in Mississippi and widespread systemic presence of Agrobacterium spp. in symptomless cultivated muscadine, there is need to determine pathogenicity of the vascular isolates from such plants. Muscadine is the only known host for muscadine isolates of AT. Failure of repeated screening techniques to free rooted softwood cuttings of AT led to production of muscadine plants through tip culture. Muscadine plants for determining pathogenicity of AT isolates have been successfully produced from shoot tips on variations of Murashige-Skoog and Woody Plant Medium amended with benzylaminopurine. The effectiveness of different growth regulators on shoot proliferation will be discussed.

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EVALUATION OF 'BULGARIA 12' TOMATO SOMACLONE PROGENY FOR RESISTANCE TO CLAVIBACTER MICHIGANENSIS SUBSP. MICHIGANENSIS. R.M. De Vries-Paterson and C.T. Stephens. Department of Botany and Plant Pathology, Michigan State University, East Lansing, MI 48824.

A total of 191 somaclones were generated from leaf explants of 'Bulgaria 12', a tomato cultivar with partial resistance to Clavibacter michiganensis subsp. michiganensis (CMM), causal organism of bacterial canker of tomato. Thirty-two percent of the somaclones were discarded due to abnormal phenotype or poor growth. One aberrant R_1 line produced currant-type fruit on a vine. Fifty-five R_1 progeny were screened for resistance to CMM in a growth chamber. There were some variations in reaction to CMM among progeny regenerated from the same individual callus. A few R_1 lines showed an increased level of resistance to CMM and could prove useful to plant breeders.

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Epidemiology and spread of Clavibacter michiganensis subsp. michiganensis on tomato. R. J. Chang, S. M. Ries and J. K. Pataky. Dept. of Plant Path., Univ. of Illinois, Urbana.

Ten rifampin-resistant mutants of Clavibacter michiganensis subsp. michiganensis (CMM) were used to study the epidemiology and spread of bacterial canker. CMM spread from infected to healthy plants in beds of 10,000 direct seeded plants clipped six times with a rotary mower. Initial disease incidences of 0.01, 0.05, 0.1 and 0.5% resulted in 4, 8, 12 and 83% infected plants, respectively, when clipped plants were transplanted. CMM also was spread during transplanting when healthy and diseased plants were pulled, shaken, and mixed together in shipping crates. Twelve, forty-two and fifty-two per cent of the transplanted plants showed canker symptoms when initial proportions of diseased plants placed in crates were 1, 5, and 10%, respectively. A susceptible and a tolerant cultivar supported epiphytic populations of CMM of 10^7-10^9 CFU/g fresh weight, although symptoms of secondary infection, marginal scorch of leaflets, were more severe on the susceptible cultivar.

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Survival of wildtype and gentically-altered Erwinia carotovora subsp. carotovora (ECC) strains in soil. H.K. Austin, G.H. Lacy and J. Cairns, Jr., UCE&HMS and the Laboratory for Molecular Biology of Plant Stress, VPI&SU, Blacksburg, VA 24061-0331.

We assessed survival of genetically-altered, kanamycin-resistant L-864 and wildtype, rifampin-resistant L-863 strains of ECC in non-amended soil; in soil amended with germinating seeds of radish, carrot, tomato (all hosts), or grass (non-host); and in soil amended with potato tuber pieces or enrichment medium (EM; Phytopathology 66:367-370, 1976). Survival of a genetically-altered, pectate lyase-deficient ECC strain L-872 in soil amended with potato tuber pieces or EM was also studied. L-863 and L-864 did not differ significantly ($P \geq 0.05$) in response to seedlings, potato pieces, or EM; their densities increased > 500-fold with potato pieces ($P < 0.01$). Indigenous bacteria increased >30-fold in soil amended with potato pieces or the polygalacturonic acid EM. Survival and detection of the genetically-altered ECC was enhanced in soil amended with potato tuber pieces or EM.

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COLLOIDAL GOLD/PROTEIN A IMMUNOBLOT ASSAY FOR Clavibacter xyli subsp. xyli CAUSE OF RATOON STUNTING DISEASE OF SUGARCANE. K. E. Damann, Jr. and W. J. Todd. Dept. of Plant Path. & Crop Physiol., and Dept. of Vet. Science, LAES, LSU Agricultural Center, Baton Rouge, LA 70803.

A one-step assay for detection of Clavibacter xyli subsp. xyli was developed. Application of 1 ul of vascular extract or sugarcane sap from ratoon stunt-diseased or healthy cultivar L 62-96 to a nitrocellulose strip gave a positive red-purple color in response to infected plants. This occurred when strips were incubated for 1 hr with 1 ml of protein A/gold and 10 ul of a 100-fold dilution of antiserum to either C. x. subsp. xyli or C. x. subsp. cynodontis. The technique was also used in tissue blots by centrifuging vascular contents from 1 cm stalk sections onto a nitrocellulose filter. The distribution of colored spots on the filter was compared with the distribution of vasucular bundles exhibiting alkaline-induced metaxylem autofluorescence in the stalk section. The results will be discussed.

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NEW PERENNIAL HOSTS OF EPIPHYTIC POPULATIONS OF XANTHOMONAS CAMPESTRIS PV. TRANSLUCENS. D. C. Thompson[1], N. W. Schaad[2], and R. L. Forster[3]. [1]Dept. PSES, University of Idaho, Moscow, ID 83843 and [3]Kimberly, ID 83341; [2]Harris-Moran Seed Co., San Juan Bautista, CA 95045.

Black chaff of gramineacious crops, caused by Xanthomonas campestris pv. translucens (Xct) develops from seedborne inoculum. A program was established in Idaho to produce Xct-free seed. In 1987 and 1988, black chaff occurred in spring wheat fields grown from Xct-free seed at Tetonia and Aberdeen. Epiphytic populations of Xct were found on leaves of known hosts (Bromus inermis, Agropyron repens) and on previously undescribed hosts (Poa pratensis, Festuca arundinaceae, F. rubra, Hordeum leporinum, Medicago sativa) near these fields. Black chaff symptoms were not observed, but they contained populations of 13 cfu to 7.5×10^6 cfu per gram fresh tissue. Pathogenicity tests indicated a broad and variable host range, with high levels of virulence on wheat and barley. Xct was not isolated from plants in southwest ID where black chaff does not occur frequently.

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PSEUDOMONAS VIRIDIFLAVA, THE CAUSE OF A STEM CANKER OF POINSETTIA PLANTS. Arthur W. Engelhard and Jeffrey B. Jones. Univ. of Florida, IFAS, Gulf Coast Research & Education Center, Bradenton, FL 34203.

A stem canker occurred in December 1984 on potted poinsettia plants grown outdoors near Bradenton, Florida. A fluorescent bacterium was isolated that caused a positive reaction for tobacco hypersensitivity, DL-lactate, D(-) tartrate, erythritol and a negative one for arginine dihydrolase, oxidase and levan. It, thus, was identified as Pseudomonas viridiflava. Two strains of the bacterium tested were pathogenic on all seven cultivars of poinsettia plants evaluated, although the cultivars varied in susceptibility. The disease was most severe at 10 and 15°C, mild at 27.7°C, and no disease occurred at 32.2°C. These Florida strains of P. viridiflava are not expected to become a serious problem in poinsettia plant production in Florida because of their low optimum temperature requirement for their pathogenicity.

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CYPROCONAZOLE: A NEW SYSTEMIC FUNGICIDE FOR TURF DISEASE MANAGEMENT. G. G. Thomas, W. B. O'Neal, and P. Schmid. Sandoz Crop Protection Corporation, 1300 East Touhy Avenue, Des Plaines, IL 60018.

Cyproconazole, a-(4-chlorophenyl)-(1 cyclopropylethyl 1)-1 1,2, 4-triazole-1-ethanol, is a new broad spectrum fungicide with excellent systemic activity. Cyproconazole has rapid plant tissue penetration and is translocated primarily acropetally. At low dosages(0.1 to 0.4 kg a.i./ha) cyproconazole has demonstrated control of a wide range of fungi important in turf grass disease management. Common diseases, brown patch caused by Rhizoctonia solani and dollar spot caused by Sclerotinia homoeocarpa have been effectively controlled for a period of three to five weeks following application. In addition to foliar disease control, cyproconazole has shown potential to control turf grass root diseases; necrotic ring spot caused by Leptosphaeria korrae and summer patch caused by Magnaporthe poae.

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CONTROL OF IRIS RUST WITH FUNGICIDES. Albert O. Paulus and Robert D. Raabe. Departments of Plant Pathology, University of California, Riverside 92521 and Berkeley 94720.

Rust of iris, resulting from infection by *Puccinia iridis*, is common in coastal California. Bearded iris (*Iris germanica*), Dutch iris (*I. xiphium* and *I. filifolia* x *I. xiphium*) and a Pacific coast native, *I. munzii*, are particularly susceptible. In experiments to control rust on Dutch iris cv. Blue Ribbon in southern California, plants were sprayed 3 times at 2-week intervals. Diniconazole gave excellent control, Mobay 1608 gave very good control and flusilazole and myclobutanil gave moderately good control. In northern California, sprays were applied 3 times at 3-week intervals to seedlings of bearded iris. Best control resulted with Ciba Geigy 453 and myclobutanil. Moderately good control resulted with oxycarboxin, benodanil and Nor Am SN 596. Control with Nor Am SN 39865, penconazole, triadimefon, prochloraz, and benomyl was not satisfactory.

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FLOWERING OF FLORIBUNDA ROSES IN RESPONSE TO WEEKLY APPLICATIONS OF A COMBINATION AND SINGLY APPLIED FUNGICIDE SPRAY. P. F.

Colbaugh. Texas Agricultural Experiment Station, Texas A&M Univ. Res. and Ext. Center, 17360 Coit Rd., Dallas, TX 75252.

Replicated field plantings of four varieties of floribunda rose were sprayed weekly with a combination benomyl 50W + mancozeb 80W or triforine 16.5 EC protective fungicide spray to determine their influence on flowering. Both fungicide spray programs are in use by commercial Texas rose growers for foliar disease control. Counts of flower blossoms and opened flower buds were made on treated plants each week prior to the application of fungicides. The flowering of treated roses during August 1985 varied by rose variety; however, the mean weekly flowering of all varieties was increased on roses receiving triforine sprays. The increased flowering response of triforine treated rose varieties over the four week period was as follows: Charisma (65%), Angel Face (55%), Bahia (62%), and Red Gold (74%). Observations of disease activity during the study indicated both fungicide sprays were effective for controlling powdery mildew and blackspot diseases.

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CHEMICAL CONTROL OF PHOMOPSIS VITICOLA ON VITIS VINIFERA IN CALIFORNIA. G. M. Leavitt, W. J. Moller (deceased), University of California Cooperative Extension, 328 Madera Avenue, Madera, CA 93637.

Post bud break spring rains are necessary for the spread of Phomopsis viticola. All materials registered for control were tested on Vitis vinifera cultivars Thompson seedless and Grenache. All were effective in reducing disease incidence. Two well timed foliar applications on Grenache at bud break to 1 inch and 8 days later at shoots 4-6 inches tended to be superior in control. Application of one foliar captan at bud break equaled dormant treatment control levels but was not as effective as the two treatments. On Thompson seedless, one foliar application at shoots 2-4 inches after a post bud break rain reduced further infections. Significant crop loss on Grenache to Phomopsis viticola was measured.

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EFFECT OF FOLIAR IRRIGATION ON THE ACCUMULATION OF METALAXYL IN POTATO TUBERS. R. J. Young and Thomas Basden. Dept. of Plant Pathology, West Virginia University, Morgantown, WV 26506-6057.

Below normal rainfall and soil moisture in 1987-88 were related to non-detection of metalaxyl (met) activity in tubers. Rainfall, May through August (87-88), averaged 38% below normal. In irrigated plots (1988) soil moisture was kept at 20% and plants received a short intensive watering 8 and 24 hours after met (one or two) treatments. Metalaxyl activity was determined by measuring growth of Phytophthora infestans on tuber slices inoculated with zoospores, and incubated for 4 and 7 days at 19 C. No met activity was detected in tubers from met/non-irrigated plots. Tubers from met/irrigated plots showed activity beginning 7 days after treatment, and continued through five weekly harvest. Both 8 and 24 hr. irrigations were effective, and 2 met treatments were more effective than a single treatment. At harvest #5, 23% of the tubers showed no metalaxyl activity.

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EVALUATION OF NATURALLY-OCCURRING TOLERANCE AND RESISTANCE TO METALAXYL IN PHYTOPHTHORA MEGASPERMA F.SP. GLYCINEA. K. M. Howard and A. F. Schmitthenner, Dept. of Plant Pathology, Ohio State Univ., Wooster, OH 44691.

Sensitive, tolerant and resistant isolates of P. megasperma f.sp. glycinea (Pmg) which can grow at 0, 1 and 10 ppm metalaxyl, respectively, were evaluated for growth rate in vitro and virulence in vivo in the presence of metalaxyl. Resistant isolates were significantly different ($P=0.05$) from sensitive or tolerant isolates in all tests and able to grow and cause disease at concentrations up to 100 µg/ml. Tolerant isolates varied in response to metalaxyl and were similar to sensitive isolates in both assays. Oospore progeny from tolerant isolates segregated for resistance, tolerance and sensitivity to metalaxyl whereas resistant and sensitive isolates appeared to be homozygous. Results suggest that metalaxyl resistance in Pmg is homozygous dominant as reported for P. infestans.

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FUNGICIDAL AND FUNGISTATIC EFFECTS OF CARBOXIN ON MYCELIAL GROWTH OF USTILAGO NUDA. G. Newcombe and P.L. Thomas. Research Station, Agriculture Canada, 195 Dafoe Road., Winnipeg, Manitoba, R3T 2M9

Carboxin, at concentrations of from 0.01 to 1000 µg/mL, was applied to 24-h sporelings of three isolates of Ustilago nuda growing in liquid shake culture. The cultures were examined by light microscopy after further incubation. The number of apical cells per sporeling was used as a measure of growth. Death of apical cells of sporelings was determined with Evans blue. Sporelings were not affected by 24 h of exposure to carboxin concentrations below 0.03 µg/mL. Carboxin concentrations of 1 µg/mL or higher stopped growth of sporelings without causing apical cell death. However, carboxin concentrations between 0.03 and 1 µg/mL reduced growth rate and killed apical cells. An agar growth test showed that most sporelings were dead after 5 days at 0.125 µg/mL while few sporelings were killed by 10 µg/mL for 5 days. Carboxin has a fungicidal effect on U. nuda at concentrations below those at which it has a fungistatic effect.

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SUPPRESSION OF SOUTHERN STEM ROT OF PEANUT WITH GRANULAR SOIL INSECTICIDES. A. K. Hagan, and J. R. Weeks. Auburn University, Al 36849.

Peanuts treated with Chlorpyrifos 15G (2.2 kg a.i./ha), ethoprop 15G (3.3 kg a.i./ha), or fonofos 10G (2.2 kg a.i./ha) were compared with PCNB 10G (11.2 kg a.i./ha) for southern stem rot suppression (Sclerotium rolfsii) in on-farm trials, 1985-1987. Stem rot counts compared to the non-treated control were reduced each year by the use of chlorpyrifos and two of three years by ethoprop and fonofos. Ethoprop and chlorpyrifos suppressed disease as well as PCNB each year, and fonofos two of three years. Only in 1986 did the insecticide-treated plots outyield the untreated plots while PCNB treatments increased yields in all years. Yields in PCNB-treated plots were higher than those treated with fonofos or ethoprop but not chlorpyriofos. Pooled results show that all insecticides and PCNB suppressed stem rot but only PCNB and chlorpyriofos increased yield. Yield differences between PCNB and chlorpyrifos over 3 years were significant

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FUNGICIDAL CONTROL OF TAKE-ALL PATCH. Gary A Chastagner and John M. Staley, Wash. State Univ., Puyallup, WA 98371.

During 1984-85, fenarimol and triadimefon (0.29 gm ai/m²) and propiconazole (0.63 gm ai/m²) were applied to 'Highland' bentgrass turf in 79.9 ml of water/m² during December or December and April. During 1985-86, fenarimol (0.15 - 0.59 gm ai/m²) and triadimefon (0.07 - 0.29 gm ai/m²) were applied in December or April. Applications of propiconazole (0.29 and 0.59 gm ai/m²) were only made in December. In June 1985, 28% of the turf area in the check plots had take-all patch (TAP), caused by Gaeumannomyces graminis var. avenae. The level of TAP in the treated plots ranged from 0-6% and there were no significant differences between December or December and April applications. Data collected during June 1986 indicated that December applications significantly reduced the level of TAP. The level of reduction was similar for all three fungicides and was dependent upon the rate of active ingredient applied. Applications in April were not as effective as the applications in December. Some phytotoxicity was observed on the turf treated with propiconazole.

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LY211795 - A Novel Foliar Applied Fungicide for Control of Powdery Mildew in Cereals and Grapes. W. R. Arnold, M. J. Coghlan, H. R. Hall, E. V. Krumkalns, Lilly Research Laboratories, A Division of Eli Lilly and Company, P. O. Box 708, Greenfield, IN. C. Longhurst, Lilly Research Centre Ltd., Erl Wood Manor, Windlesham, Surrey GU20 6PH, UK.

Lilly 211795 is highly effective as a foliar fungicide on wheat and barley powdery mildew (Erysiphe graminis; f. spp. tritici and hordei, respectively) and grape powdery mildew (Uncinula necator). The compound penetrates into the plant tissue very rapidly and is translocated acropetally. Compound 211795 shows excellent protectant activity against mildew resistant to DMI chemistry. When used in mixtures with other fungicides, a wide range of diseases can be controlled.

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THE EFFECTS OF PENCONAZOLE AND PROPICONAZOLE ON THE ULTRASTRUCTURE OF *PYTHIUM ULTIMUM* AND *PHYMATOTRICHUM OMNIVORUM*. J. R. Anciso and S. D. Lyda. Department of Plant Pathology and Microbiology, Texas Agricultural Experiment Station, College Station, TX 77843.

The accepted mode of action of penconazole (Topas^r) and propiconazole (Tilt^r) is the inhibition of ergosterol biosynthesis. A time course study on effects of the two chemicals on *Pythium ultimum* and *Phymatotrichum omnivorum* was investigated. The ED_{50} for *P. ultimum* is 25 ppm with penconazole and 98 ppm with propiconazole, while the ED_{50} for *P. omnivorum* is 9 ppm with penconazole and .01 ppm with propiconazole. The ED_{50} (minimum inhibitory concentration) was chosen as the concentration to observe ultrastructural changes. The primary observation was the loss of normal-appearing endoplasmic reticulum and the abundant appearance of circular endoplasmic reticulum-like material in the treated for both species. *P. ultimum* which does not synthesize or require sterols has shown "moderate sensitivity" to penconazole in greenhouse and *in vitro* tests. These similar ultrastructural changes found in the nonsterol-synthesizing *P. ultimum* and the sterol-synthesizing *P. omnivorum* suggest that another mode of action exists for both of these triazoles.

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RESPONSE OF CYST-NEMATODE RESISTANT AND SUSCEPTIBLE SOYBEAN CULTIVARS TO IN-FURROW APPLICATIONS OF ALDICARB. P. M. Phipps, Tidewater Agr. Exp. Sta., VPI&SU, Suffolk, VA 23437-0099.

Soybean cultivars were evaluated with and without applications of aldicarb (Temik 15G) in fields naturally infested with soybean cyst nematode (SCN) in 1987 and 1988. Cultivars were main plots consisting of six 10.7-m rows spaced 0.9-m apart. Treatments were applied to the seed furrow of two-row subplots at planting. The experimental design employed four randomized complete blocks. The effect of cultivars on nematode populations and yield was significant (P=0.05), however, aldicarb only had a significant effect on yield. The overall yield of SCN-resistant cultivars (Centennial, Forrest, Pioneer Brand P9581) averaged 2576 kg/ha (38.3 bu/A) compared to 1917 kg/ha (28.5 bu/A) for susceptible cultivars (Essex, York). Aldicarb at 0.56 and 1.12 kg/ha improved yield of susceptible cultivars 276 and 390 kg/ha (4.1 and 5.8 bu/A) and yield of resistant cultivars 363 and 356 kg/ha (5.4 and 5.3 bu/A), respectively.

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EFFECTS OF FUNGICIDE RH3486 ON SCLEROTINIA BLIGHT OF PEANUT IN FIELD AND MICROPLOT TESTS. F. D. Smith, P.M. Phipps and R. J. Stipes, Tidewater Agr. Exp. Sta., VPI & SU, Suffolk, VA 23437.

The fungicide, RH3486, and several other fungicides were evaluated for control of Sclerotinia blight of peanut, caused by *Sclerotinia minor*. Two applications of RH3486 at 0.56 or 1.12 kg a.i./ha significantly (P=0.05) suppressed disease 69 and 81% and increased yields 2191 and 2243 kg/ha, respectively. Similar applications of iprodione (1.12 kg/ha), vinclozolin (0.84 kg/ha), dicloran (3.36 kg/ha) or PCNB at (5.60 kg/ha) suppressed disease only 42, 47, 21 and 18%, respectively. Microplots, 76-cm-dia., were infested with *S. minor* sclerotia from strain S-2 (wild-type) or B-83-T2 (dicarboximide-resistant) and planted to Florigiant peanut. Plots treated three times with RH3486 at 1.12 kg/ha averaged 0 and 1.0 lesions/plot with strain S-2 and B-83-T2, respectively. Untreated plots likewise averaged 27.0 and 31.5 lesions/plot. Three applications of iprodione at 1.12 kg/ha did not significantly suppress disease caused by either strain. Two years of similar field data have shown RH3486 to be an extremely active fungicide against wild-type and dicarboximide-resistant strains of *S. minor*.

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CYPROCONAZOLE: A NEW SYSTEMIC FUNGICIDE FOR CONTROL OF SOIL BORNE AND FOLIAR PATHOGENS IN PEANUT. H. S. McLean and P. Schmid. Sandoz Crop Protection, 1300 East Touhy Avenue, Des Plaines, IL 60018.

Cyproconazole, a-(4-chlorophyenyl)-(1 cyclopropylethyl 1)-1 1, 2,4-triazole-lethanol, is a new broad spectrum fungicide with excellent systemic activity. Cyproconazole systemic activity is characterized by rapid plant tissue penetration and acropetal translocation. In field trials conducted 1984-1988, cyproconazole has consistently provided control of important foliar and soil borne diseases in peanut when applied at relatively low rates (0.061-0.098 kg ai/ha) on a 14-21 day spray schedule. Early and Late leafspot (Cercospora arachidicola and Cercosporidium personatum) are easily controlled with cyproconazole. Southern Blight (Sclerotium rolfsii) and Rhizoctonia limb rot (Rhizoctonia solani) are also controlled by foliar applications of cyproconazole within the same rate range as listed for leafspot. Peanuts have excellent tolerance to Cyproconazole and display virtually no plant growth regulation effects.

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Production of hydroxyl radical in photodynamic biocidal reaction of methionine riboflavin mixture. D. D. Tzeng, and M. H. Lee. Department of Plant Pathology, National Chung Hsing University, Taichung, 40227 Taiwan, R.O.C.

Production of hydroxyl radical (·OH) was detected from the methionine riboflavin mixture (MR) under continuous illumination by the method developed by Baker and Gebicki. The rate of ·OH production from MR was much greater at pHs 4.0-5.0 than at pHs 6.0-8.0, which indicated the possible involvement of iron contaminant in the reaction. The addition of exogenous iron was stimulatory to the ·OH formation of MR. However, the presence of iron chelators like desferal, or phenanthroline, and free radical scavengers like thiourea, all greatly reduced the radical forming activity. At pH 4.0, it was also noted that addition of ascorbic acid or H_2O_2 at certain concentrations greatly enhanced the generation of the test radical. The rapid increament of ·OH formation via the iron catalyzed Haber-Weiss reaction was apparently a major factor which contributed to the photodynamic biocidal activity of MR.

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THE EXPRESSION OF RESISTANCE OF *USTILAGO AVENAE* TO TRIADIMENOL IS AN INDUCED RESPONSE. Wolfram Köller and Franzine D. Smith, Department of Plant Pathology, N Y State Agricultural Experiment Station, Geneva, NY, 14456.

A strain of *U. avenae* sensitive to triadimenol (sen) and a resistant laboratory mutant (r1) were treated with triadimenol (2mg/L) after 15 h of growth in liquid culture. Initially, reproduction of both strains was almost completely blocked; however, the inhibitory phase was transient for r1, and full growth resumed after 10 h. This pattern of initial growth inhibition and subsequent recovery was correlated with a decline of sterol precursors, as analyzed by GC-MS. Although precursors (pre-dominantly 24-methylenedihydrolanosterol) accumulated during the phase of growth inhibition, and also were still prominent at the onset of renewed growth, they were absent after 24 h of treatment with triadimenol. Pulse-labeling of sterols at various time intervals after treatment with the inhibitor revealed that the continuous disappearance of precursor sterols is not explained by a dilution of the inhibitor from the target site.

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WATER-DISPERSIBLE GRANULES TECHNOLOGY. P.R. Larson and J.M. Denis. UCB Chemicals Corp., 5365-A Robin Hood Rd., Norfolk, VA 23513 and UCB Chemical Sector, Ave. Louise 326, B-1050 Brussels, Belgium.

UCB is one of the world's leading producers of methylamines and their derivatives such as Thiram and Ziram fungicides. In efforts to improve worker safety, UCB evaluated other methods for formulating Thiram and Ziram and concluded that the water-dispersible granule (WG) formulation was the most appropriate. The WG technology developed by UCB has been registered under the trade name "GRANUFLO." The high quality granules readily disperse in water and offer many advantages over traditional formulations including being virtually dust-free, easy to package, easy to handle, insensitive to temperature variations and having improved product efficacy. The Granuflo technology allows opportunities for formulating associations of fungicides which are useful in developing anti-resistance strategies.

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BASELINE-SENSITIVITY OF THREE POPULATIONS OF *VENTURIA INAEQUALIS* TO FLUSILAZOLE. Franzine D. Smith, Wolfram Köller and Diana M. Parker, Department of Plant Pathology, Cornell University, New York State Agricultural Experiment Station, Geneva 14456.

One hundred monoconidial isolates of *V. inaequalis* were collected from each of two abandoned orchards (orchards 1 and 2), where no sterol demethylation inhibitors (DMI) had been used, and from a research orchard where DMI fungicides had been used for 12 years (orchard 3). The mean ED_{50} values based on colony diameter were 0.0083 µg flusilazole/ml, 0.0072 µg/ml, and 0.0105 µg/ml for orchards 1, 2, and 3, respectively. ED_{50} values for individual isolates ranged from 0.002 to 0.0654 µg/ml, 0.0001 to 0.0469 µg/ml, and 0.0011 to 0.1108 µg/ml, in orchards 1, 2, and 3, respectively. There was no significant difference between the mean of the $\log_{10}$ transformed ED_{50} values of any orchard. Our results indicate that the three populations examined had similar mean ED_{50} values, and that highly tolerant isolates occurred in natural populations prior to flusilazole exposure. The range and variance of ED_{50} values observed in our study also indicates that small sample sizes are unlikely to represent accurately the sensitivity of populations of *V. inaequalis* to DMI fungicides.

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SULFUR DIOXIDE RESIDUES IN TABLE GRAPES AFTER SO_2 FUMIGATION. J. L. Smilanick, J. M. Harvey, P. L. Hartsell, D. J. Henson, C. M. Harris, D. C. Fouse, and M. Assemi. USDA, ARS, HCRL, 2021 South Peach Avenue, Fresno, CA 93727

Grapes were fumigated to evaluate factors that influence sulfur dioxide (SO_2) residues. Residues with SO_2 decreased rapidly after fumigation; the SO_2 half-life was 24-48 hr. Grapes fumigated at warmer temperatures accumulated higher but less persistent residues than those fumigated at low temperatures. Immature grapes accumulated more SO_2 than mature grapes. SO_2 residues were located on or near the berry surface. Grapes infected with Botrytis cinerea or injured by cuts accumulated more SO_2 than sound grapes. Sulfur dust applied before harvest left no SO_2 residues. The decay suppression with low (312-1250 ppm) SO_2 doses was only slightly inferior to that obtained by standard doses (2500 ppm). SO_2 residues after low doses did not exceed 10 ppm. High residues can be avoided by low-dose fumigations, good temperature management, and use of grapes free from rot or injuries for storage.

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EVALUATION OF FUNGICIDES FOR CONTROL OF LEAF RUST AND SEPTORIA BLOTCH OF WHEAT. R. T. Gudauskas, A. K. Hagan, E. L. Carden, and N. R. McDaniel, Dept. of Plant Pathology and Gulf Coast Substation, Auburn University, AL 36849

During 1983-1988, 24 fungicide treatments were evaluated for control of leaf rust and Septoria blotch on highly susceptible cultivars 'Blueboy' and 'McNair 1003' and a less susceptible cultivar 'Fla. 301'. One or two applications of each fungicide were made between growth stages 8 and 10.2. Most of the fungicides tested reduced the severity of both diseases as compared to the unsprayed control. Yield increases associated with fungicide treatments in the highly susceptible cultivars ranged from 268 to 2016 kg/ha; yield increases in 'Fla. 301' ranged from 0 to 806 kg/ha. Fungicides giving the highest levels of disease control and yield increases included diniconazole, propiconazole, mancozeb, terbutrazole, and triadimefon.

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USES OF FUNGICIDE COMBINATIONS CONTAINING TETRAMETHYLTHIURAM DISULPHIDE OR THIRAM ON APPLES, PEARS AND STRAWBERRIES. J.M. Denis and P. Creemers. UCB Chemical Sector, Ave. Louise 326, B-1050 Brussels, Belgium and Opzoekingsstation van Gorsem, Brede Akker 3, B-3800 Sint-Truiden, Belgium.

Field trials with Thiram alone or in combination with other fungicides were conducted at the Fruit Research Station of Gorsem. The use of the compound Thiram in fungicide combinations showed an increase of preventive activity, a broadening of the spectrum of activity, and a decrease of the selection pressure of the fungicide with which it was combined. It is concluded that Thiram is an effective fungicide partner when used on apples (scab), pears (scab) and strawberries (grey mould).

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VARIABILITY OF DISEASE DEVELOPMENT IN NECTARINE CULTIVARS FOR EXPORT. B. T. Manji, J. M. Ogawa, J. E. Adaskaveg, and J. M. Osorio. Department of Plant Pathology, University of California, Davis, CA 95616.

Nectarine fruit in bins were preconditioned to a pit (inner mesocarp) temperature of 21 C and fumigated with methyl bromide, 48 g/m³ for 2 hr at 21 C. After fumigation, non-treated and fungicide-treated fruit were packed into boxes and stored for 12-14 days at 1 C to simulate transit by ship to oversea markets. In non-fungicide treatments, early-maturing cultivars (May Glo, May Grand, and Spring Red) generally developed less than 5% brown rot (Monilinia fructicola), whereas, later-maturing cultivars (Red Diamond and Firebrite) developed as high as 80% brown rot during the 5 day ripening period at 21 C. Generally, fruit washed with 100 µg/ml chlorine had less brown rot than non-chlorinated fruit. Postharvest treatment with iprodione in a water soluble wax further extended the shelf-life of the fruit by 3-5 days.

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EFFICACY OF COPPER RESINATE AND TRI-BASIC COPPER SULFATE IN PEANUT DISEASE CONTROL. W.W. Osborne and J. D. Taylor. IAI, Inc., South Boston, VA 24592; and J & S Consultants, Inc., Skippers, VA 23879.

Laboratory and field studies conducted during the past four years with copper resinate and tri-basic copper sulfate show an inverse relationship between chemical rates and control of major peanut diseases. Copper resinate was the most economical and effective treatment when compared with tri-basic copper sulfate and other fungicides currently being used for the control of major peanut diseases.

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EVALUATION OF AGRI-CHEMICALS ON SPORES AND MYCELIUM OF STIGMINA CARPOPHILA AND MONILINIA FRUCTICOLA. A.J. Feliciano, J.E. Adaskaveg, and J.M. Ogawa. Univ. of California, Davis, 95616.

Spores of S. carpophila (Sc) and M. fructicola (Mf) were exposed to (chemical/concentrations-µg/ml a.i.): NaOCl/400; Hg/32; Cu/1800; ziram /1800, captan/50, 500, 1200; iprodione/1200; and captafol/10, 100, 500, using the cellophane-transfer technique. Spores were exposed for 2 or 24 hr, rinsed for 0, 24, 48, 72 or 96 hr, and plated on PDA. Colony formation (cf) was determined after incubation for 10 da at 25C. Inhibition of cf for each fungus at chemical concentrations tested was: Sc - Hg, NaOCl, or captafol/2 hr (chemicals/exposure); captan/24 hr; Mf - Hg or NaOCl/2 hr; and captan or captafol/24 hr. Ziram, Cu, or iprodione did not inhibit cf of either fungus at both exposures. Rinsing increased the rate of growth but not % cf. Increasing concentrations of captan decreased the time of exposure that inhibited cf: Mf - 16 hr exposure/50 µg/ml, 12/500, 8/1200; and Sc - 4/10, 4/100, and 2/500. In a 24-hr, direct exposure study using mycelium growing on cellophane, 1200 µg/ml of captan or 500 µg/ml of captafol did not inhibit mycelial growth of either fungus. Current concepts of defining chemicals as fungicides may need re-evaluation based on fungal tissue, chemical concentration, exposure time, and duration of incubation on a medium.

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MITOCHONDRIAL PLASMIDS IN ENDOTHIA PARASITICA. N. Mahanti and D.W. Fulbright. Dept. Botany and Plant Pathology, Michigan State University, East Lansing, MI 48824.

Hypovirulent strains of Endothia parasitica found in Europe and North America are associated with dsRNA. One strain of E. parasitica (CL25) from Michigan has all the characteristics of dsRNA-associated transmissible hypovirulence but harbors no detectable dsRNA. Studies with CL25 show plasmids are present in mitochondria. These plasmids are in low titer and have been difficult to isolate consistently. The plasmid DNA was isolated by electrophoresing total mitochondrial DNA in an agarose gel, cutting the plasmid band from this gel, followed by electroelution. The restriction enzyme SauIII-A was used to clone the plasmid into vector PRL 498. Southern hybridization studies show that this plasmid is present in some virulent and other hypovirulent strains even though the plasmid bands are not visible in gels after ethidium bromide staining. Therefore these plasmids may not have a direct effect on hypovirulence but may be affecting the mitochondrial genome.

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IDENTIFICATION OF SEQUENCES WITH PROMOTER ACTIVITY FROM GIBBERELLA PULICARIS (FUSARIUM SAMBUCINUM) TRANSFORMANTS. Yangkyo P. Salch and Marian N. Beremand, USDA, Agricultural Research Service, Northern Regional Research Center, Peoria, IL 61604

G. pulicaris (GP) is a heterothallic ascomycete and causes dry rot on potato tubers. GP protoplasts were transformed with a cosmid cosHyg1 containing hygromycin B phosphotransferase (hygB) fused to promoter 1 from Cochliobolous heterostrophus. Transformation occurred by random integration of the cosmid into the GP genome. Based on restriction digestion and Southern hybridization analyses, one of the transformants with a single copy insertion, 63C3, had the recombination event occurring between the 5' end of hygB coding sequence and the 3' end of promoter 1. Expression of hygB resistance most likely resulted from endogenous GP promoter-like sequences. Cloning and analysis of the GP promoter-like sequences will be discussed.

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CAROTENOID-OVERPRODUCING TRANSFORMANTS OF NEUROSPORA CRASSA ARE NOT RESISTANT TO CERCOSPORIN. C. J. Cooperman, M. E. Daub, R. G. Upchurch, and G. A. Payne. Dept. of Plant Pathology, North Carolina State University, Raleigh, NC 27695-7616.

The photoactivated toxin cercosporin produces singlet oxygen and superoxide. Resistance of Cercospora spp. to cercosporin appears to act at several levels. Carotenoids, which are potent singlet oxygen quenchers, are associated with resistance to cercosporin. A Cercospora nicotianae genomic library constructed in the cosmid vector pSV50 was used to transform cercosporin-sensitive Neurospora crassa. A clone (B5) was identified that conferred increased carotenoid production to the transformants. Eight additional B5 transformants also overproduced carotenoids. Southern blot analysis indicated the insertion of C. nicotianae DNA into the genome. The carotenoid-overproducing transformants did not show increased resistance to cercosporin. These results support the previous hypothesis (Phytopathology 79:180) that carotenoids are not the sole mechanism of cercosporin resistance.

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REGULATION OF NITROGEN METABOLISM IN FUSARIUM BY A HOMOLOGOUS NEUROSPORA GENE. Martin B. Dickman* and John F. Leslie, *Dept. of Plant Path., Univ. of Nebraska, Lincoln 68583, and Dept. of Plant Path., Kansas State Univ., Manhattan 66506.

The nit-2 gene of N. crassa encodes an activator protein that governs overall nitrogen metabolism in the cell. We have used the cloned gene as a heterologous probe to survey various fungal phytopathogens for homologues. A wide variety of genera showed homology as determined by Southern blotting. To further study nitrogen regulation, we have chosen Fusarium spp. Genomic libraries of Fusarium moniliforme and Fusarium sacchari have been constructed. Following screening, we have isolated a 6kb fragment from both fungi with homology to the nit-2 gene from N. crassa. We also used the nit-2 gene to complement a mutant of Fusarium graminearum that is defective in overall nitrogen metabolism. Following transformation colonies were obtained which now could utilize nitrogen sources similar to the wildtype. This suggests that this heterologous regulatory gene is expressed in Fusarium and functions to activate expression of nitrogen regulated genes in this organism.

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STRUCTURAL AND FUNCTIONAL ANALYSES OF TWO β-TUBULIN GENES IN COLLETOTRICHUM GRAMINICOLA. D. G. Panaccione and R. M. Hanau. Dept. of Botany and Plant Pathology, Pudue University, West Lafayette, IN 47907.

In organisms with multiple tubulin genes, expression of individual tubulin genes is often developmentally regulated. Two β-tubulin genes, TUB1 and TUB2, were cloned from Colletotrichum graminicola with the interest of studying their involvement in conidial development. Southern hybridization and DNA sequencing demonstrated that although the two genes are considerably divergent, they both have the capacity to encode β-tubulin. RNA blots indicated that the level of TUB2 message relative to TUB1 message increased in conidiating cultures. To study the functional significance of this, the TUB2 gene was replaced with a truncated copy of the gene by site-specific integrative transformation. Transformants carrying the truncated TUB2 allele did not display any abnormalities in conidial development.

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TRANSFORMATION OF TRICHODERMA SPP. TO HYGROMYCIN B RESISTANCE. A. Sivan, T.E. Stasz, G.E. Harman and M. Hemmat, Dept. of Horticultural Sciences, Cornell Univ., Geneva, NY, 14456.

Trichoderma protoplasts were obtained by digesting mycelium with Novozyme 234. Protoplasts were treated with the plasmid pH1B (obtained from O.C. Yoder) containing an Escherichia coli hygromycin B phosphotransferase gene, which was used with the permission of Eli Lilly & Co., fused to a Cochliobulus heterostrophus promoter. Treated protoplasts were plated in a molten agar medium which later was covered with a second medium layer containing the antibiotic. The application time of the antibiotic was critical and differed between strains. Southern analysis of putative transformants showed integration into the genomic DNA. The frequency of transformed nuclei in hygromycin B resistant isolates was usually lower than 1%. Therefore, nuclei from all putative transformants were allowed to segregate through conidiation. Single spore isolates were obtained that were mitotically stable on selective and non selective media.

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HEAT SHOCK-INDUCED DEVELOPMENT OF INFECTION STRUCTURES BY THE RUST FUNGI: EXPRESSION OF THE INF GENES. S. Bhairi and R.C. Staples, Boyce Thompson Institute, Cornell University, Tower Road, Ithaca, NY 14853.

Germlings of Uromyces appendiculatus induced by exposure to 28.5°C heat for 1.5 hr developed infection structures similar to those induced thigmotropically and at about the same rate. After heat shock, appressorium development was accompanied by the appearance of at least six heat-shock proteins, but synthesis of the thigmotropic-specific proteins which occurs during contact-induced development was not observed. Thigmotropically-induced appressorium development is accompanied by an upshift in the expression of a small group of INF genes, and we have identified six of these by now. Here we have examined the expression of four ds-genes after inducing infection structure development by heat shock. Genes INF56 and INF24 were induced only moderately. INF64 was induced in germlings heat shocked for 4 hr but not when heat shock was applied for only 2 hr. INF88 was not induced.

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RESTRICTION FRAGMENT LENGTH POLYMORPHISMS BETWEEN SOYBEAN AND ADZUKIBEAN PHIALOPHORA GREGATA ISOLATES. L. E. Gray, and A. Hepburn, USDA, Agricultural Research Service, and

Department of Agronomy, University of Illinois, Urbana, IL 61801.

Phialophora gregata causes a vascular disease of soybean and adzukibean. We have been using RFLP analysis to study genome relationships between soybean and adzukibean strains of the fungus. A Library of BamHl fragments from a soybean isolate was made in puC8. Selected clones were then used to probe BamHl and EcoRl digested DNA of both soybean and adzukibean strains of the fungus. In general all soybean isolates show the same hybridization pattern or a band deletion. With adzukibean isolates, with a given soybean isolate probe, different adzukibean isolates show different hybridization patterns. In some cases no hybridization is observed. These results indicate that the adzukibean Phialophora isolates are not closely related to the soybean isolates.

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TRANSFORMATION OF COLLETOTRICHUM GLOEOSPORIOIDES F. SP. AESCHYNOMENE. D.O. TeBeest, and M.B. Dickman. Dept. of Plant Path., Univ. of Arkansas, Fayetteville, AR 72701 and Dept. of Plant Path., Univ. of Nebraska, Lincoln, NE 68583.

Colletotrichum gloeosporioides f. sp. aeschynomene, incitant of an anthracnose on Aeschynomene virginica, was transformed to resistance to MBC (methyl-2-benzimidazole carbamate). Protoplasts were exposed, in the presence of PEG, to the vector, pSV50, encoding a gene for B-tubulin from Neurospora crassa The gene confers resistance to MBC. Transformants were isolated by overlaying regeneration media with nutrient media containing MBC (1 ug/ml). Southern blot hybridizations of genomic DNA from resistant strains confirmed integrative transformation. Resistance to MBC was maintained in all isolates after passage through plants and after serial transfer to non-amended media and thus was mitotically stable. Comparison of a wild-type isolate to transformed isolates suggested transformation reduced aggressiveness and fitness on A. virginica.

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A RESTRICTION FRAGMENT LENGTH POLYMORPHISM MAP OF COCHLIOBOLUS HETEROSTROPHUS. Tzeng, T., C. R. Bronson and C. Ford, Departments of Plant Pathology and Genetics, Iowa State University, Ames, IA 50011.

C. heterostrophus, the causal agent of southern leaf blight of maize, is a model for reseach on how fungi infect plants. We are developing a genetic map of this pathogen to facilitate the cloning of pathogenicity genes and to characterize a chromosome rearrangement hypothesized to be associated with the virulence locus Toxl. Restriction fragment length polymorphisms (RFLPs) and phenotypic markers are being used to construct the map. To date, of 99 markers analyzed, 89 RFLP markers and 4 phenotypic markers have shown significant linkage, indicating the coverage of about 94% of the genome. The map length at present is about 1200 cM. Several differences in chromosome arrangement between the two parents of our cross have been identified. Markers have been found tightly linked to Toxl. These markers should be useful for cloning this pathogenicity gene by chromosome walking.

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CHARACTERIZATION OF DOUBLE-STRANDED RNA IN MEXICAN, EUROPEAN, AND PERUVIAN ISOLATES OF PHYTOPHTHORA INFESTANS. J. R. Newhouse and P. W. Tooley, USDA-ARS, Frederick, MD 21701.

Double-stranded RNA (ds-RNA) recently was found in Mexican isolates of P. infestans. Additional isolates of the fungus from diverse populations were evaluated for ds-RNA. Isolates from Mexico (15, 83%), Europe (6, 29%), and Peru (1, 3%) were positive for ds-RNA, while those from the United States (35) were negative. Both A1 and A2 mating type Mexican isolates contained ds-RNA, but among European isolates, only A2 types were positive. Ten banding patterns were distributed among the isolates tested. Sizes of the bands in kilobase pairs (kbp) were determined by denaturing and electrophoresing the ds-RNA on formaldehyde gels along with known standards. The bands ranged in size from 1.35 to 11.35 kbp, and some Mexican and European isolates were found to have ds-RNA bands of the same size. This preliminary evidence suggests that European A2 mating type isolates of P. infestans may have originated in Mexico.

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APPLICATION OF RESTRICTION FRAGMENT LENGTH POLYMORPHISM ANALYSIS AS A TAXONOMIC TOOL TO DIFFERENTIATE PYRENOPHORA SPECIES. B. M. Baltazar, A.L. Scharen, and V. Raboy, USDA, ARS, Plant Pathology Dept, Montana State University, Bozeman, MT 59717-0002.

Pyrenophora species cause economically important diseases of barley, wheat, rice and other crop species. Little is known concerning the phylogenetic relationships among species in this genus. Restriction fragment length polymorphism (RFLP) analysis provides one way to estimate the evolutionary distance between species. A genomic library was made from P. teres f. sp. maculata DNA in pUC12. Individual clones from this library have been screened to identify those which hybridize to polymorphic sequences among the various species. As an example, a 2.4 kb genomic clone hybridized to a 3.7 kb band in EcoRI digested genomic DNA of P. teres f. sp. maculata, and to 5.0 and 5.9 kb bands in EcoRI digested genomic DNA of P. teres f. sp. teres. This clone did not hybridize to P. graminea or barley genomic DNAs. We plan to employ PCR (polymerase chain reaction) to amplify fungal DNA in infected plant tissue, which might be used to detect and identify Pyrenophora species or subspecies.

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A FAMILY OF CONSERVED REPETITIVE DNA ELEMENTS FROM THE FUNGAL PLANT PATHOGEN *GLOMERELLA CINGULATA* (TELEOMORPH OF COLLETOTRICHUM LINDEMUTHIANUM). R. J. Rodriguez. Dept. of Plant Pathology, Univ. of California, Riverside, CA 92521.

Glomerella cingulata (*Colletotrichum lindemuthianum*), transformed with the hygromycin B phosphotransferase gene, and the DNA flanking the site of integration was isolated for analysis. This flanking DNA contained a sequence which was repeated many times in the genome. The repetitive DNA was dispersed in the genome and showed a high level of conservation of genomic locations among different isolates of this fungus. The sequence of the repeated element consists of tandemly arranged CAX triplets, in which X is most commonly G or A. Sequences of three independently isolated copies of the repeated element indicated that it represents a family of repeats, each varying in length and in the nucleotide representing X in the CAX triplet. A probe representing this family of repeats was found to hybridize with genomic DNAs of several different fungal genera in a species-specific manner. The potential for using this repetitive element in disease diagnoses and identification of fungal species will be discussed.

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QUANTIFICATION OF BLUEBERRY SHOESTRING VIRUS RNA AND ANTIGEN IN ITS APHID VECTOR, Illinois pepperi, DURING ACQUISITION, RETENTION AND TRANSMISSION. B.T. Terhune, D.C. Ramsdell, and K.L. Klomparens. MI State Univ. E.L., MI 48824.

Blueberry shoestring virus (BBSSV) was monitored in late instars of Illinoia pepperi by dot-ELISA, a silver enhanced-colloidal gold-immunosorbent assay, and dot-hybridization. Aphids acquired BBSSV-antigen at a rapid rate during a 12 hr acquisition access period (AAP) from sachets containing BBSSV or BBSSV-infected blueberry plants, but the acquisition rate declined between AAPs of 12 and 96 hr. BBSSV-RNA was acquired at higher levels, and the acquisition rate did not decline over a 4 day AAP. Levels of BBSSV-antigen and RNA retained by aphids declined rapidly 1 day after acquisition, but remained constant during the next 3 to 4 days. BBSSV antigen and RNA were retained after a molt, and both were detected in aphid hemolymph after 1 to 4 day AAPs. Aphids were able to transmit BBSSV to blueberry plants 8 to 10 days after a 24 hr AAP on sachets or BBSSV-infected plants.

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DETECTION OF TOBACCO MOSAIC AND TOBACCO NECROSIS VIRUSES IN WATER USING POSITIVELY CHARGED MEMBRANE FILTERS. V. Jacobi, and J.D. Castello. Faculty of Environmental and Forest Biology, State University of New York, College of Environmental Science and Forestry, Syracuse, NY 13210.

Ten liters of distilled water were seeded with tobacco mosaic virus at 50 ng/ml and passed through Zeta Plus 50 S filter disks (90 mm). Following elution percent virus recovery determined by local lesion assay was 75%. Reducing elution time to 5 min. increased recovery to 90%. Prefiltration through 5, 1, or 0.5 um depth filters reduced recovery to 0-10%. Optimum virus binding to the filter occurs at pH 5.5. 100% recovery of TMV and tobacco necrosis virus (TNV) was demonstrated by ELISA when 10 l of distilled water was seeded with these viruses at 0.1 ng/ml and eluted with 0.5 M NaCl or 0.1 M NaCl (pH 8.0), respectively. This corresponds to a virus detection sensitivity of 1 pg/ml and 2 pg/ml for these two viruses, respectively. This system will be used to recover plant viruses from natural waters.

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SYSTEMIC RESISTANCE TO TOBACCO MOSAIC VIRUS INDUCED IN SUSCEPTIBLE PLANTS AFTER LOCALIZED INFECTIONS BY TOBACCO NECROSIS VIRUS. D. A. Roberts, Univ. of Florida, Gainesville, FL 32611.

The lowest three expanded leaves of plants of Turkish tobacco (Nicotiana tabacum L. 'Samsun'), susceptible to tobacco mosaic virus (TMV) but hypersensitive to tobacco necrosis virus (TNV), were inoculated with TNV. Leaves of comparable control plants were mock inoculated with juice from healthy plants. One week later, two expanded leaves above the TNV-inoculated and mock-inoculated ones were challenge inoculated with TMV. TMV-inoculated leaves and those systemically infected were harvested and frozen, respectively, one and two weeks after the challenge inoculation. Infectivity of TMV in the juice from thawed leaves was assayed by the half-leaf method in Samsun NN Turkish tobacco. In six experiments, infectivity of TMV in TMV-inoculated leaves was significantly ($P = 0.01$) reduced below that in the controls by an average of 19%, in the systemically infected leaves, by 32%. Thus, replication of TMV was slowed in plants of Turkish tobacco previously inoculated with TNV.

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PURIFICATION OF THE RMV AND SGV ISOLATES OF BARLEY YELLOW DWARF VIRUS FOR ANTISERUM PRODUCTION. G.N. Webby and R. M. Lister, Purdue Univ., W. Lafayette, IN 47907, and S. M. Gray, Cornell Univ., Ithaca, NY 14853.

Past attempts to purify the RMV and SGV isolates of barley yellow dwarf virus (typifying those transmitted specifically by Rhopalosiphum maidis and Schizaphis graminis, respectively) have resulted in very poor yields (e.g. less than 50 $\mu g.Kg^{-1}$ plant tissue for SGV). Moreover, production of useful virus-specific antisera has proved difficult due to contaminating plant antigens. We have investigated enhancing yields and purity of these isolates for the production of specific antisera of high titer. Propagation factors examined included host, environmental conditions, harvest time, and plant parts used. For purification, a procedure previously developed at Purdue for other isolates was evaluated and modified for use with RMV and SGV. Improved yields (2-400 $\mu g.Kg^{-1}$) of each were obtained using oat shoots, ground in liquid nitrogen, and extracted by repeated blending in 0.5 M phosphate, pH 6.0. Incorporating macerating enzymes during extraction reduced yields slightly. When injected into rabbits the preparations yielded polyclonal antisera readily capable of discriminating RMV and SGV from other isolates by DAS-ELISA.

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EPITOPE DIVERSITY AMONG CITRUS TRISTEZA VIRUS ISOLATES. S. M. Garnsey[1], T. Kano[2], T. A. Permar[1], M. Cambra[3], M. Koizumi[2], and C. Vela[4]. [1]USDA, ARS, Orlando, FL, [2]Fruit Tree Research Station, MAFF, Okitsu, Japan, [3]Instituto Valenciano Investigaciones Agrarias, Moncada, Spain, and [4]Ingenasa, 41 Hermanos Garcia Nobelas, Madrid, Spain.

At least five distinct epitopes were detected in citrus tristeza virus (CTV) when 28 selected CTV isolates from Japan, Florida, and Brazil were tested against a polyclonal antiserum (PCAS) and four monoclonal antibodies (MCA). IgG from the PCAS was used as trapping antibody, and the Spanish MCAs, 3DF1 and 3CA5, and the Florida MCAs, MCA13 and MCA14, were used as intermediate antibodies in double-sandwich indirect ELISA. Binding specificity of each MCA among the isolates was distinct. None of the four MCAs reacted with all isolates tested, but all isolates reacted to the PCAS in double-antibody sandwich direct ELISA. Many field sources of CTV apparently contain complexes of CTV serotypes.

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CROSS PROTECTION AND RELATIONSHIPS AMONG BARLEY YELLOW DWARF VIRUSES. F. Wen and R. M. Lister. Department of Botany and Plant Pathology, Purdue Univ., W. Lafayette, IN 47907.

Studies on cross protection in barley yellow dwarf viruses (BYDV) using ELISA and cDNA probes (Phytopathology 78: 1587) were extended to include additional isolates representing different serological groups and serotypes. The serotypes were: PAV (non-specifically transmitted by Sitobion avenae and Rhopalosiphum padi); MAV (specifically transmitted by S. avenae), and SGV (specifically transmitted by Schizaphis graminum), regarded as Group 1 serotypes, and RPV (specifically transmitted by R. padi) and RMV (specifically transmitted by R. maidis), regarded as Group 2 serotypes, together with two closely related serotypes derived by subculturing from an MAV source. Cross protection was most efficient and persistent between the two serotypes derived from MAV, and undetectable between RPV and either MAV or PAV. Other combinations also showed that the degrees of cross protection obtained were consistent with serological relationships as indicated by ELISA, and with genomic relationships as indicated by cDNA hybridizations and sequencing information.

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INHIBITION OF TOMATO BUSHY STUNT VIRUS BY DEFECTIVE INTERFERING PARTICLES IN TOBACCO PROTOPLASTS. R. W. Jones, A. O. Jackson and T. J. Morris, Dept. of Plant Pathology, Univ. of California, Berkeley, CA 94720.

Defective interfering (DI) particles of tomato bushy stunt virus (TBSV) are small RNAs generated from genomic RNA, which attenuate disease symptoms. The effect of DI presence on TBSV replication was determined by PEG-mediated protoplast inoculation of TBSV genomic RNA, with or without DI RNA. Protoplasts were derived from Nicotiana edwardsonii, N. benthamiana and N. tabacum. In each tobacco, DI replication, based upon tritiated uridine incorporation, was directly correlated with the quantity of DI inoculum added. Inoculation of equal amounts (1:5 molar ratio) of genomic and DI RNA markedly inhibited TBSV genomic RNA accumulation within 18 hr. In contrast, DI RNA continued to accumulate between 18 and 39 hr and reached a 20-fold greater molar ratio over that of genomic RNA. Data indicates that the disease attenuation found in the presence of DI RNA may be directly related to a suppression of genomic RNA synthesis.

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ANTISERA TO CYTOPLASMIC INCLUSION PROTEINS OF POTYVIRUSES CONTAIN CROSS-REACTIVE ANTIBODIES. John Hammond. USDA-ARS, Florist and Nursery Crops Laboratory, Beltsville, Md. 20705.

Antisera against the cytoplasmic inclusion proteins (CIPs) of sweet potato feathery mottle (SPFMV), iris severe mosaic (ISMV), bean yellow mosaic (BYMV), clover yellow vein (CYVV), and turnip mosaic (TuMV) viruses were used to probe Western blots and dot-blots of potyvirus CIPs. On Western blots antisera to SPFMV, BYMV, CYVV and TuMV CIPs each reacted with 17 potyvirus CIPs; ISMV-CIP antiserum reacted with fewer. Cross-reactivity was much reduced on dot-blots; specific reactions above background were not observed with all sera. The cross-reactivity is presumably due to antibodies reactive with conserved sequences not exposed on the native subunit but accessible on denatured proteins; this would explain the superior activity on Western blots compared to dot-blots, and the greater virus-specificity previously observed by others in gel-diffusion tests with antisera against potyvirus CIPs. Thus potyvirus CIPs and coat proteins may both have virus-specific exterior epitopes, and conserved interior sequences.

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COMPARISON OF ELISA AND DOT BLOT HYBRIDIZATION FOR DETECTING TOMATO RINGSPOT VIRUS IN NECTARINE TISSUE. C.A. Powell, A. Hadidi, and J.M. Halbrendt. AREC, Univ. of Florida, Fort Pierce, FL 34954; USDA, ARS, Beltsville, MD 20705; and Fruit Research Lab, Penn State Univ., Biglerville, PA 17307.

Approximately 1 g of leaf, bark, or root tissue from 20-year-old nectarine trees with symptoms of the Prunus stem pitting (PSP) disease was frozen in liquid nitrogen, triturated with a mortar and pestle, and thawed in PBS. One-half of each sample was analyzed for TmRSV antigen by DAS ELISA. Total nucleic acid was extracted from the remainder of each sample using phenol/chloroform and analyzed for TmRSV-specific RNA by dot blot hybridization with a ^{32}P-labeled cRNA probe. DAS ELISA detected TmRSV in 0 of 17 leaf samples, 8 of 17 bark samples, and 12 of 17 root samples. Dot blot hybridization detected TmRSV RNA in 0 of 17 leaf samples, 17 of 17 bark samples, and 3 of 17 root samples. Total nucleic acid was extracted from bark collected from various locations on two young nectarine trees with PSP. TmRSV RNA was detected by dot blot hybridization from bark where PSP symptoms were visible.

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THEORY OF TESTING FOR SEROLOGICAL IDENTITY IN QUANTITATIVE ELISA. P. M. Burrows and O. W. Barnett. Clemson University, Clemson, South Carolina 29634.

Curves of optical density response to dilutions of antigen preparations are characterized by parametric functions which provide a definition of serological identity for virus isolates in terms of hypothesized parametric identities that can be tested for each antiserum. The necessity of allowing for unknown and different antigen concentrations, in virus preparations before dilution, reduces the number of vulnerable parametric identities by one. Combined analysis of response curves from different antisera changes the definition of serological identity and increases the sensitivity of the test. This method can be validated by 'blind' testing of different preparations of the same virus isolate for which the hypothesis of serological identity should not be rejected.

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SEROLOGICAL NONIDENTITY OF IRIS SEVERE MOSAIC VIRUS AND ITS BEARDED IRIS MOSAIC STRAIN BY QUANTITATIVE ELISA. O. W. Barnett and P. M. Burrows, Clemson University, Clemson, South Carolina 29634.

Bearded iris mosaic virus, once considered a separate virus, now is considered a strain of iris severe mosaic virus. The bulbous iris and bearded iris strains are serologically indistinguishable by enzyme-linked immunosorbent assay (ELISA) in their homologous and heterologous antisera. Serological identity of the strains was tested by comparisons of curves of optical density responses to dilutions of antigen preparations in indirect, double antibody sandwich ELISA. When undiluted and half strength preparations of virus were compared in bulbous iris and bearded iris antisera, the hypothesis of serological identity was not rejected as required for a valid test. When preparations of the two strains were compared in the two antisera in four experiments, the hypothesis of identity was always rejected. Thus by quantitative ELISA comparisons the bulbous iris and bearded iris strains are closely related but serologically nonidentical.

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EFFECTS OF PLANT SAP ON ANTIGEN CONCENTRATIONS CALIBRATED BY ELISA. S. W. Scott, P. M. Burrows, and O. W. Barnett, Clemson University, Clemson, South Carolina 29634.

Calibration of optical density response to standard antigen concentrations enables estimation of unknown antigen concentrations and formulation of detection rules for future samples tested in a quantitative ELISA system. But calibrations performed with purified virus preparations (bean yellow mosaic, clover yellow vein, peanut stunt, red clover mosaic and southern bean mosaic viruses) are not reliable for estimation of concentrations in preparations that include plant sap. Associations of saps with virus can have disruptive or cooperative effects on optical density responses depending on the sources of plant sap. Disruptive associations lead to underestimation of virus concentration while cooperative associations lead to overestimation. Therefore it is necessary to perform ELISA calibrations in sap of the plant species for which the assay is intended subsequently.

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DETECTION OF APPLE SCAR SKIN AND DAPPLE APPLE VIROIDS WITH SP6-GENERATED cRNA PROBES. A. Hadidi, ARS-USDA, Beltsville, Maryland 20705.

Scar skin and dapple diseases are among the most damaging fruit blemishing apple diseases in Japan and China. The occurence of these two viroid diseases in North America or Europe is rare. Recombinant plasmids composed of an pSP 65 vector containing sequences derived from apple scar skin viroid (ASSV) were used to generate high specific activity ^{32}P labeled ASSV cRNA probes by SP6 RNA polymerase. In Northern blot and dot blot hybridization assays, probes hybridized with RNA from ASSV or dapple apple viroid (DAV)-infected, but not uninfected apple tissue. DAV or ASSV was detected from apple seed, fruit, leaf, bark, or root tissue. These assays are rapid, accurate, and sensitive. Testing periods for either viroid can now be reduced from several years by observing the fruit symptoms on grafted woody indicators to a few days by recombinant DNA assay. This advancement will make monitoring ASSV and DAV world-wide possible.

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PARTIAL CHARACTERIZATION OF A VIRUS-LIKE PARTICLE ASSOCIATED WITH A HYPOVIRULENCE IN *Leucostoma* sp.
C. J. P. Jensen and G. C. Adams, Department of Botany and Plant Pathology, Michigan State University, East Lansing, MI, 48824

Abundant isometric virus-like particles were visible in the hyphae of isolate 14.4a, a hypovirulent isolate of *Leucostoma* sp. Our objective was to purify and characterize these particles. Mycelia from 14 to 21 day old liquid cultures were homogenized in 0.1 M phosphate buffer pH 7.0, and centrifuged to remove cellular debris. The particles were precipitated from the supernatant with 8% polyethylene glycol and clarified by differential centrifugation. Spectrophotometric analysis of sucrose and CsCl density gradients revealed two peaks with an absorbance at 254 nm. Only the top peak consistently yielded both protein and nucleic acid. The top peak contained isometric particles with diameters of 40 nm. The particles had a buoyant density in CsCl of 1.313 g/cm^3 and had a sedimentation coefficient of 104S as determined from linear-log sucrose gradients. Extraction of protein from the top peak yielded one major coat protein in SDS-PAGE with a molecular weight of 32,000. Extraction of nucleic acid from the top peak yielded one major segment of dsRNA that is slightly larger than the three dsRNA segments normally associated with the presence of the particles in hyphae.

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DEMONSTRATION OF THE SATELLITE NATURE OF VIRUS-LIKE PARTICLES ASSOCIATED WITH MAIZE WHITE LINE MOSAIC VIRUS. L. Zhang, T. A. Zitter, and P. F. Palukaitis, Department of Plant Pathology, Cornell University, Ithaca, NY 14853.

Virus-like particles (17 nm diam.) associated with maize white line mosaic virus (MWLMV, 35 nm diam.) were separated from MWLMV by two cycles of 10-40% sucrose gradient centrifugation. A dilution series of both virus-like particles and MWLMV preparations was made and used separately to inoculate sweet corn. No plants inoculated with the virus-like particles developed symptoms and no virus-like particles could be detected. In almost every case the virus-like particle RNA was detected in plants inoculated with MWLMV. These results show that the virus-like particles associated with MWLMV cannot infect plants without MWLMV and hence are a satellite virus of MWLMV (SV-MWLMV).

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WHY UREDINIOSPORE GERM TUBES OF PUCCINIA SORGHI DO NOT ADHERE TO MAIZE LEAVES WITHOUT EPICUTICULAR WAX. R. Chaubal, V. A. Wilmot, and W. K. Wynn, Plant Pathology Department, University of Georgia, Athens 30602.

Germinating urediniospores of most cereal rusts are appressed to leaves with epicuticular wax but not to those without wax. To understand this phenomenon, the extracellular mucilage produced by germ tubes of Puccinia sorghi was visualized ultrastructurally after adding cationic compounds (cetylpyridinium chloride, ruthenium red) to conventional fixation solutions. On waxy leaves the mucilage flowed from the lower surface of the germ tubes into the spaces around the wax projections, anchoring the fungus to the jagged cuticle. On leaves without epicuticular wax, the adhesive material spread to the outside of the germ tubes but was not present beneath the fungus to attach it to the smooth cuticle. The role of the mucilage in adherence was confirmed by treatments with dilute alkalies, pronase E, and laminarinase which removed the mucilage and also detached germ tubes from artificial surfaces.

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INFECTION PROCESS OF CERCOSPORA ARACHIDICOLA ON PEANUT LEAVES. H. A. Melouk, and S. S. Aboshosha. USDA-ARS, Dept. of Plant Pathology, Oklahoma State Univ., Stillwater, OK 74078-9947, and Dept. of Plant Pathology, College of Agriculture, Alexandria, Egypt.

Leaflets of peanut Tamnut 74 (susceptible) and PI 276235 (resistant) were inoculated with C. arachidicola (CA) by applying 0.1 ml conidial suspension ($2X10^4$ /ml) on adaxial surface. Leaflets were incubated on moist filter paper in petri plates at 25±1C in darkness. Samples were collected at various times after inoculation, and processed for SEM. CA conidia germinated after 24 hr on both genotypes. Germ tubes did not enter open stomata of Tamnut 74. Smooth surfaced, round or club shaped appressoria formed on mycelia of CA on both genotypes within 3 days. Infection pegs emerged from the appressoria at 4 to 5 days on Tamnut 74. Most appressoria on PI 276235 collapsed and disintegrated at 4 to 6 days incubation. Infection of peanut leaves by CA appears to occur by inter-stomatal penetration.

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FIELD TEST FOR PHYSIOLOGIC SPECIALIZATION AMONG ISOLATES OF THE SOYBEAN STEM CANKER DISEASE PATHOGEN. B. L. Keeling, USDA-ARS, Jamie Whitten Delta States Research Center, Stoneville, MS 38776

The virulence of twenty four isolates of Diaporthe Phaseolorum var. caulivora isolated from soybean plants symptomatic of the stem canker disease in 1979, 1983, 1984, and 1986 was measured and compared. Virulence of the isolates was accessed by measuring lesion development 30 days after inoculating 60-day old field grown plants of the cultivars Tracy-M, S-100, Arksoy, Centennial, and J77-339 using infested toothpicks. Results demonstrate a wide difference in isolate virulence from very weakly pathogenic to very virulent. The virulence of isolates to different cultivars do not support a hypothesis of physiologic specialization among isolates.

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NUCLEAR NUMBER AND BEHAVIOR IN SCLEROTINIA SCLEROTIORUM. E. J. Ford, D. C. Sands, and K. Adkisson, Dept. of Plant Pathology, Montana State University, Bozeman, MT 59717.

Fluorescent microscopy was used to study nuclear number and behavior in asci, ascospores, germinating ascospores, hyphae, protoplasts and microconidia of S. sclerotiorum. Specimens were stained with hydroethidine, DAPI, propidium iodide, Hoerst, and calcofluor either singly or in various combinations to reveal details of nuclear number, meiosis, mitosis, and cell wall development. Classical patterns of meiosis were observed in asci with 8 nuclei being formed followed by wall formation and then a final nuclear division to yield two nuclei per mature ascospore. Nuclear division in ascospores was evident after 2 hrs incubation in a nutrient broth at room temperature, which was prior to germination of the ascospores. Young sporelings had up to 16 nuclei prior to cross wall formation. A nuclear generation time of ca. 2.4 hrs was determined for sporelings. Hyphae from young shake cultures had 20-30 nuclei/cell. Spheroplasts formed from active hyphae averaged 3.5 nuclei per spheroplast. Microconidia were uninucleate.

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ON HOST FAMILIES AND GENERA OF PERONOSPORA AND PLASMOPARA DOWNY MILDEWS AND ON GEOGRAPHIC PROCLIVITIES OF THE PATHOGENS. R.Kenneth, Hebrew Univ. Fac. of Agric., Rehovot 76100, Israel

Data from host-downy mildew (dm) check-lists provided information on the two most common genera of Peronosporaceae. Of the ca 51 host families with Peronospora, 8 are stricken in single countries only; of the 22 with Plasmopara, at least 4. Despite a dearth of dms of these genera in the tropics, a few thrive e.g. Plasmopara on Vitaceae and Peronospora on Euphorbiaceae, the latter unrecorded in cold regions. Australia and New Zealand have few dms: 3 and 10 host families respectively with Peronospora and 1 and 2 with Plasmopara (vs 34 in Romania with Peronospora and ca 17 in USA and 9 in Canada with Plasmopara). Although Plasmopara is recorded on only 7 families in Britain and 8 in France, there are 20 and 26 host genera of Umbelliferae vs only 4 and 2 in USA and Canada (and none in the east Mediterranean Basin). For Plasmopara on Asteraceae, however, there are ca 20 host genera in USA vs none in Britain and 4 in France. Of the 33 legume genera with Peronospora, 8 are in single countries only e.g. Cicer in Israel, Vigna in USA.

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SCLEROTINIA BLIGHT OF PEANUT IN TEXAS: OCCURRENCE AND DETECTION OF THE PATHOGEN IN SEED. R.A. Taber, D.H. Smith, J. S. Neck, S.L. Segner, D.M. Porter, D.H. Lewis, and T.M. Omran. Dept. Plant Path. & Micro., Tx. Agri. Exp. Sta., Texas A&M University, College Station and Yoakum, Tx.; USDA, ARS, Suffolk, Va.; and Vet. Micro., TAMU, College Station, Tx. 77843

Peanut Sclerotinia blight was first observed in Texas in Mason County in 1981 on cv. 'Florunner'. Since 1981 it has been observed in other counties in Texas. The role of seed in propagule dispersal is currently under study. Sclerotia were observed in infested seed; however, the incidence was less than 1%. In spite of seed shriveling and discoloration, germinability and emergence from infested seed exceeded 90%. Presence of the pathogen was successfully detected in seed using the Agri-Diagnostics Immunoassay Kit and serial sectioning. The pathogen was most prevalent in the peanut testa. Fluorescein isothiocyanate-labeled monoclonal antibodies developed from an isolate of Sclerotinia from Texas peanuts permitted localization and verification of specific hyphae of the pathogen in the seed with the aid of immunofluorescence.

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INFLUENCE OF SPRAY SCHEDULES ON RESISTANT POPULATIONS OF BOTRYTIS CINEREA. M.L. GULLINO, C. ALOI and A. GARIBALDI Istituto di Patologia vegetale, Via Giuria 15, 10126 Torino, Italy.

In the presence of benzimidazole and dicarboximide resistant strains of Botrytis cinerea Pers. and of high disease incidence, treatments with dicarboximides provided only partial control of grey mould on grape, strawberry and tomato and increased the percent of dicarboximide resistant strains. The combination of a benzimidazole with diethofencarb (alternated or not with a dicarboximide) provided satisfactory control of grey mould and decreased the percent of benzimidazole resistant strains. The mixture of procymidone with thiram controlled grey mould of grape, while the combination of procymidone and chlorothalonil was only partially effective on strawberry. Both mixtures increased dicarboximide resistance. Thiram or chlorothalonil used alone did not provide satisfactory grey mould control, but did decrease the incidence of dicarboximide resistant strains.

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HYBRID PERFORMANCE AND YIELD LOSSES ASSOCIATED WITH GRAY LEAF SPOT DISEASE OF CORN IN VIRGINIA. P. J. Donahue and E. L. Stromberg, Departments of Agronomy and Plant Pathology, Physiology and Weed Science, VPI&SU, Blacksburg, VA 24061-0331.

Commercial corn hybrids were evaluated for 7 years at Wythe and Shenandoah Counties and 1 year at Montgomery Co. under field conditions for response to gray leaf spot disease, caused by Cercospora zeae-maydis. Plants were scored 3 times during the growing season. Disease severity indices were regressed against grain yield and harvest moisture, and lodging by location and year. Significant grain loss due to disease occurred in 2 of 6 years at the Shenandoah Co. site and 1 of 6 years at the Wythe Co. site. A significant increase in lodging occurred in 2 of 6 years at the Shenandoah Co. site and 5 of 6 years at the Wythe Co. site. These data represent the effects of C zeae-maydis on a range of genotypes differing in yield potential. The fact that significant associations occurred indicate the strong effect gray leaf spot can have when environmental conditions are favorable.

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EVALUATION OF METHODS FOR SAMPLING, RECOVERY AND ENUMERATION OF BACTERIA APPLIED TO THE PHYLLOSPHERE. C. A. Matyac[1], K. Donegan[1], R. Seidler[2], V. Prince[1] and A. Porteus[2]. N.S.I. Technology Services[1] and E.P.A.[2], Corvallis Environmental Research Laboratory, Corvallis, OR. 97333.

Erwinia herbicola or Enterobacter cloacae were sprayed on oat or bean leaves. Bacterial counts from single leaf and bulk leaf samples were similar 1-7 days after application, but after 14-35 days counts from bulk samples were significantly larger. Bulk sample values could be adjusted to those of single leaves using estimates of the single leaf variance. More bacteria were removed by stomacher blending than sonication, blending, or washing leaves in buffer. Stomacher blending was reliable over a wide range of bacterial populations and when 2-100% of the leaf sample carried bacteria. The surface drop technique for enumeration of bacteria uses four 10 ul aliquots and showed no differences from the 100 ul aliquot spread plate method. These methods proved to be rapid, efficient and precise in estimating parameters of bacterial populations.

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DIFFERENTIATION OF TOMATO RACES 1 AND 2 OF VERTICILLIUM DAHLIAE USING VEGETATIVE COMPATIBILITY ANALYSIS. I. R. Joaquim and R. C. Rowe, Dept. of Plant Pathology and W. A. Erb, Dept. of Horticulture, Ohio State Univ., Wooster, OH 44691.

Sixteen strains of Verticillium dahliae, designated as either tomato races 1 or 2, were tested for vegetative compatibility. Compatibility was assessed by pairing complementary, nitrate-nonutilizing (nit) mutants derived from each strain with nit mutants of tester strains representing several vegetative compatibility groups (VCGs) (Phytopathology 73:1305-1308). Nit mutants were isolated from each wild-type strain by selecting for chlorate resistance on corn meal agar with dextrose (Difco)

amended with 25 g/L of $KClO_4$. Preliminary results indicate that all 12 race 1 strains from Ohio, North Carolina, Japan, Canada and Australia belong to the same VCG. The remaining four race 2 strains from North Carolina and Australia were in a distinctly separate VCG. Vegetative compatibility analysis may be a useful tool for rapid differentiation of these races of V. dahliae in place of pathogenicity tests.

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PARTIALLY AUTOMATED BIOASSAY TO DETECT SPORANGIUM GERMINATION AND GROWTH IN PYTHIUM ULTIMUM AND GROWTH IN RHIZOCTONIA SOLANI. K. K. Kim, R. D. Lumsden, and S. Mischke. USDA-ARS, Biocontrol of Plant Diseases Laboratory, Beltsville, MD 20705.

In vitro assays for sporangium germination and growth of Pythium ultimum and growth of Rhizoctonia solani following exposure to metabolites of Gliocladium virens in fermentation extracts were developed. The assays used a Bio-Tek model EL-307 ELISA plate reader or a Titertek Fluoroskan II. The bioassays were carried out in liquid medium (100-200µl) in each well of 96 well plates after incubation for 24 hr. Sample density was measured with the EL-307. Also, the samples were incubated for additional time periods with treatments. Aliquots of fluorescein diacetate were added and fluorescence was determined with the Fluoroskan II. These bioassays results correlated well with those obtained from microscopic counts of P. ultimum germination and with visual observation of growth 2 days later for P. ultimum and R. solani.

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COMPARISON, USE AND MANUFACTURE OF A CYLINDRICAL IMPEDANCE LEAF WETNESS SENSOR. R.E. Pitblado, and T.J. Gillespie, RCAT, Ridgetown, NOP 2CO and University of Guelph, N1G 2W1, Ontario, Canada.

Development of weather-timed fungicide spray programs in vegetable crops depend on the accuracy and ease of recording critical weather parameters. Leaf wetness duration has been identified as one of those essential elements. Commercially manufactured instruments for recording surface wetness are available ranging from the mechanical types to electircal-resistance sensors. The need for rapid recovery and use of timely data has shifted interest towards the electrical-resistance sensors which can be connected to electronic recording devices for real time data analysis. In the development of TOM-CAST a weather-timed fungicide spray program for field tomatoes a comparison was made between several flat-plate and cylindrical impedance sensors. The method of manufacturing the cylindrical impedance leaf wetness used for TOM-CAST will be described.

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AN IMPROVED SELECTIVE MEDIUM FOR ISOLATION OF GAEUMANNOMYCES-LIKE FUNGI. M. L. Elliott, University of Florida, Fort Lauderdale Research and Education Center, Fort Lauderdale, Fl. 33314

A medium has been developed for the isolation and differentiation of Gaeumannomyces-like fungi from plant root tissue. It is based on medium SM-GGT3 (Juhnke et al. 1984. Plant Disease 68:233-236) and is composed of 500 mg L-β-3,4-dihydroxyphenylalanine (L-DOPA), 100 mg streptomycin sulfate, 10 mg metalaxyl, 10 mg dicloran, 10 mg flutolanil, 10 mg vinclozolin and 1 mg CGA-449 in 1 liter potato dextrose agar. Thus far, the medium has been used to isolate G. incrustans and Magnaporthe poae from bermudagrass roots. Based on pure culture studies, the medium should also be useful in the isolation of Phialophora spp., the G. graminis group and other related fungi associated with cereal grains or turfgrasses.

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LONG-TERM EFFECT OF SOIL SOLARIZATION ON CONTROLLING ROOT-KNOT NEMATODES IN VEGETABLES. C. Stevens, V. A. Khan, A. Y. Tang, C. Bonsi and M. A. Wilson, Dept. of Agricultural Sciences, Tuskegee University, Tuskegee, AL 36088.

A three year study involving solar heating of soil (soil solarization) with polyethylene mulch demonstrated for two years control of root knot nematodes (Meloidogyne incognita). The population of M. incognita was reduced >90% in the 0-30 cm depth of solarized soil from 1985 vegetables crops i.e Vates collard greens, Market Topper cabbages, Early Sprouting Calabbrese broccoli and Georgia Jet sweet potatoes. The number of eggs per gram root recovered and the root gall index from these crops were reduced (92 - 98%) by soil solarization. Growth and yield of these crops were enhanced in solarized soil. The beneficial effects of solarization was observed in the second year following two additional cropping cycles of collard greens and sweet potatoes.

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EFFECT OF THE BASIDIOMYCETE ATHELIA BOMBACINA ON DEVELOPMENT OF VENTURIA INAEQUALIS PSEUDOTHECIA. C. S. Young and J. H. Andrews. Department of Plant Pathology, University of Wisconsin-Madison, 1630 Linden Drive, Madison, WI 53706

McIntosh apple leaves, naturally infected with V. inaequalis, were inoculated with the antagonist A. bombacina, incubated in an orchard from November 1986 to May 1987 and sampled monthly. An immunochemical stain based on polyclonal antibodies to A. bombacina was sufficiently specific to detect hyphae of the fungus in situ. A. bombacina grew endophytically and epiphytically. It did not prevent growth of V. inaequalis hyphae into the interior of leaves, or initiation of pseudothecia. There was no spatial association between hyphae of the two fungi, nor any sign of direct parasitism of hyphae or pseudothecia of V. inaequalis. Pseudothecia in leaves inoculated with the antagonist did not mature further than to produce pseudoparaphyses. Pseudothecia in leaves without the antagonist developed asci and enlarged normally.

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A PUTATIVE NULL GENE OF *PSEUDOMONAS SYRINGAE* PV. *SYRINGAE* BLOCKS PATHOGENICITY ON TOMATO. D. L. McCoy and J. V. Leary. Dept. of Plant Pathology, Univ. of California, Riverside, CA 92521.

A cosmid clone containing an approximately 22 kb insert of chromosomal DNA from a strain of *Pseudomonas syringae* pv. *syringae* which gives a null response on tomato was moved by triparental mating into a strain which is strongly pathogenic on tomato. When the transconjugant was inoculated to tomato, variety Pakmor, the pathogenicity was greatly reduced or absent. The *in planta* growth of the transconjugant was significantly reduced when assayed over 5 days post-inoculation. Two restriction fragments of 12.4 kb and 8.6 kb were ligated into pLAFR5, transformed into *E. coli* HB101 and mobilized into the pathogenic tomato strain. Each of the subclones blocked pathogenesis and restricted the growth of the transconjugants in Pakmor tomato. The intact cosmid clone and the 12.4 kb subclone did not inhibit the pathogenicity of the transconjugants on Faba bean, a host susceptible to both strains.

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MOLECULAR BASIS OF IAA PRODUCTION IN ERWINIA HERBICOLA pv. GYPSOPHILAE. S. Manulis[1], E.M. Clark[2], Y. Ophir[2], I. Barash[1] and Y. Gafni[2] Depts of Plant Pathology[1] and Plant Genetics[2], ARO, The Volcani Center, Bet Dagan 50250, Israel.

Erwinia herbicola pv. gypsophilae (Ehg) is a bacterial phytopathogen which generates galls on the ornamental Gypsophila paniculata. A correlation between IAA production in culture and gall formation was found. Twenty two isolates of Ehg were examined for their plasmids content and found to contain between 1 to 4 plasmids. A DNA fragment that exhibits homology to the IAA genes of Pseudomonas savastanoi was cloned

from a lambda library of Ehg (isolate 713) plasmid DNA. A 7.5 kb fragment was further subcloned into pUC118 plasmid in both orientations. Both plasmids direct IAA biosynthesis in transformed E. coli cells, as can be seen on TLC. Two new proteins were shown to be made by these plasmids in a minicell system.

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GENETIC ANALYSIS OF SYRINGOTOXIN, (ST), PRODUCTION IN PSEUDOMONAS SYRINGAE PV. SYRINGAE (PSS) STRAIN B457. R.O. Nordeen, G. Somlyai and A.K. Chatterjee, Department of Plant Pathology, University of Missouri, Columbia, MO 65211, U.S.A.

Previous analysis of 11 ST⁻ Tn5 mutants of B457 suggested that the insertions mapped to adjacent 21.8 and 10.1 kb EcoRI fragments. The wild-type alleles were isolated by complementing the ST⁻ mutants with a cosmid (pSF6) library of the Pss (B452) genome. Clone pNC1021 complemented 10 of the 11 mutants. This observation and homology between restriction sites in the pNC1021 insert and flanking regions of the Tn5 insertions associate a contiguous stretch of about 32 kb DNA in ST production. Tn3 HoHo mutagenesis of pNC1021 resulted in the isolation of over 200 lacZ fusions. ß-galactosidase activity specified by 21 of these in B457, and the position of the insertions in pNC1021, suggest the presence of two promoters separated by approximately 15 kb DNA. ß-galactosidase assays of these promoter fusions in potato dextrose broth, syringomycin minimal (SRM) and minimal salts media indicated maximum activity in SRM.

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CO-REGULATION OF pTIC58 CONJUGAL TRANSFER AND OPINE CATABOLIC FUNCTIONS. S. Beck von Bodman, G. T. Hayman, and S. K. Farrand. Department of Plant Pathology, University of Illinois, Urbana, Il. 61801.

Conjugal transfer of *Agrobacterium tumefaciens* Ti plasmid pTiC58 is induced by agrocinopines A and B, two opines produced by crown gall tumors incited by strain C58. These compounds also induce the *acc* locus which encodes their catabolism and sensitivity to agrocin 84. A fragment of pTiC58 was subcloned that represses both functions when *in trans* to an *acc*-constitutive, Tra-constitutive mutant of pTiC58. The gene encoding this activity was mapped to a 2 kb fragment located within the 3 kb region separating Tra region I from *acc*. *Trans*-repression of the two phenotypes is relieved by addition of the conjugal opines. A *cis*-acting region within TraI was identified, which, when mutated, also leads to constitutive conjugal transfer. In these mutants *acc* remains inducible. Genetic analysis shows this mutation to be *cis*-dominant. These results show that the opine plant signals regulate Ti plasmid conjugal transfer through a single repressor that acts on Tra and agrocinopine catabolic operons.

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CHARACTERIZATION OF avrl0, AN AVIRULENCE GENE ISOLATED FROM XANTHOMONAS CAMPESTRIS PV. ORYZAE. S. Kelemu, F.F. White, M. L. Ryba-White, and J. E. Leach. Department of Plant Pathology, Kansas State University, Manhattan, KS 66506 U.S.A.

An avirulence gene from a race 2 isolate of Xanthomonas campestris pv. oryzae altered the phenotype of race 1 and race 6 transconjugants in rice cultivar Cas 209 (Xa-10 resistance gene) from compatible to incompatible. The gene was located to a 2.5 kb fragment. Southern analysis with the 2.5 kb fragment revealed sequence similarity to all races of X. c. pv. oryzae and other pathovars of X. campestris. Other species of Xanthomonas and Pseudomonas did not show sequence similarity by DNA hybridization. A second clone (pSK11-33) from the race 2 strain conferred incompatibility to Cas 209 when present in the race 1 stains. The cloned DNA did not hybridize with the avrl0 clone. Preliminary data indicate that a near isogenic rice line containing Xa-10 is susceptible to the race 1 strain with pSK11-33. This data suggest that Cas 209 contains a previously unidentified resistance gene. The sequence data for avrl0 will also be discussed.

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MOLECULAR CHARACTERIZATION OF A GENE THAT REGULATES VIRULENCE AND EXTRACELLULAR POLYSACCHARIDE (EPS) SYNTHESIS IN PSEUDOMONAS SOLANACEARUM. Y. Huang and L. Sequeira, Dept. Plant Path., 1630 Linden Dr., U.W.-Madison, Madison, WI 53706.

An 8 kb DNA fragment from P. solanacearum that specifies both virulence and EPS biosynthesis was identified from genomic libraries of the wild-type strain K60 (race 1) (Vir⁺, EPS⁺) and the spontaneous mutant B1 (Vir⁻, EPS⁻). The 8 kb fragment was cloned into pLAFR3 to yield the plasmid pBE6. When conjugated into strain K60, pBE6 caused loss of both virulence and EPS production. Mutagenesis of pBE6 with Tn3-GUS indicated a functional DNA region of approximately 1.0 kb. The direction of transcription of the gene was determined; the 1.0 kb fragment encoded a protein of about 25.5 kDa in maxicell assays. The results suggest that over-expression of this gene in P. solanacearum has a negative regulatory effect on both virulence and EPS production.

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MONOCLONAL ANTIBODIES (MAS) AGAINST XANTHOMONAS CAMPESTRIS PVS. BEGONIAE (XCB) AND PELARGONII (XCP). J. B. Jones, GCREC, 5007 60th St. E., Bradenton, FL, 34203, J.W.L. Van Vuurde, IPO, The Netherlands, and A. Karu, Univ. of California, Berkeley.

In an attempt to produce highly specific MAS, different antigen preparations (whole cell or two membrane fractions) from XCB and XCP were injected into two Balb/c and B10.Q mice. The M2 fraction, a mixture of inner and outer membranes, responded better serologically than the outer membrane fraction and was compared with the whole cell antigen for production of MAS. Balb/c and B10.Q mice responded best to whole cell and M2 antigens, respectively, and were used in the fusion. Resulting hybridoma supernates were screened against the antigen preparations using ELISA. Supernates with high specificity were observed from the XCB whole cell preparation, but not from either M2 or XCP whole cell preparations. ELISA was more sensitive than Indirect Immunofluorescence for detecting positive supernates in secondary screening. Whole cell preparations produced more specific MAS than the M2 fraction.

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CHARACTERIZATION AND CLONING OF ZINC RESISTANCE FROM PSEUDOMONAS FLUORESCENS. D. Kobayashi, A. Moayeri and T. Suslow. DNA Plant Technology, 6701 San Pablo Avenue, Oakland, CA 94608.

Foliar applications of agrichemicals containing Zn or Cu ions reduce populations of epiphytic bacteria. Strains of Pseudomonas fluorescens originally isolated from almonds were shown to be resistant to $ZnSO_4$ and $CuSO_4$ at concentrations up to 2.5 mM in casitone-yeast extract media (CYE). Pre-exposure of strains to subinhibitory concentrations of Zn or Cu induced growth in CYE broth at normally inhibitory levels of these heavy metals. Strain 484AL was selected for subsequent studies to determine the genetic basis of the resistant phenotype. A genomic library was constructed in the cosmid vector pLAFR3 and maintained in E. coli. Several cosmid clones were identified which conferred novel resistance to $ZnSO_4$ in the E. coli host. These cosmids, when mobilized into various Zn^S P. fluorescens and P. syringae conferred resistance to $ZnSO_4$ at concentrations up to 1.0mM. One cosmid was further subcloned to a 4 kb EcoR1-Kpn1 fragment. Cloned Zn^R from the original or subcloned fragments did not confer Cu^R in E. coli nor Pseudomonas.

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A LOCUS REQUIRED FOR LESION FORMATION BY *PSEUDOMONAS SYRINGAE* PV. *SYRINGAE* ON BEAN AFFECTS SYRINGOMYCIN PRODUCTION *IN VITRO*. E. M. Hrabak[1], J. J. Rich[1], C. J. Kennedy[1] & D. K. Willis[1,2], Dept. of Plant Pathology[1] & ARS-USDA[2], Univ. of Wisconsin-Madison, 53706.

Pseudomonas syringae pv. *syringae* B728a is a causal agent of bacterial brown spot of bean (*Phaseolus vulgaris*). We are analyzing a genetic locus, designated as *lemA*, required for lesion formation on pods and leaves of bean. A mutation in the *lemA* locus does not affect colonization of bean leaves or the ability to incite the hypersensitive response on the non-host tobacco (Phytopathology 75:1320). In an effort to identify the functional product(s) affected by *lemA*, the wild-type strain, B728a, and mutant derivative strain, NUVS1 (*lemA1*::Tn*5*), were bioassayed for syringomycin (SR) production *in vitro*. B728a produced SR *in vitro*, but NUVS1 did not. Additional Tn*5*-induced SR⁻ mutants of B728a fell into three classes: pathogenic, non-pathogenic, and intermediate. The fact that some SR⁻ mutants were capable of causing brown spot symptoms indistinguishable from those induced by the wild-type suggests that SR production is not required for lesion formation.

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GENETIC AND DNA SEQUENCE ANALYSIS OF THE INTERVAL BETWEEN TWO DIVERGENTLY TRANSCRIBED PHYTOTOXIN BIOSYNTHESIS GENES FROM *PSEUDOMONAS SYRINGAE* PV. *SYRINGAE*. N. B. Quigley, Y.-Y. Mo, and D. G. Gross, Department of Plant Pathology, Washington State University, Pullman, WA 99164-6430.

P. s. syringae genes involved in syringomycin biosynthesis have been subcloned on cosmids and mapped by Tn3HoHo1 mutagenesis.

lacZ gene fusions have been used to determine transcriptional direction and to demonstrate that certain plant extracts induce expression of some of these genes. Tn*5* insertions in one gene (*syrD*) were found to prevent transcriptional induction of *lacZ* fusions in an adjacent gene (*syrB*). It is thought that the *syrD* product positively regulates expression of the *syrB* gene. The *syrB* and *syrD* genes map approximately 1 kb apart and are transcribed divergently. A fragment carrying the 5' ends of both genes and the DNA between them has been subcloned and sequenced. The important features of this intergenic interval and the regulatory interaction between the *syrB* and *syrD* genes will be discussed.

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CONSTRUCTION OF LACZ FUSIONS WITH COPPER RESISTANCE GENES OF PSEUDOMONAS SYRINGAE PV. TOMATO AND CELLULAR LOCALIZATION OF PROTEIN PRODUCTS. J.-S. Cha and D. A. Cooksey. Dept. of Plant Pathology, University of California, Riverside, CA 92521.

LacZ fusions were constructed with each of the four open reading frames (ORFs) of the copper resistance operon of pPT23D by subcloning into the lacZ translational fusion vectors pUR190-192. Antibodies were raised to the lacZ fusion proteins obtained from Escherichia coli clones. Western blot analysis of cytoplasmic, periplasmic, and membrane fractions of P. s tomato and P. s. syringae containing the cloned copper resistance genes indicated that the products of the first two ORFs of the operon are periplasmic proteins. These proteins were only detected when cells were grown in media supplemented with cupric sulfate. An additional copper-inducible periplasmic protein encoded by chromosomal genes was detected in P. s. tomato using antiserum raised to the lacZ-ORFA fusion product, but this protein was not detected in P. s. syringae.

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HOMOLOGY BETWEEN THE COPPER RESISTANCE OPERON OF PSEUDOMONAS SYRINGAE PV. TOMATO AND PLASMIDS IN COPPER-RESISTANT STRAINS OF XANTHOMONAS CAMPESTRIS PV. VESICATORIA and ERWINIA HERBICOLA. D. A. Cooksey and H. R. Azad. Department of Plant Pathology, University of California, Riverside, CA 92521.

Copper-resistant strains of Xanthomonas campestris pv. vesicatoria and Erwinia herbicola were isolated from a tomato leaf sample with bacterial spot disease. The X. c. vesicatoria strain grew on media supplemented with up to 1.5 mM cupric sulfate, and the E. herbicola isolate grew on media with 2.6 mM cupric sulfate. Southern blot experiments showed homology between the copper resistance operon of Pseudomonas syringae pv. tomato and a 100 kilobase plasmid in the X. c. vesicatoria strain. A larger plasmid of about 200 kilobases in the E. herbicola strain hybridized with the P. s. tomato copper resistance operon. No homology was detected between the P. s. tomato copper resistance operon and DNA of copper-sensitive strains of either X. c. vesicatoria or E. herbicola.

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REGULATION OF AVIRULENCE GENE D (avrD) FROM *PSEUDOMONAS SYRINGAE* PV. *TOMATO* STUDIED USING A NOVEL Tn7-LUX SYSTEM. H. Shen and N. T. Keen. Dept. of Plant Pathology, University of California, Riverside, CA 92521.

An expression vector was constructed for investigating the regulation of avirulence genes from *Pseudomonas syringae*. A promoterless bacterial luciferase (Lux) operon was made as a reporter system and flanked by Tn7 border sequences which permit its stable, single copy insertion into the bacterial chromosome [Barry, Gene 71 (1988)]. The promoter regions of avrD from *P.s.* pv. *tomato* and its homolog from *P.s.* pv. *glycinea* (*Psg*) R4 were transcriptionally fused to the Lux operon. These constructs were then inserted into the *Psg*R4 chromosome. Little or no light was produced when the cells were grown in rich or minimal culture media, but high level expression was induced from both promoters when the cells were inoculated into soybean leaves. Preliminary results have shown that addition of soybean leaf intercellular fluids to minimal culture medium also induces Lux expression. These results indicate that the avrD gene is plant inducible, possibly by specific plant constituents.

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CHARACTERIZATION OF INDIGENOUS PLASMIDS IN PSEUDOMONAS SYRINGAE PV. MORSPRUNORUM AND P. S. PV. SYRINGAE FROM CHERRY. J.M. Paterson and A.L. Jones. Department of Botany and Plant Pathology. Michigan State University, East Lansing, MI 48824.

Pseudomonas syringae pv. syringae (Pss) and P. s. pv. morsprunorum (Psm) from cherry orchards in Michigan were examined for plasmid diversity. Psm isolates contained 4-8 plasmids and Pss isolates contained 0-2 plasmids. Plasmid DNA ranged from 25-121 kilo basepairs among the isolates. Plasmid profiles of Psm isolates were found to vary both within and among orchard locations, and up to five distinct profiles were identified among Psm isolates from one orchard. Enumeration and molecular weight estimates were used to further characterize plasmid DNA content. Sequence homology among plasmids from Psm and Pss isolates were demonstrated with DNA/DNA hybridizations.

351 Withdrawn

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ASSESSING LATE LEAFSPOT DISEASE OF PEANUT WITH A MULTISPECTRAL RADIOMETER. F. M. Shokes, D. W. Gorbet, and F. W. Nutter. N. Fla. Res. and Educ. Ctr., University of FL, Quincy, FL; Dept. of Plant Pathology, University of Georgia, Athens, GA 30602.

In 1986 and 1987 a multispectral radiometer was tested for assessment of the severity of late leafspot on peanut (Arachis hypogaea) cultivars and breeding lines. A total of 19 tests were compared with varying levels of disease within tests. At least four assessments were made using visual ratings (1-10 scale) and radiometer ratings (800 nm band). Correlation coefficients were calculated for visual vs radiometer ratings, ratings vs defoliation, and ratings vs yield. Correlations increased with disease severity. Visual ratings were highly correlated with radiometer ratings (-0.85 to -0.94) by late September. From results of these tests the radiometer appears to be as effective as visual ratings for determining severity of late leafspot disease of peanut. It has an added advantage of greater precision of measurement as was evidenced by lower coefficients of variation than were obtained with visual assessments.

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RELATIONSHIP BETWEEN PEANUT LEAFSPOT-INDUCED YIELD LOSS AND HEALTHY LEAF AREA DURATION (HAD). V. M. Aquino, R. D. Berger, F. M. Shokes and D. W. Gorbet. Department of Plant Pathology, University of Florida, Gainesville, 32611; N. Florida Res. and Educ. Center, Quincy 32351.

A field trial was conducted in Marianna, Florida to test the hypothesis that healthy leaf area duration (HAD) predicts yield loss due to late leafspot disease. The peanut cultivar Florunner was used with spray treatments of chlorothalonil to establish different levels of disease. Treatments (14-day interval sprays were; i) beginning 35 days after planting, ii) an unsprayed control, and iii) four treatments corresponding to 0.13X, 0.25X, 0.50X, and 1.00X times the rate of 1.5 pts/-acre of Bravo 720 initiated when late leafspot incidence reached 1%. Disease severity, defoliation, and leaf area index (LAI) were measured eight times during the season. Healthy leaf area duration was calculated from LAI and total disease. Pod yields for all treatments increased with HAD. Results support the concept that HAD can be used to predict yield loss due to late leafspot disease.

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EFFECT OF FUNGICIDES ON THANATEPHORUS CUCUMERIS (FRANK) DONK IN BEAN CULTIVARS. B.Mora; F.Villalobos,and G.Galvez. Dpto, Fitopatologia. MAG & CNP San Jose, Costa Rica. CIAT, Cali, Colombia.

The effect of fungicide was studied in the bean varieties, Porrillo 70, Huetar, Ica Pijao and Diacol Calima to control T. cucumeris. Four treatements were evaluated: pentachloronitrobenzene(PCNB) at 15 kg a.i/ha; PCNB at 15 kg i.a/ha + benomyl 0.6 g a.i/l; benomyl 0.6 g a.i/l and control. PCNB was applied to soil 7 days after planting, and benomyl was sprayed onto foliage after 20, 30, and 45 days. Porrillo 70 and Huetar averaged 323 kg/ha and 308 kg/ha respectively; whereas Diacol, Calima, and Ica Pijao yielded 41 and 33 Kg/ha. Plants with benomyl yielded 365 kg/ha, and plants treated with PCNB produced 49 kg/ha. The interaction of cultivars with fungicides showed that Porrillo 70, yielded 753 kg/ha, had low disease severity, and an apparent infection rate (r) of 0 14; in contrast, Diacol Calima, had a yield of 1.7 kg/ha, higher disease severity, and an r value of 0 26. The disease progress curve fit a logistic transformation, in which the epidemic began slowly, but rapidly increased 30–50 days after planting.

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RELATIONSHIP OF IRRIGATION REGIME TO POPULATIONS OF MACROPHONIMA PHASEOLINA MICROSCLEROTIA IN ROOT TISSUE AND YIELD OF SOYBEAN. S.R. Kendig and J.C. Rupe, University of Arkansas, Fayetteville, Arkansas 72701.

The relationship of populations of M. phaseolina microsclerotia (ms) in root tissue to final yield was determined for the soybean cvs., Davis and Lloyd, grown under various irrigation regimes. Irrigation treatments included: none, full-season, until flowering, and after flowering. Biweekly, ms populations were determined in all roots collected in a randomly selected 0.61 m section of row (av. 12 plants/section). Recovery of ms began as early as 4 weeks after planting. Final soybean yields were positively correlated (P=0.01) with irrigation and negatively correlated (P=0.01) with populations of ms from root tissue at weeks 16 (R4, pod development) and 18 (R5, early seed development). Microsclerotia were negatively correlated with irrigation regimes (P=0.01). There were no cultivar differences in populations of ms or yield.

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EFFECT OF RUST ON FORAGE YIELD AND DIGESTIBILITY OF DWARF AND TALL PEARL MILLET CULTIVARS. J.P. Wilson, R.N. Gates, and W.W. Hanna, USDA-ARS, Coastal Plain Expt. Stn., Tifton, GA, 31793.

Effects of infection by Puccinia substriata var. indica on yield and digestibility of pearl millet forage were examined in 1988. Dwarf hybrids, Tifleaf 1 (susc) and Tifleaf 2 (res), and tall hybrids, Gahi 3 (susc) and Gahi 4 (res) were treated with chlorothalonil or inoculated after the first harvest to establish different levels of disease. No differences in yield or digestibility were detected between disease-free plots of susceptible and resistant cultivars for either dwarf or tall types. Rust severities of inoculated plots of Gahi 3 were 54% of those of Tifleaf 1. Green yield, dry matter yield, and in vitro digestibility were negatively correlated with final disease severity and area under the disease progress curve of both cultivars. Dry matter concentration was unaffected by disease. Digestible dry matter yield (Y, percent of healthy control) as a function of percent disease severity 10 days before harvest (X), could be expressed as Y=97.6-0.75X, and Y=101.6-1.01X for dwarf and tall cultivars, respectively.

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A POSSIBLE TOBAMOVIRUS ASSOCIATED WITH RINGSPOT AND OAKLEAF PATTERN IN EPIMEDIUM X YOUNGIANUM CV. NIVEUIM. M. L. Putnam and S. T. Nameth. Maryland Dept. of Agriculture, Annapolis, MD 21401 and Dept. of Plant Path., The Ohio State Univ., Ohio Agr. Res. and Dev. Center, Columbus, OH 43210.

Sap extracts from Epimedium leaves taken from a nursery in Maryland, showing chlorotic ringspots and oak leaf patterns were rub inoculated onto the leaves of indicator hosts. Symptoms on indicator hosts included local lesions on the inoculated leaves of Nicotiana tabaccum cv. Samsun and Gomphrena globosa and a systemic infection and eventual death of Nicotiana benthamiana. Chenopodium quinoa did not react. Sap extracts from infected 'Samsun' leaves tested negative for TMV (common strain), TSWV, TSRV and TomRSV using an indirect-sandwich ELISA but positive for viral-associated dsRNA. Electrophoresis of dsRNA in infected 'Samsun' extracts revealed the presence of a replicative form of dsRNA with an estimated molecular weight of 4.2 x 10^6 and 4 subgenomic dsRNA's with molecular weights ranging from 2.3 - 0.4 x 10^6. This appears to be the first report of virus associated with Epimedium.

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PREDISPOSITION OF LATE EMERGING CORN PLANTS TO EAR ROTTING FUNGI. D. M. Huber, H. L. Warren, T. S. Roseman, and C. Y. Tsai. Botany & Plant Pathology Dept., Purdue University, W. Lafayette, IN 47907.

The effect of delayed emergence on growth, yield, and disease of four maize hybrids (A632xLH39, Pioneer Brand 3165, B73xMo17, and Callahan Brand C773) was evaluated under high fertility conditions on a Brookston silt loam soil. Treatments consisted of all plants emerging uniformly early, uniformly 10 days late, or a continuous mix of two early and one plant 10 days late. Final plant populations were 52,000/ha. No differences in growth, barrenness, biomass, or yield were observed for uniformly early, uniformly late, or early emerging plants in the mixed emergence treatments. Delayed emerging plants, adjacent to early emerging plants in the row, were severely stunted, excessively barren (70-90%), and produced only 20-40% of the biomass and 2-10% of the grain yield of early emerging plants. Ear rotting fungi were assayed by plating kernels on PDA and incubating at room temperature. Delayed emerging plants had 40-100% more kernels infected with Fusarium moniliforme, F. roseum, and Colletotrichumgraminicola than early or late uniform emerging plants.

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DEVELOPMENT OF BIOASSAYS FOR IDENTIFICATION OF SOYBEAN GENOTYPES RESISTANT TO MACROPHOMINA PHASEOLINA. F. Beleid-El Moshaty, A. Novacky, T.D. Wyllie, and A. Karr. Department of Plant Pathology, University of Missouri, Columbia, MO 65211.

This study reports development of a rapid, sensitive, toxin bioassay with E. coli and a selection by bioassays with root cap, cotyledonary and leaf cells and whole soybean plants for identification of resistant genotypes to Macrophomina phaseolina toxin. Toxin is located by measuring the ability of fractions to inhibit growth of E. coli, strain HB101, in soft agar overlays. The samples to be tested [5 µl aliquots] in 95% (v/v) ethanol are applied to the surface of the overlay, where toxic fractions cause a concentration dependent formation of clear plaques in the bacterial lawn. For genotype selection, toxin-treated root cap, cotyledonary, or leaf cells are tested for viability with Evans blue dye. Whole plants are scored for symptoms of leaf tissue collapse. With these techniques, the soybean cultivars Chamberlain, Stine 3790, Resnik, Asgrow 4393 and Williams 82 respectively, were ranked [least sensitive to most sensitive] in their sensitivity to the M. phaseolina toxin. Identification of the toxin raises the possibility for development of a selection program for resistance to charcoal rot.

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EFFECT OF CITRUS ROOTSTOCK ON POPULATIONS OF PHYTOPHTHORA PARASITICA IN FLORIDA. J. P. Agostini,* L. W. Timmer,** W. S. Castle,** and D. J. Mitchell.* *Plant Pathology Dept., Univ. of Florida, Gainesville, FL 32611; **Citrus Research and Education Center, 700 Expt. Station Rd., Lake Alfred, FL 33850.

The effect of rootstock on the population of P. parasitica was determined by plating soil from the root zone from two Valencia sweet orange rootstock trials and one pot test with seedlings on selective media periodically from Dec. 1987 to Feb. 1989. The average number of propagules per cm^3 of soil over 4 to 5 sampling dates was: (St. Cloud, Avon Park, and pot test): Palestine sweet lime (29,10,...), C. volkameriana (30,...,...), Ridge Pineapple sweet orange (...,13,102), Cleopatra mandarin (17,10,87), trifoliate orange (14,8,14), and Swingle citrumelo (4,5,14). Populations were significantly higher in the summer (32 propagules/cm^3) than in the winter (3.4 propagules/cm^3) due to the greater root biomass in summer. Soil populations generally reflected the susceptibility of the rootstocks to P. parasitica with the lowest population on trifoliate orange and Swingle citrumelo and the highest on sweet orange.

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EFFECT OF TEMPERATURE ON DISEASE SEVERITY OF CORKY ROOT OF TOMATO CAUSED BY PYRENOCHAETA LYCOPERSICI. N. Shishkoff, USDA-ARS, Frederick, MD 21701 and R. N. Campbell, Dept. Plant Pathology, Univ. Calif., Davis, CA 95616

Tomatoes were sown in infested soil in microplots at monthly intervals from Feb. to May 1988 to provide varying temperature regimes. The disease progress curves were linear and significantly lower only for the last planting date. Disease severity was greater at 16 or 21 C than at 27 C when tests were done in infested vermiculite in controlled environment chambers. In other experiments seedlings were grown in infested vermiculite at 16 or 27 C and switched to sterile vermiculite and grown at 16 or 27 C for a lesion development period. Disease was more severe at 16 C than at 27 C for the lesion development period, regardless of the infection temperature.

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PATHOGENICITY OF ISOLATES OF RHIZOCTONIA SOLANI AG-3 COLLECTED FROM POTATO PLANTS AND SOIL. D.E. Carling and R.H. Leiner. University of Alaska, 533 E. Fireweed, Palmer, AK 99645.

Sclerotial, hymenial and lesion isolates of Rhizoctonia solani were collected from 20 plants from each of three fields in southcentral Alaska. Also, isolates were collected from soil, directly from pegs placed on KHP media, and indirectly via beet seed baiting. Additionally, isolates were collected (directly) from soils associated with several crops other than potato. All isolates were characterized as to anastomosis group (AG). Pathogenicity testing was done on all isolates of AG-3 in soil on developing sprouts at 50 F. Nearly 95% of sclerotial, hymenial and lesion isolates but only 52% of soil isolates belonged to AG-3. Most isolates of AG-3 were moderately to highly pathogenic on potato sprouts. Pathogenicities of isolates collected from lesions, sclerotia, hymenia and soil were the same, P = 0.05. Pathogenicities of isolates collected from soils associated with potato, carrot, barley, bluegrass or fallow were the same, P = 0.05. These data suggest that source and associated plant are not indicative of pathogenicity of R. solani AG-3.

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INFLUENCE OF INOCULUM LEVEL AND IRRIGATION ON PHYTOPHTHORA ROOT ROT OF PROCESSING TOMATO. D. A. Neher and J. M. Duniway.

Department of Plant Pathology, University of California, Davis, CA 95616.

Effects of initial Phytophthora parasitica populations and duration of furrow irrigation on root rot development and yield loss in processing tomato variety 6203 were quantified. Field plots in which P. parasitica was not detected initially were inoculated to give 0, 1, 4 and 12 propagules per gram soil. Disease incidence and severity increased significantly with increased inoculum levels in both 1987 and 1988. However, there was significant yield loss at the highest inoculum level in one of two years. Moderately severe symptoms on roots and shoots were required before yield loss was observed. Extending irrigation episodes from 4 to 24 hours significantly increased root and shoot symptoms in one year at one intermediate level of inoculum. The results suggest that early season inoculum levels must exceed a threshold for disease to develop and that a higher threshold is necessary for significant yield losses to occur.

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FACTORS INFLUENCING THE DEVELOPMENT OF FUSARIAL WILT OF BANANA (PANAMA DISEASE). R.C. Ploetz, University of Florida, IFAS, 18905 SW 280th Street, Homestead 33031.

Conidial suspensions of Fusarium oxysporum f. sp. cubense (incitant of Fusarial wilt or Panama disease), and tissue-culture-derived plantlets of race differentials of banana were used to characterize virulence and resistance/susceptibility in this pathosystem. Experiments were conducted in autoclaved silica sand which was recolonized by aerial microflora for at least two wk prior to use; calcareous soil from south Florida (autoclaved or nonautoclaved) or freshly autoclaved or nonautoclaved sand were responsible for less consistent disease development. Light intensity was positively correlated with disease severity in growth chamber (photosynthetic photon fluxes $\leq$ 130 μmols sec^{-1} m^{-2}) and in greenhouse environments ($\leq$ 1,200 μmols sec^{-1} m^{-2}). Root size (displacement volume) and inoculum density (conidia ml^{-1}) were also positively correlated with disease severity. When these factors were optimized, differential responses were obtained for compatible and incompatible combinations of races 1, 2, and 4 and race differentials.

365 Withdrawn

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RELEASE OF HYDROXYPROLINE RICH PROTEINS FROM POTATO CELL WALLS BY ENZYMES FROM ERWINIA CAROTOVORA SUBSP. CAROTOVORA (Ecc). J. Lewosz, A. Kelman and L. Sequeira. University of Wisconsin-Madison, 1630 Linden Drive, Madison, WI 53706

Cell walls were freed of a) cytoplasmic proteins by repetitive extraction with deoxycholate and b) noncovalently bound proteins by extraction with 2 N NaCl and phenol:acetic acid:water (2:1:1). Additional proteins and pectic materials were released by extracting with 0.5% EDTA, hot ammonium oxalate or 5 M ammonia. Most hydroxyproline-rich glycoproteins were released by boiling the cell walls at pH 1.0. Extensins were partially released after splitting isodityrosine linkages with Na-chlorite. Solubilized extensins were not degraded significantly by bacterial proteases freed of pectinolytic enzymes by affinity chromatography on cross-linked polygalacturonate. Release of hydroxyproline-containing compounds by culture filtrates of Ecc is attributed to pectin degradation that results in liberation of tightly-linked proteins.

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DRY HEAT SEED TREATMENT FOR XANTHOMONAS CAMPESTRIS PV. TRANSLUCENS. D. C. Sands, E. Fourrest, and L. Rehms. Dept. of Plant Pathology, Montana State University, Bozeman, MT 59717.

Bacterial black chaff and bacterial leaf streak of cereal grains are caused by Xanthomonas campestris pv. translucens, a seed transmitted pathogen. This bacterium has apparently spread via seed to a number of countries in the past decade, as evidenced by appearance of the pathogen first in breeder's seed and later in commercial fields. A dry heat treatment (72°C for 7 days) of severely infested barley is sufficient to reduce the infestation rate by seven orders of magnitude to undetectable levels. Germination of wheat and barley is reduced by dry heat, but still remains above 80% after seven days.

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GROWTH OF BACTERIA ON PLANTS: DETECTION AND QUANTIFICATION. Joe J. Shaw, Department of Botany and Microbiology, Auburn University, Auburn AL, 36849

Bacterial pathogens of plants are ubiquitous in nature. Plant pathogenic bacteria are often found living in the soil, on non-host plants and as epiphytes on host plants, and in these cases do not cause disease, although these sources may serve as primary sources of disease inoculum. Thus, it is important to know where bacteria are what they are doing when they grow upon plants without causing disease. Bacteria which have been genetically engineered to bioluminesce may be easily detected upon plants and enumerated. A bioluminescent isolate of Xanthomonas campestris pv. campestris is studied during stages of plant colonization on crucifers. Factors such as humidity, temperature and host are examined as they affect host colonization.

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UNUSUAL CHARACTERISTICS OF Agrobacterium tumefaciens STRAINS ISOLATED FROM WILD BLACKBERRY. M.L. Canfield and L.W. Moore. Department of Botany and Plant Pathology, Oregon State University, Corvallis, Oregon 97331-2902

Interest in the distribution and ecology of A. tumefaciens led to a study of naturally occurring galls in agriculturally undisturbed areas. As part of this investigation, an unusual group of agrobacteria was isolated from aerial galls of wild blackberry. Thirty-one of the 43 isolates induced galls on tomato plants and 16 of these were also highly virulent on Emla 7 apple rootstock, a host which has been difficult to infect with A. tumefaciens strains. The bacteria grow well only on a mannitol glutamate medium supplemented with a large variety of salts, trace elements and yeast extract. Although the strains formed a homogeneous group, attempts to identify them using classical biochemical tests failed to place them into A. tumefaciens biovars 1, 2, or 3, nor did they fit the description for A. rubi strains. The strains were, however, presumed to be A. tumefaciens because of their ability to induce tumors and the strong homology between their DNA and ^{32}P labeled probes for vir region genes of the Ti plasmid. These characteristics illustrate again the diversity found within the genus Agrobacterium.

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A toothpick assay to determine the ice nucleation activity of bacteria. D. E. Legard and J. E. Hunter. Cornell University, New York State Agricultural Experiment Station, Geneva, NY 14456

A rapid assay was developed for the routine determination of ice nucleation activity (INA) of *Pseudomonas syringae*. The method involves placing a mass of cells from a 24 hr culture that have been prechilled at 4C on the end of a sterile toothpick and inserting the bacteria-laden end into a prechilled 1 ml glass culture tube containing 650 ul of sterile distilled water. After incubation at the desired temperature for 20 min, the tubes can easily be evaluated for INA by determining if the protruding toothpick is still mobile. The effect of growth temperature, media and, prechilling of cultures and tubes were evaluated with 93 strains of *Pseudomonas syringae* pv. *syringae* from numerous hosts. Several other pathovars of *P. syringae* were also evaluated. Strains of *P. syringae* known to be INA at -3.0C when grown at 22C for 24 hr would occassionaly fail to be INA at -3.0C when grown at 25C, and very few strains were INA when grown at 28C or 31C. Prechilling of the cultures and testing apparatus at 4C for 30 min before transferring bacteria to culture tubes restored INA of strains grown at 25C, 28C, or 31C. Growth of strains on three media (KB, NA, KBC) did not affect INA at -3.0C. The toothpick assay used with the prechilling protocol provides a reliable and rapid means of evaluating large numbers of bacterial strains for INA.

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DIFFERENCES IN FATTY ACID PROFILES OF PSEUDOMONAS SYRINGAE PV. TOMATO AND PSEUDOMONAS SYRINGAE PV. SYRINGAE DUE TO PHYSIOLOGICAL AGE AND CULTURE MEDIUM. A. E. Voloudakis, R. D. Gitaitis, and R. W. Beaver. Univ. of Georgia, Tifton, GA 31793-0748.

Strains of Pseudomonas syringae pv. tomato and P. s. pv. syringae were isolated from tomato transplants; grown on KMB and NA for 2, 4, and 6 days; and analyzed for total cellular fatty acids by gas-liquid chromatography. The percentage of 9-octadecenoic acid (18:1w9) was significantly lower in cultures grown on KMB. The relative levels of dodecanoic (12:0) and 2-hydroxydodecanoic (2-OH-12:0) acids increased

over time, whereas hexadecanoic (16:0) acid decreased in both pathovars regardless of growth medium. The ratio of saturated to unsaturated fatty acids was higher in cultures grown on KMB. The ratio initially increased, but decreased between days four and six for both pathovars and media.

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MOLECULAR CLONING OF XANTHOMONADIN PIGMENT SYNTHESIS GENES FROM XANTHOMONAS CAMPESTRIS FOR IDENTIFICATION OF XANTHOMONADS. Kawalek, M.D.[1], Poplawsky, A.R., and Schaad, N.W.[2]. Dept. PSES, Division of Plant Pathology, University of Idaho, Moscow, ID 83843. Present address: [1]Oregon State University; [2]Harris Moran Seed Co.

Xanthomonads are the cause of many seed transmitted diseases. However, proper identification of these bacteria requires lengthy pathogenicity testing. Because the xanthomonadin pigment is unique to Xanthomonas species, genes for pigment synthesis could be very useful for identification purposes. Eleven pigment minus mutants of Xanthomonas campestris pv. campestris B-24 were produced by exposure to ethyl methanesulfonate. All mutants were typical in colony morphology on YDC agar, positive for starch hydrolysis, and virulent when tested in cabbage seedlings. Nine of the mutants were prototrophic when tested on basal M9 glucose agar. A genomic library of B-24 DNA was constructed in cosmid vector pLAFR3 and mobilized into one of the mutants via the helper plasmid pRK2013. Two different cosmids with B-24 DNA inserts of 27 and 32 kb complemented this mutation for pigment production. In subsequent matings of these cosmids, all eleven of the mutants were complemented. Experiments are underway to further subclone these inserts and determine their specificity to various xanthomonads.

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LETTUCE CORKY ROOT CAUSED BY STRAINS OF A GRAM-NEGATIVE BACTERIUM FROM MUCK SOILS OF FLORIDA, NEW YORK, AND WISCONSIN. A. H. C. van Bruggen, P. R. Brown, and K. N. Jochimsen. Dept. of Plant Pathology, University of California, Davis, CA 95616.

Slow-growing bacteria similar to a Californian strain (CA1) causing lettuce corky root (CR) were isolated from muck soils of Florida, New York, and Wisconsin, using lettuce seedlings as bait. All isolates were tested for reaction with polyclonal antibodies produced against strain CA1, and for pathogenicity on a CR susceptible (Salinas) and resistant (Green Lake) lettuce cultivar in a greenhouse. Five strains from Florida, three from New York, and three from Wisconsin induced severe CR symptoms on Salinas and mild symptoms on Green Lake. All strains were gram-negative, aerobic, oxidase, positive, catalase positive, and reduced nitrate to ammonia. Whole-cell fatty acid compositions were similar for all isolates, indicating that CR of lettuce is caused by strains of the same bacterium in Florida, New York, Wisconsin, and California.

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PERSISTANT, NON-LYTIC BACTERIOPHAGE IN STRAINS OF XANTHOMONAS CAMPESTRIS PATHOVARS PRUNI AND VESICATORIA . D.F. Ritchie. Department of Plant Pathology, N.C. State Univ., Raleigh, NC 27695.

More than 25 strains of each pathovar were assayed for persistant, non-lytic phage by centrifugally removing bacteria from 18-24 h broth cultures and spotting 1 µl of supernatant on the lawn of each bacterial strain in 0.6% top agar. Phage were detected in cultures of all strains. Detection in some strains depended on the indicator strain used. Titers among strains ranged from 10^3 to 10^{10} pfu/ml. Attempts to "cure" a strain using acridine orange were unsuccessful. Phage infectivity was destroyed by 10 min exposure to > 1% chloroform but most phage strains were not inactivited by 10 min exposure to 90C. Phage nucleic acid was not sensitive to RNase A but was sensitive to mung bean nuclease. This suggests these phages contain ssDNA.

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VARIATION IN AGGRESSIVENESS OF XANTHOMONAS CAMPESTRIS PV. CITRUMELO ASSOCIATED WITH CITRUS BACTERIAL SPOT (CBS) IN FLORIDA. J. H. Graham and T. R. Gottwald, University of Florida, Lake Alfred 33850 and USDA-ARS, Orlando, FL 32803.

Wound-inoculated detached leaves of Swingle citrumelo and Duncan grapefruit were used to characterize strains of Xc pv. citrumelo from citrus nurseries and to distinguish these strains from Xc pv. citri causing Asiatic citrus canker. CBS strains varied in aggressiveness based on the extent and persistence of watersoaking and the development of necrosis. Aggressiveness on detached leaves was correlated with that on wound-inoculated leaves in the greenhouse and field. In vitro inoculations clearly distinguished the flat-spreading lesions of CBS from the erumpent, callus-like reaction produced by Xc pv. citri. In 4 nursery outbreaks, strain aggressiveness was related to severity of leaf symptoms and spatial distribution of CBS; only the highly aggressive strains were spread naturally whereas less aggressive strains were spread mechanically. Of 25 independent outbreaks since 1984, aggressive strains have appeared in only 4 nurseries.

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INOCULUM PRODUCTION FROM ASIATIC CITRUS CANKER LESIONS AND EPIPHYTIC SURVIVAL OF XANTHOMONAS CAMPESTRIS PV. CITRI IN ARGENTINA. L. W. Timmer, Univ. of Florida, Citrus Research and Education Center, 700 Expt. Station Rd., Lake Alfred, FL 33850.

Inoculum production was studied by placing water in plastic reservoirs over canker lesions and measuring bacterial populations in exudates by plating on selective media. Upon wetting new, fully developed lesions, 10^4 to 10^5 bacteria/ml were detected immediately and populations rose slightly with further wetting to 24 hr. Old lesions produced 10^3 bacteria/ml or less and extended wetting did not increase populations. A leaf-swab technique was used to assay epiphytic populations on symptomatic leaves and on asymptomatic leaves of trap seedlings. Populations on symptomatic leaves varied from 10^4 to 10^6 per leaf in the early morning and declined to 10^2 to 10^5 per leaf, respectively, by mid-afternoon. The bacterium was consistently detected on trap plants, but populations were always low (< 10^3 per leaf). Most inoculum for spread of citrus canker is produced by exudation of bacteria from new lesions. Epiphytic bacteria on asymptomatic leaves are of little epidemiological significance.

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FREQUENCY, DISTRIBUTION AND CHARACTERISTICS OF ENDOPHYTIC PSEUDOMONAS SYRINGAE IN PEAR TREES. **S. K. Whitesides and R. A. Spotts. Oreg. St. Univ., Mid-Columbia Agric Research and Extension Center, Hood River, OR 97031.**

Internal stem and root tissues of pear trees were sampled for presence of Pseudomonas syringae (Ps) at 7 orchard sites in Oregon. Endophytic Ps were found in 76% (45/59) of trees sampled with 140 of 195 isolates found in root tissues. Electrophoretic banding profiles of total bacterial DNA were used to compare isolates. Visual evaluation showed identical profiles between isolates found at different sites on a single tree and between isolates from adjacent trees. However, different profiles were observed between root and stem isolates in one tree. Ps inoculations into internal tissues of potted trees resulted in limited, detectable Ps movement, 2-3 cm in stems and no movement above the crown from root inoculations. All isolates were tested for ice nucleation activity with only 3 isolates testing positive. These 3 were from 4 year old stem tissues of 2 trees from different orchards.

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PATHOGENICITY AND BIOTYPE DETERMINATION OF AGROBACTERIUM ISOLATES FROM MUSCADINE. K. L. Thies, D. E. Griffin, and C. H. Graves. Dept. of Plant Pathology and Weed Science, Mississippi State University, Mississippi State, MS 39762.

Although Agrobacteria are classified on the basis of pathogenicity, three distinct groups (Biovars 1, 2, and 3) are recognized on the basis of biochemical tests. Crown gall on grapes (Vitis vinifera) is considered to be due to Agrobacterium tumefaciens biovar 3. A high incidence of Agrobacteria were isolated from vascular fluids, galls, and roots of muscadine (Vitis rotundifolia) throughout Mississippi. Selective media and diagnostic tests were used to determine biovars, while lateral bud cultures, detached muscadine leaf assays, and whole plants were used to determine pathogenicity of isolates. All three biovars were isolated. Biovar 3 was the predominant biovar isolated from vascular fluids. Gall induction by both biovar 1 and 3 was demonstrated. Additional isolates are being assayed to determine the relationship between biovar and crown gall development in muscadine.

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TRANSMISSION AND REISOLATION OF A BACTERIUM ISOLATED FROM GREENING-INFECTED CITRUS IN SOUTH AFRICA. R-J Chippindall and V H Whitlock, Department of Microbiology, University of the Witwatersrand, P O Wits, 2050, South Africa.

A culture of a bacterium repeatedly isolated from greening-infected citrus in South Africa was shown to induce characteristic greening symptoms when mechanically inoculated into citrus and the organism reisolated from test plants, thereby fulfilling Koch's postulates. The bacterium was also isolated from symptomatic cucumber connected via dodder to naturally infected citrus. Based on these findings, this bacterium was thought to be the

causative agent of greening, however, insect vector transmission of the isolate was negative, and inoculated plants did not exhibit the presence of the gentistic acid marker of greening disease. Also, antisera raised against the isolate failed to consistently detect greening in citrus known to be infected with the disease. Results suggest that this organism, identified as a Clavibacter spp. on the basis of morphology and fatty acid profiles, is not the causative agent of citrus greening per se, but a probable component of the "greening syndrome".

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MORPHOLOGICAL COMPARISONS OF MALES OF ONE ISOLATE EACH OF HETERODERA GLYCINES, H. CRUCIFERAE AND ONE OF THEIR HYBRIDS. L. I. Miller, Dept. of Plant Path., Phys., & Weed Sci, VPI&SU, Blacksburg, VA 24061-0331.

Comparisons were made of male characters of 21 specimens each of one isolate of Heterodera glycines(M) cultured on 'Lee' soybean, one isolate of H. cruciferae(C) cultured on 'Market Prize' cabbage and one of their hybrids(MC) cultured on soybean. Dimensions in µm were as follows-length(LTH) :M 1275-1600 (mean 1428, s.d. ± 106.5), C 930-1270 (1095 ± 100.7), MC 1050-1510 (1256 ± 111.6); stylet knobs to dorsal gland orifice (DGO) :M 3.0-4.9 (3.7 ± 0.6), C 3.9-4.9 (4.3 ± 0.3), MC 2.3-3.5 (2.7 ± 0.3); breadth of stylet knobs(KBS) :M 4.0-5.2 (4.6 ± 0.4), C 3.7-5.0 (4.4 ± 0.4), MC 4.4-5.7 (5.0 ± 0.3). Characters LTH and DGO were significantly different (P=0.05) for M, C and MC. Character KBS for M and C was not significantly different; however dimensions of the MC hybrid differed significantly from the M and C parents. The MC hybrid reproduced on soybean, cabbage and 'US75' sugarbeet.

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RESISTANCE TO WHEAT LEAF RUST OF LAND CULTIVARS AND THEIR DERIVATIVES. Beatriz A. Perez and A. P. Roelfs, Department of Plant Pathology, University of Minnesota and Cereal Rust Laboratory, USDA/ARS, St. Paul, MN 55108.

Many wheats (Triticum aestivum) have been derived from Americano 25c, Americano 26n, and/or Americano 44d. Durable resistance to leaf rust in several modern wheats has been related directly to resistance from Americano 44d. Seedlings and adult plants of these wheats, selected cultivars, near-isogenic lines used for race identification, and Thatcher were tested with isolates of Puccinia recondita f. sp. tritici. Some cultures differentiated Americano 25c from Americano 26n and Americano 44d. Seedlings of Americano 25c were resistant to avirulent cultures on TcLr16. Seedlings and adult plants of Buck Manantial, an Americano 25c derivative, were immune to all cultures tested. Seedling and adult plants of Americano 26n and Americano 44d were susceptible. The resistance of Americano 44d was moderately severe in adult plants. Seedlings of Marcos Juarez Inta, an Americano 44d derivative, had a high infection type but was low to moderately severe in adult plant stage.

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In vitro screening for resistance to gray leaf spot in maize. VM Best, JM Perkins, AS Wang, ME Coker, JB Milcic, DS Cheng. Sungene Technologies Corp., 2050 Concourse Dr., San Jose, CA 95131

Isolates of Cercospora zeae-maydis were collected from the field and cultured in liquid media. Cell-free filtrates were prepared that proved 50% toxic to immature embryos and 90% toxic to callus cultures of maize. Over 250 plants were regenerated from the surviving callus and grown to maturity in the greenhouse. Progeny of these plants showed a 60% increase in resistance to the filtrate. Field resistance to gray leaf spot is being tested.

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PREDICTION OF PARTIAL RESISTANCE TO RICE BLAST USING MULTILOCATION TRIALS. S.W. Ahn, D.V. Seshu, and J.M. Bonman. International Rice Research Institute, P.O. Box 933, Manila, Philippines.

Assessment of partial resistance to blast is often impossible for some cultivars because virulent races of Pyricularia oryzae are not present at the screening site. Thirty-nine rice cultivars were evaluated for partial resistance in upland miniplots. Results were analyzed to determine the relationship between the partial resistance assessment and disease severity index (DSI). The DSI is the average score of compatible reactions for a cultivar in the International Rice Blast Nursery (IRBN) trials. IRBN is a multilocation trial conducted at more than 20 sites in different countries yearly. DSI was positively correlated with diseased leaf area (r=0.77) and log diseased leaf area (r=0.80). Seventeen cultivars with DSI less than 5.5 had 10% diseased leaf area, while the susceptible check had more than 70%. DSI may help breeders predict partial resistance of rice genotypes that do not encounter virulent races of P. oryzae during tests.

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PROTECTION OF YIELD BY COMBINING RESISTANCE AND TOLERANCE TO SEPTORIA TRITICI BLOTCH OF WHEAT. Z. Eyal, Department of Botany, Tel Aviv University, Tel Aviv 69978, Israel.

Selection for high 1000-kernel weight (TKW >45g) was performed in early generations of crosses between the moderate-resistant cultivar Musala"S" (CIMMYT, CM16780) and the susceptible, high-TKW cultivar Lakhish and with the tolerant cultivar Miriam, under sever epidemics induced by a mixture of Septoria tritici isolates. "Frontal" resistance from Musala"S" was detected in selected, tolerant F_5 lines using isolate ISR398, and the "background" tolerance by using ISR8036 to which Musala"S" is susceptible. Some Musala"S"/Lakhish lines expressed the resistance of Musala"S" to ISR398 and retained the high TKW of Lakhish under ISR8036 epidemic. Some Musala"S"/Miriam lines expressed resistance to ISR398 and TKW as high or higher than that of Miriam when subjected to ISR8036. Lines were selected which combine "frontal" resistance to ISR398 and high kernel weight retention to ISR8036, together with earliness and high-yieldind potential.

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COMPONENTS OF RESISTANCE OF TOBACCO TO PHYTOPHTHORA PARASITICA VAR. NICOTIANAE. Keith Jones and H. D. Shew, Department of Plant Pathology, North Carolina State University, Raleigh, NC.

Four cultivars of tobacco with field resistance ranging from highly resistant to susceptible were used. Root growth was quantified in a controlled environment and in the field using root observation chambers. Data were collected using computer based digitizing equipment. The highly resistant cultivar produced roots more slowly than the other cultivars, reducing the number of pathogen propagules encountered. Inoculum efficiency was observed by inoculating zoospores onto undisturbed roots of plants in controlled and field conditions, and was least on roots of the highly resistant cultivar. Lesion expansion on roots also was followed visually. Maximum lesion expansion was measured by plating root samples onto selective medium, and with an ELISA system. The ELISA system was used to quantify the amount of fungal tissue in root lesions on the four cultivars. These results show that root production and inoculum efficiency are important components of field resistance.

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GENETIC CONTROL OF IMMUNITY TO TURNIP MOSAIC VIRUS IN WINTER OILSEED RAPE (Brassica napus ssp. oleifera) AND THE EFFECT OF FOREIGN ISOLATES OF THE VIRUS. J. A. Walsh, AFRC Institute of Horticultural Research, Wellesbourne, Warwick, CV35 9EF, UK.

Immunity to a UK isolate of turnip mosaic virus (TuMV) was studied in eight lines of oilseed rape. Six of these lines were homozygous for the immunity factor and two were from heterozygous parents. Segregation ratios in the F_2 generations of reciprocal crosses between homozygous immune lines and homozygous susceptible cultivars showed that immunity was controlled by a single dominant nuclear gene. The immunity was confirmed by the inability to detect virus particles in mechanically inoculated plants by back inoculations, ELISA, and ISEM tests. Plants were immune to repeated inoculations and aphid transmissions. The immunity was effective against two German isolates of TuMV. However, a Greek isolate partially overcame the immunity causing local infection only and Canadian and Danish isolates overcame the immunity completely causing systemic mosaic-type symptoms.

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COMPARATIVE STUDIES OF RESISTANCE IN PEACH GENOTYPES TO MONILINIA FRUCTICOLA. J.E. Adaskaveg, A.J. Feliciano, and J.M. Ogawa. Dept. of Plant Pathology, University of California, Davis. 95616

Blossoms and fruit of peaches were evaluated for resistance to M. fructicola. Blossoms were spray inoculated with a spore suspension (2 X 10^4 conidia/ml) and incubated with free-moisture for 48 hr in the laboratory (20 C) or 48-72 hr in the field (16-20 C). In both tests, percentage of anthers infected was 10-30% less in resistant (Bolinha, Kakamas) than in susceptible genotypes (Starn, Loadel, Tufts, Flavorcrest) after 48 hr. Incubation > 72 hr resulted in no differences between genotypes. Non-wounded fruits were spray inoculated with a spore suspension and incubated as above for 2-16 hr at 20 C, air-dried, and evaluated after 72 hr. Percentage of infected, inoculated fruits

increased from 0% (Bolinha) and 60% (Corona) after 2 hr of wetness to 12.5-25% (Bolinha) and 100% (Corona) after 8 hr of wetness. Histological studies of fruit, in similar stages of development, demonstrated morphological differences in the epidermal tissue between resistant (Bolinha, Kakamas) and S-genotypes (Corona, UCD18-8-11). Generally, R-genotypes had a thicker cuticle, compact epidermal cells, and fewer trichomes that originated from the first cell layer of the epidermis.

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VARIATION IN TOLERANCE TO BENOMYL AMONG COLLETOTRICHUM GLOEOSPORIOIDES ISOLATES FROM MANGO. R.T. McMillan, Jr., Michael M. Moss, L.R. Bowling, and L. Stempel, University of Florida, IFAS, Trop. Res. & Educ. Center, Homestead, Florida USA, 33031.

In 1987 and 1988 a mango grove located in Dade County Florida USA lost over 50% of the crop to mango anthracnose caused by C. gloeosporioides. The grove was sprayed with 1 1/2 lbs of benomyl weekly in flower and every 3 to 4 weeks after fruit set by the owner in 1987 and by a professional grove management company in 1988, with no noticeable control of anthracnose in both years. In the summer of 1988, 100 infected fruits were harvested randomly from the grove from which 84 single spore colonies were isolated. The isolates were screened at 0, 1, 10, and 100 ppm of benomyl. Out of 84 single spore colonies, nearly 40% were tolerant to 10-100 ppm of benomyl, while 60% were sensitive, showing little or no radial growth. These results may explain the lack of anthracnose control by benomyl in the grove.

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INFECTION OF PEPPER MESOPHYLL PROTOPLASTS WITH PEPPER MILD MOTTLE VIRUS USING ELECTROPORATION. J.L. Jacobs and C.T. Stephens. Michigan State University, East Lansing, MI 48824.

Mesophyll protoplasts of pepper (Capsicum annuum) were inoculated with pepper mild mottle virus (PMMV) by electroporation. The protoplasts, derived from cotyledons of seedlings grown in vitro, were obtained by treatment with an enzyme solution containing 1.0% (w/v) Cellulysin, 0.5% (w/v) Driselase, 0.1% (w/v) Macerase, CPW salts and 0.4 M sorbitol at pH 6.0. Infection was achieved with several direct current pulses of 10 msec at a field strength of 0.36 to 0.57 kV/cm and a virus concentration of 100 µg/ml in a 0.7 M mannitol solution. The proportion of infected protoplasts was quantified by staining with viral coat protein-specific antibodies conjugated to fluorescein isothiocyanate. The percentage of viable protoplasts after electroporation was determined by Evan's blue exclusion. Variables that influence the optimum uptake of whole virus particles (PMMV) into protoplasts of pepper are being investigated.

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HERITABILITY OF RESISTANCE TO WINTER STRESS FACTORS IN DACTYLIS GLOMERATA. Anne Marte Tronsmo, Norwegian Plant Protection Institute, P.O.Box 70, N-1432 ÅS-NLH, Norway.

In Norwegian climate winter survival is a critical stage in the cultivation of perennial grasses. Winter injury is caused by snow molds and several abiotic factors. Improved winterhardiness is an important objectivein our grass breeding, and we try to obtain this by artificial testing. The progress depends on the variation and the heritability of the traits. Earlier investigations have shown that there exists a great variation in snow mold resistance and freezing resistance. The heritability was studied in half sib families from polycross fields of Dactylis glomerata. The broad sense heritability (h^2) for the polycross progeny were 0.69 for freezing resistance, 0.49 for resistance to Typhula ishikariensis, and 0.42 for resistance to Fusarium nivale, which indicates good possibility for progress by selection for these traits.

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RESIDUAL BLAST RESISTANCE OF NEAR-ISOGENIC RICE LINES. J.M. Bonman, T.I. Vergel de Dios, and D.J. Mackill, International Rice Research Institute, P.O. Box 933, Manila, Philippines.

A set of near-isogenic rice lines (NI) was developed through backcrossing and recurrent selection for resistance to specific isolates of Pyricularia oryzae. The recurrent parent, C039, is widely susceptible to tropical races of the pathogen. NIs were tested for complete resistance against a range of isolates, and 7 groups were identified based on reaction patterns. C039 and NIs with different resistance genes were tested for partial resistance against two compatible isolates. NIs C103TTP, C104LAC, C101PKT, and C105TTP-4 showed 29-61% fewer lesions than C039. NIs C104PKT and C102PKT differed little from C039. Thus, certain NIs have residual resistance compared to the recurrent parent. Efforts are underway to pyramid resistance genes from the NIs showing residual resistance.

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EVIDENCE FOR CYTOPLASMIC OR MATERNAL DETERMINANTS IN THE REACTION OF WHEAT CULTIVARS TO TAN SPOT. Shabeer, A., W. W. Bockus, and B. L. Norman, Dept. of Plant Pathology, Kansas State Univ., Manhattan, KS 66506.

Two tan spot-resistant winter wheat cultivars, Red Chief (RCH) and Auburn (ABRN), were crossed with susceptible TAM105. Parent and F_1 seed of reciprocal crosses was produced in the greenhouse. Plants were grown in a randomized block design with 9 replications, 10 plants/entry/replication, inoculated with a conidial suspension (13,000/ml) of Drechslera tritici-repentis, and placed in a mist chamber. The top 3 leaves of each plant were rated on a 0-5 scale. Average leaf ratings using ABRN as the resistant parent were: ABRN = 0.79, ABRN X TAM105 (F_1) = 1.80, TAM105 X ABRN (F_1) = 2.18, and TAM105 = 2.50. Average ratings using RCH were: RCH = 0.91, RCH X TAM105 (F_1) = 2.10, TAM105 X RCH (F_1) = 2.35, and TAM105 = 2.75. Ratings of F_1 plants when the resistant parent was used as a female were significantly ($P = 0.05$) less than when it was used as a male indicating reciprocal effects.

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INDEPENDENT SELECTION OF RESISTANCE TO USTILAGO SCITAMINEA AND SUGARCANE MOSAIC VIRUS (SCMV) IN SUGARCANE. M. P. Grisham, B. L. Legendre, and J. W. Dunckelman, USDA, ARS, Sugarcane Research Unit, Houma, Louisiana 70361.

Sugarcane smut caused by Ustilago scitaminea and sugarcane mosaic (SCM) caused by SCMV are important diseases of Louisiana sugarcane. One-hundred sugarcane clones from each of four biparental crosses of parents with different combinations of reactions to U. scitaminea and SCMV were evaluated for resistance to the two pathogens in separate field tests. No association was found between resistance or susceptibility to U. scitaminea and to SCMV within or among the progeny of the four crosses. The breeder can therefore concurrently select for resistance to U. scitaminea and SCMV, or independently select for resistance to one without increasing the probability of selecting for susceptibility to the other. Among the four crosses, the number of clones that developed smut was greater in one cross in which the female parent was susceptible to U. scitaminea, but the number of clones with SCM did not differ.

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The Effect of Ethylene and Silver Thiosulfate on the Disease Expression of Colletotrichum lagenarium on Cucumber Leaves. C. Biles, F. Abeles, and C. Wilson. USDA-ARS, Appalachian Fruit Research Station, 45 Wiltshire Road, Kearneysville, WV.

Cucumber seedlings were sprayed with 1mM silver thiosulfate (STS) or gassed with 100 ppm ethylene. After 24 hr, the first true leaves were inoculated with Colletotrichum lagenarium. Ethylene and water-treated control leaves exhibited high levels of lesion development, whereas, STS-treated plants had significantly fewer lesions. Tests on culture media showed that STS was not fungitoxic to C. lagenarium. STS is known to be an inhibitor of ethylene action. These data suggest that ethylene may play a role in lesion development and tissue senescence in cucumbers.

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THE REGULATION OF THE PEA DISEASE RESISTANCE RESPONSE GENE, DRRG-49, VIA BOTH AP-1 DNA ATTACHMENT SITES AND TOPOISOMERASES AND THEIR DNA CONSENSUS SITES. C. C. Chiang, A. Pettinger and L. A. Hadwiger, Department of Plant Pathology, Washington State University, Pullman, WA 99164.

Topoisomerase II consensus site clusters, which specify scaffold attachment, exist on regions 3′ and 5′ to the DRRG-49 structural gene providing the potential for a chromosomal loop. Also, AP-1 sites are present that recognize specific transcriptional proteins. In Fusarium solani challenged pea endocarp, there is a sharp increase in topoisomerase I activity and a release of chitosan heptamer. DRRG gene regulation in terms of topography and torsional stresses of chromosomal loops and by transcription factors, topoisomerases

and chitosan heptamer will be discussed. We propose the regulation of the DRRG-49 can result from major alterations in chromatin as well as from the more specific transcription factor-interaction involving DNA elements in the gene's promoter regions.

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REGULATION OF CAPSULAR POLYSACCHARIDE SYNTHESIS IN *ERWINIA* SP. BY *rcsA*. K. F. Poetter, F. R. Bernhard, and D. L. Coplin. Dept. of Plant Pathology, The Ohio State University, Columbus, OH 43210

The *rcsA* gene product activates capsular polysaccharide biosynthesis in enteric bacteria. The nucleotide sequence of the *rcsA* gene from *E. stewartii* was determined and its predicted amino acid sequence had substantial homology with the homologs of the *E. coli* (64%) and *K. aerogenes* (58%) *rcsA* genes. The 3' end of the proteins were highly conserved (79-91%). A cosmid clone from an *E. amylovora* library complemented *E. stewartii* and *E. coli rcsA* mutants and stimulated expression of *cps::lac* fusions and EPS production in these species. An *rcsA::Tn5* mutation from *E. stewartii* was crossed into the *E. amylovora* chromosome and the resulting mutant was avirulent and nonmucoid. Preliminary nucleotide sequence comparisons of the *rcsA* genes in *E. stewartii, E. amylovora,* and *E. herbicola* suggest strong conservation of *rcsA* in the genus *Erwinia*.

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QUANTITATIVE GENETIC ANALYSIS OF NORTHERN LEAF BLIGHT AND LEAF FRECKLES AND WILT RESISTANCE IN A CORN SYNTHETIC. Hidayat Rahman, M. L. Carson and Z.W. Wicks, III, SDSU, Plant Science Department, Box 2109, Brookings, SD 57007.

Two hundred North Carolina Design I progenies from SDPPS maize synthetic were evaluated during the summer of 1988 in two separate experiments for northern leaf blight (NLB) and leaf freckles and wilt (LFW) resistance at Brookings, SD. For LFW, plants were inoculated at 4 and 6 leaf stages with a mixture of four isolates of C. michiganese subsp. nebraskense. In the NLB experiment, ground corn leaf tissues infested with Exserohilum turcicum (Race I) were placed in the plant whorls at the six and eight leaf stages. Disease progress was recorded at weekly and biweekly intervals for LFW and NLB respectively. Variance component analysis of the Design I progenies was conducted to estimate type and magnitude of gene action involved in the inheritance of resistance to these two diseases. Heritability estimates and genetic correlations between reactions to these two diseases will be discussed.

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HETEROKARYOSIS AND VEGETATIVE COMPATIBILITY IN THE THREE VARIETIES OF *LEPTOGRAPHIUM WAGENERI*. P. J. Zambino and T. C. Harrington. Department of Botany and Plant Pathology, University of New Hampshire, Durham, NH 03824.

Nitrate non-utilizing mutants were selected from strains of the indigenous forest pathogen *Leptographium wageneri* on chlorate-containing media. Pairings were made between mutants of complementing phenotype on a minimal (nitrate) medium (MM). In some pairings, dense hyphal growth (complementation) occurred along the area of contact between the mutants. Subcultures from complementing areas grew both faster and denser than auxotrophic mycelia on MM. Rarely, hyphal tip (HT) transfers from complementing subcultures were prototrophic on MM, and these prototrophs had isozyme markers of both of the original mutant strains. All examined hyphal tips were multinucleate. Complementation occurred in pairings among mutants from 19 tested strains of var. *wageneri*, indicating one vegetative compatibility (VC) group. There were three geographically-separated VC groups among 20 tested strains of var. *ponderosum*. The greatest variability was found in var. *pseudotsugae*, which had 10 VC groups among 28 strains. No complementation was found between varieties.

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BIOGEOGRAPHY OF PHYTOPHTHORA CINNAMOMI MATING TYPES IN SOUTH AFRICA. S. L. von Broembsen, Dept. of Plant Pathology, Oklahoma State Univ., Stillwater, OK 74078-9947.

Mating types were determined for more than 3000 P. cinnamomi isolates from agricultural and natural ecosystems in South Africa. Certain biogeographic patterns emerged from analysis of the distribution and host associations of the A1 and A2 mating types. In indigenous forests, the A2 mating type was most prevalent, but both mating types frequently occurred together at the same sites. All isolates from native fynbos vegetation and rivers draining fynbos mountain catchments were found to be exclusively the purportedly rare A1 mating type. More than 80 native fynbos plants were recorded as new A1 hosts. Isolates from cultivated forests and agricultural crops were predominately the A2 mating type and occurred on hosts similar to those previously recorded elsewhere. The occurrence of the A1 mating type on cultivated crops was limited to proteas, grapevines and pines in regions adjacent to native fynbos vegetation.

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ISOZYME COMPARISONS OF EIGHT PYTHIUM SPECIES. W. Chen, R. W. Schneider, and J. W. Hoy, Dept. of Plant Pathol. and Crop Physiol., La. Ag. Exp. Station, La. State Univ. Ag. Center, Baton Rouge, LA 70803.

Starch gel electrophoresis of 13 enzymes was used to compare eight Pythium species (total 204 isolates) collected from North America, Hawaii, Japan, Korea and Australia. Banding patterns were determined for each isolate and similarity coefficients were calculated for each pair of isolates. The matrix of similarity coefficients was then subjected to principle component analysis. Isolates of morphological species generally formed clusters. Clusters of P. aphanidermatum and P. deliense, and P. arrhenomanes and P. graminicola could not be distinquished. P. irregulare and P. spinosum, and P. myriotylum and P. ultimum clustered independently, but overlapped. Isozyme variation within species was sometimes associated with differences in geographic origin or morphology. Host differences did not affect variation. The significance of the findings in regard to Pythium taxonomy will be discussed.

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NON-RANDOM DISTRIBUTION OF VIRULENCE AND PHENOTYPIC DIVERSITY IN TWO POPULATIONS OF PUCCINIA RECONDITA IN CANADA. J.A. Kolmer, Agriculture Canada, Winnipeg MB R3T 2M9 Canada.

The eastern and prairie populations of Puccinia recondita in Canada were examined for non-random distributions of virulence and levels of phenotypic diversity. In the prairie region where resistant cultivars have been grown and polymorphisms for unneeded virulences are low, virulences to Lr1 and Lr2a appeared to be positively associated from 1960-1974, but was negatively associated from 1975-1980. Non-random distributions of other virulences in the prairie region were also observed. In the eastern region where susceptible cultivars are grown and unneeded virulences are common, positive associations were found among virulences to Lr2c, LrB, Lr3ka, Lr11, and Lr18. Based on the infection types of the UN differentials, the eastern population had a higher level of diversity as measured by the Shannon index than the prairie population in the three years examined. The non-random distributions of virulence observed in both populations are characteristic of asexual cereal rust populations.

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INTROGRESSION OF DISEASE RESISTANCE FROM WILD RICES TO ORYZA SATIVA. R. Nelson, A. Amante, N. Oliva, R. Dalmacio, L. Sitch and H. Leung. Depts. of Plant Pathology and Plant Breeding, International Rice Research Institute, Los Baños, Philippines

Resistance to blast and bacterial blight was evaluated in 3 tetraploid wild rices - O. longiglumis, O. minuta, and O. ridleyi. Each species was resistant to a blast fungus isolate and to six races of the bacterial blight pathogen. By back-crossing and embryo rescue, BC1, BC2, and BC3 plants with different numbers of O. minuta chromosomes were obtained from O. sativa X O. minuta crosses. Five BC1 and one BC2 plants showed complete resistance to bacterial blight. Since O. longiglumis and O. ridleyi were sexually incompatible with O. sativa, attempts were made to introduce wild species DNA by transformation via the pollen tube pathway. Repetitive DNA sequences specific to the wild species were isolated as probes to follow the introgression of alien DNA. Dispersed wild species-specific sequences might eventually be used to isolate closely-linked disease resistance genes in interspecific recombinants.

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INHERITANCE OF VIRULENCE IN PHYTOPHTHORA MEGASPERMA F.SP. GLYCINEA. Ravindra G. Bhat, A. F. Schmitthenner and B. A. McBlain*, Dept. of Plant Pathology and *Dept. of Agronomy, Ohio State Univ., Wooster, OH 44691.

The genetics of host-pathogen interaction in soybean-Phytophthora megasperma f.sp. glycinea (Pmg) system has been

hampered by lack of suitable technique to clarify the pathogen's genotype. A simple and efficient method was developed to obtain inbred single oospore cultures of Pmg. Field isolates of Pmg races 1 and 4 were selfed to study the segregation of virulence genes. Host differentials for a hypocotyl inoculation test included two universal suscepts and cultivars with single resistance genes Rps1, Rps1-b, Rps1-k or Rps6. Progeny data indicated that race 1 is conditioned by a single dominant gene and avirulence is recessive. Race 4 virulence seems to be conditioned by two genes, one of which appears to be the same gene as for race 1. The dominant gene for race 1 and another gene which is recessive appear to be required for race 4 virulence.

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GLYCEOLLIN ELICITATION BY AVIRULENT SINGLE OOSPORE PROGENY OF PHYTOPHTHORA MEGASPERMA F.SP. GLYCINEA. A. F. Olah and R. G. Bhat, Dept of Plant Pathology, Ohio State Univ., Wooster, OH 44691.

Virulent and avirulent single oospore progeny of Phytophthora megasperma f.sp. glycinea were hypocotyl-inoculated into normally suceptible or resistant soybeans. After 48 hrs, growth of the fungus was measured using ELISA and glyceollin elicited was determined by HPLC. All avirulent isolates produced no disease but only some of these elicited glyceollin in amounts that would account for the lack of disease. Familial patterns for glyceollin elicited will be presented and comparison made to interactions of soybean with P. cactorum and Aspergillus sp.

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EVALUATING TOBACCO GENOTYPES FOR RESISTANCE TO *PHYTOPHTHORA PARASITICA* VAR. *NICOTIANA* USING DETACHED LEAVES. E.C. Tedford, T.L. Miller, and M.T. Nielsen. Department of Agronomy, University of Kentucky, Lexington, Ky. 40546.

A nondestructive technique to screen tobacco germplasm for resistance to *Phytophthora parasitica* Dast. var. *nicotiana* (Breda de Haan) was developed to facilitate selection in a breeding program. Leaves (7-9 cm long) were excised, sterilized for 30 sec. in 0.05% NaOCl3, and inoculated with four 1-cm-diameter mycelial plugs of 2-d-old *P. parasitica* grown on oatmeal agar. Leaves were incubated under high humidity in a growth chamber at 27 C with constant light. Number of infections and percent leaf area infected were recorded daily for six days after inoculation. Genotype rankings for infection number and percent infection were similar to field resistance rankings of the same genotypes. The new technique was not effective for detecting race specific resistance derived from *Nicotiana longiflora*.

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VEGETATIVE COMPATIBILITY GROUPS IN ASPERGILLUS FLAVUS. P. Bayman and P. J. Cotty, USDA, ARS, Southern Regional Research Center, P.O. Box 19687, New Orleans, LA 70179

Aspergillus flavus causes aflatoxin contamination of cottonseed, corn and other crops. This fungus occurs in both soil and living plant tissues. Populations of A. flavus are morphologically and physiologically diverse. However, little is known about genetic relatedness of A. flavus strains from different crops or localities. We isolated more than a hundred strains of A. flavus from cottonseed and from soils of various crops in southwestern Arizona. To estimate genetic diversity and distribution in A. flavus populations, we selected nitrate non-utilizing mutants of these strains and assigned them to vegetative compatibility groups (VCGs) via complementation tests. Most VCGs were represented by more than one isolate. Several VCGs included both soil and cottonseed isolates, or isolates from fields planted to different crops. A. flavus populations from Georgia corn were compared to those from Arizona cotton. These data provide a preliminary measure of the population structure of A. flavus and may result in strategies for control of aflatoxin.

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SEQUENCE ANALYSIS OF RIBOSOMAL RNA GENES FROM *RHIZOCTONIA SOLANI*. D. Gonzalez and R. Vilgalys. Department of Botany, Duke University, Durham NC 27706.

Restriction analyses have revealed little variation between the ribosomal RNA genes of different anastomosis groups (AG's) in *R. solani*, suggesting that these fungi are closely related. Analysis of nearly complete sequences obtained for 17S and 25S RNAs from AG 4 show high overall similarity with other fungi, and decreased similarity when compared to plants, animals and more primitive eukaryotes. We have applied the recently developed polymerase chain reaction (PCR) to enzymatically amplify and sequence phylogenetically informative DNA segments from each AG within *R. solani*. Preliminary data based on partial sequences from 25S RNA show as much as 4% nucleotide divergence between different AG's. A preliminary phylogenetic analysis based on partial PCR sequences from 6 rDNA segments (including genic as well as non-genic regions) will be presented.

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HERITABILITY OF HYGROMYCIN RESISTANCE IN TRANSFORMED STRAINS OF *FUSARIUM MONILIFORME*. John F. Leslie* and Martin B. Dickman**, *Department of Plant Pathology, Kansas State University, Manhattan, KS 66506; and **Department of Plant Pathology, University of Nebraska, Lincoln, NE 68543.

Fusarium moniliforme strains transformed with the hygromycin B phosphotransferase gene conferring resistance to 100 mg/L hygromycin B were crossed with strain FKMA-59 which is auxotrophic for both nicotinic acid (*nic*) and pyridoxine (*pdx*). Both *nic* and *pdx* segregated in Mendelian ratios following meiosis, but hygromycin B resistance segregated in a 2:1 ratio of sensitive to resistant. Some of the progeny were resistant to 250 mg/L hygromycin B, even though the parental transformants were all sensitive to this level of the drug. Hygromycin-resistant progeny from one cross were backcrossed to a wild-type strain. Progeny of these second generation crosses also showed the 2:1 segregation of sensitive to resistant, indicating that resistant progeny were not more genetically stable than the original transformants.

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ANALYZING THE GENETIC STRUCTURE OF *SEPTORIA TRITICI* POPULATIONS USING RESTRICTION FRAGMENT LENGTH POLYMORPHISMS. B. A. McDonald and J. P. Martinez. Dept. Plant Pathology and Microbiology, Texas A&M University, College Station, TX 77843-2132.

RFLPs are under development as tools for studying the population genetics of the wheat pathogen *Septoria tritici*. Probes were developed by cloning 0.5-2.4 kb fragments from a total DNA digest of *S. tritici*. Random *Sau*3A fragments cloned into the vector pGEM4 were used to probe total DNA extracted from six *S. tritici* isolates digested with six different restriction enzymes. A high level of genetic variation was observed among the six isolates tested using 23 individual probes. RFLPs were detected for 91 of the 130 probe-enzyme combinations tested. Two probes detected apparent deletions. Four probes hybridized to repetitive DNA; two of these probes may be useful for DNA fingerprinting. An rDNA probe from yeast did not detect useful polymorphisms. These RFLP probes will be useful for studying genetic variation, genetic relatedness and gene flow in *S. tritici* populations.

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TOXICITY AND TOXIN PRODUCTION OF Fusarium ISOLATES FROM NORTHEASTERN CHINA. Weiping Xie and Chester J. Mirocha, Department of Plant Pathology, University of Minnesota, St. Paul, MN 55108, USA; Hangqing Li and Xue Wang, Department of Biology, Heilongjiang University, Harbin, P.R. China.

One hundred and two isolates of Fusarium spp. were isolated from soil, corn and wheat samples collected from northeastern China.

Rice cultures of the isolates were checked for toxicity in rat feeding tests. Twenty-five isolates acutely toxic to rats were analyzed for toxin production by thin layer (TLC) and gas chromatography (GC). The mycotoxins found were T-2 toxin, HT-2 toxin, scirpentriol, monoacetoxyscirpenol, diacetoxyscirpenol, neosolaniol, acuminatin, 8-acetoxyneosolaniol, 8-acetoxy T-2 tetraol, deoxynivalenol, 15-acetyl deoxynivalenol and zearalenone. The identities of the toxins were confirmed by gas chromatography-mass spectrometry (GC-MS). All the isolates analyzed were negative for wortmannin and fusarochromanone.

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SURVIVAL OF ERWINIA CAROTOVORA ON TOMATO LEAVES. J. A. Bartz and D. E. Concelmo. Plant Pathology Dept., Univ. of Florida, Gainesville, 32607.

Erwinia carotovora survived at least 20 days in wounds on leaves of greenhouse-grown tomatoes, but seldom more than 6 days on nonwounded tissues. About 10% of 10^6 cfu deposited on a pin-puncture wound were recovered after the leaf surface had dried; only 0.4% or less were recoved from a similar deposition on nonwounded leaves. Wounds infested with 10^3 cfu did not have detectable populations (> 10 cfu) immediately after the suspension dried. However, populations up to 7 X 10^2 cfu were found after 6 days. Survival for 20 days was associated with the larger initial population, but such populations did not appear to increase. Recovery of the E. carotovora was enhanced if leaves were exposed to dew before being sampled. Asymptomatic tomato leaves can support epiphytic populations of E. carotovora that may eventually contaminate or inoculate fruit.

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GLYCOSIDE FORMATION OF THE MYCOTOXIN ZEARALENONE IN LIQUID FERMENTATION BY Rhizopus sp. J. Plasencia and C. J. Mirocha. Department of Plant Pathology, University of Minnesota, St. Paul, MN 55108.

When a Rhizopus sp. is incubated in presence of zearalenone, two major conversion products are found in the culture medium. Normal phase thin layer chromatography of the two products in chloroform-methanol (4:1) give Rf values of I (0.32) and II (0.26) indicating their high polarity as compared with zearalenone. Treatment of the products with β-glucosidase yielded zearalenone as the aglycon and a carbohydrate identified as D-glucose by mass spectrometry. Highest activity of the enzymes involved in the bioconversion is found within the mycelium. Both products are found in larger amounts intracellularly than outside the mycelium. This observation lead to the isolation of cell-free extracts which contained the most active enzyme fraction.

The bioavailability of zearalenone in its conjugate form to laboratory rats will be studied.

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BIOCONTROL OF POSTHARVEST ROTS OF PEACH AND APPLE WITH THE YEASTS HANSENIASPORA UVARUM AND DEBARYOMYCES HANSENII. R. J. McLaughlin, M. E. Wisniewski, C. L. Wilson, and E. Chalutz*, USDA-ARS, 45 Wiltshire Rd., Kearneysville, WV 25430, and *Volcani Center, Bet Dagan, Israel 50250.

Peach and apple fruit were artificially wounded, treated with 10^6-10^9 cfu/ml suspensions of eight yeast strains (H. uvarum, D. hansenii, or Zygosaccharomyces rouxii), and challenged with 10^3-10^5 spores/ml of two postharvest fungal pathogens for each fruit. Strain 138 of H. uvarum was most effective in reducing Rhizopus rot in peach. Marginal reduction of brown rot of peach and Penicillium rot of apple was conferred by several strains, including D. hansenii strain US-7. Strain 138 and D. hansenii strains US-7 and 101 significantly reduced Botrytis rot in apple. Biocontrol ability of yeast strains was affected by culture age, cell concentration of the yeast and pathogen, timing of challenge inoculation, and presence of $CaCl_2$ (0, 1, and 2%) in the yeast cell suspensions.

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CONTROL OF STORAGE ROTS OF POME FRUITS WITH PYRROLNITRIN ISOLATED FROM ANTAGONISTIC PSEUDOMONAS CEPACIA. W. Janisiewicz and L. Yourman, USDA, ARS, AFRS, Kearneysville, WV, and J. Roitman and N. Mahoney, USDA, ARS, WRRC, Albany, CA.

Pyrrolnitrin, isolated from Pseudomonas cepacia which is known to control grey-mold (Botrytis cinerea) and blue-mold (Penicillium expansum) on pome fruits after harvest, was used for control of these diseases on wounded pears and apples. Three types of wounds were made: cut, nail, and bruise (with broken skin). The fruits were dipped in a conidial suspension (1 x 10^4 conidia/ml) of the pathogen with various concentrations of pyrrolnitrin ranging from 6-200 ug/ml. Following treatment, one-half of the fruit was stored at 24 C for 6 days and the other half at 2 C for 30 days, after which the diameter of the wound originating rots was measured. Complete control of both diseases was achieved under both storage conditions. However, higher concentrations of pyrrolnitrin were required for control at 24 C. The wound type had a profound effect on the control; cut wounds were the easiest and bruise wounds most difficult to control.

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Detection of Aspergillus flavus in soil, corn debris and non-corn debris from Iowa corn fields. J. F. Shearer, N. K. Baker, L. E. Sweets and L. H. Tiffany. Iowa State University, Ames, IA 50011.

Samples of soil and of stalk and cob debris were collected from 40 corn fields in eight Iowa counties following the 1988 harvest. Non-corn debris samples were collected from fencelines, waterways or ditches adjacent to 11 of the fields. Soil samples were tested for Aspergillus flavus by sprinkling approximately 0.5 gm of soil onto M3S10B agar plates. After a three day incubation period at 37° C, plates were visually examined for A. flavus colonies. A. flavus was detected in all 40 soil samples. Pieces of cob and stalk pith approximately 1 cm in diameter were pulled from freshly broken samples and plated on M3S10B agar plates. Plates were incubated at 37° C for 3 days and visually examined for presence of A. flavus colonies. Seventy percent of the cob pieces and 42 percent of the stalk pieces were positive for A. flavus. When random pieces of non-corn debris were tested on M3S10B agar, 57 percent were positive for A. flavus.

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HYDROSTATIC PRESSURE AND WOUND DIAMETER INFLUENCE ENTRY OF FUNGAL SPORES INTO PEAR WOUNDS DURING IMMERSION DUMPING. David Sugar and R.A. Spotts, Oregon State University, Southern Oregon Experiment Station, Medford, OR 97502.

Wounds in pear fruit are important infection courts for fungi causing postharvest decay of pear, and dump tanks harbor spores which may contact wound tissue during immersion dumping. Wound diameter and hydrostatic pressure due to immersion depth affect penetration of spores of Penicillium expansum and Phialophora malorum into wounds. Pears with wounds 0.4, 0.5, 1, 2 and 6 mm diameter x 2 mm depth were immersed 2 min at depths of 0, 10, 20, 40, 60, 80 and 100 cm in water containing 10^5 spores of either fungus. At wound diameters < 2 mm, incidence of infection increased with depth. Infection of wounds 6 mm in diameter was independent of immersion depth.

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COMPARISON OF CEREAL SEEDLING BIOASSAYS FOR Fusarium TOXINS. U. Bosch, D. R. Johnson, C. J. Mirocha and J. A. Percich, Department of Plant Pathology, University of Minnesota, St. Paul, MN 55108.

Mycotoxin bioassays using seedlings of barley (Hordeum vulgare 'Larker'), rice (Oryza sativa 'Lemont'), wheat (Triticum aestivum 'McNair 701') or wild rice (Zizania palustris 'Meter') were evaluated for sensitivity to the Fusarium toxins deoxynivalenol (DON) and T-2. Ten germinating seeds of each cereal were grown in DON or T-2 solutions of 20, 10, 2, 1, 0.8, 0.6, 0.4, 0.2 or 0.1 µg/ml. Coleoptile lengths were measured after 2 days (barley, wheat and wild rice) or 3 days (rice). The no-effect level for DON and T-2, respectively, were 0.2 and 0.2 µg/ml for wild rice, 2.0 and 4.0 µg/ml for wheat, 6.0 and 4.0 µg/ml for barley and 6.0 and 1.0 µg/ml for rice. All bioassays provided simple visual indicators for the two toxins, but the sensitivity of the wild rice assay permitted detection of ng amounts.

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TOXICITY TO DUCKLINGS OF FUSARIUM NYGAMAI ISOLATED FROM MILLET SEED FROM NIGERIA. N. B. Onyike, P. E. Nelson, Dept. of Plant Pathology, Penn State Univ., University Park, PA 16802, and W. F. O. Marasas, South African Med. Res. Coun., Tygerberg 7505.

Pearl millet, Pennisetum typhoides, was collected in Nigeria from seed stored in homes, sold in markets, or left unharvested in the field. All Fusarium cultures obtained from 100 seed of each of 7 samples cultured on a pentachloronitrobenzene selective medium were identified. The most prevalent species recovered was F. nygamai which made up 42.4% of the total. These cultures consisted of strains that produced short or long chains of microconidia and 45 cultures representing both strains were selected for toxicity tests. Cultures were grown on autoclaved yellow corn kernels in 2 liter flasks & incubated at 25C for 3 wks. The medium was dried at 55C for 24 hr and ground into a fine meal. The moldy meal was mixed 50% by weight with commercial chicken mash, and fed to ducklings ad lib. Of the cultures tested, 94% were toxic, causing the death of all 4 ducklings in 2.5 days with an average feed intake of 26 g.

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A RAPID QUANTITATIVE IMMUNOASSAY FOR ERGOTAMINE. R. A. Shelby and V. C. Kelley, Departments of Plant Pathology and Botany and Microbiology, Auburn University, AL 36849.

Anti-ergotamine antibodies were produced by immunizing rabbits with bovine serum albumin-ergotamine conjugate. The resulting partially purified polyclonal antiserum was used to develop a quantitative competitive inhibition (CI) ELISA capable of detecting ergotamine at the nanogram level. In cross-reactivity tests with 22 related alkaloids there was measurable reactivity only with ergotamine, ergocristine, and ergostine. Using the assay it was possible to measure ergotamine in spiked samples of wheat, rye and millet. Ergot (Claviceps purpurea) sclerotia were detectable in wheat flour at a level of 0.1% w/w. Quantitative measurements of ergotamine in ergot sclerotia of wheat and fescue as well as endophyte (Acremonium coenophialum) infected fescue seed were in the range reported for other detection methods.

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A MODEL THAT PREDICTS ALLOWABLE STORAGE TIME OF DRY EDIBLE BEANS BASED ON FACTORS INFLUENCING INVASION BY STORAGE FUNGI. J. D. Pokorny and R. A. Meronuck, Department of Plant Pathology, University of Minnesota, St. Paul, MN 55108.

A model has been developed that predicts allowable storage time for dry edible beans (Phaseolus vulgaris) stored at 80 and 85% relative humidities and at 18, 24 and 30°C. The beans used to develop the model were collected from warehouse, cooperative and farm locations in low, middle and high altitude regions of Rwanda, Africa. The percent of surface disinfected seeds which yielded Aspergillus glaucus in culture (r^2=.6383), length of previous storage (r^2=.6459), and the relative humidity of future storage (r^2=.3393) were selected as significant predictor variables for determining allowable storage time on the basis of stepwise regression analysis. Allowable storage time was determined from when the beans became a substandard grade. The model is designed to predict storability for storage lengths up to 6 months and shows potential as a management tool to assist tropical and developing countries in the safe storage of dry edible beans.

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EFFECTIVENESS OF FUNGICIDES FOR CONTROL OF MYCOTOXIGENIC FUNGI AND MYCOTOXINS IN PEANUTS. K. L. Bowen and P. A. Backman, Dept. of Plant Pathology, Auburn University, AL.

The fungicides, terbutrazole and flutolanil, used for control of southern stem rot, (Sclerotium rolfsii), and limb and pod rot (Rhizoctonia solani) in peanuts, have also been found to reduce fungal damage affecting seed quality. Peanut pods harvested from plots treated with foliar applications of these fungicides were evaluated for levels of infestation by Aspergillus spp., other seed-borne fungi, and aflatoxins. Flutolanil-treated peanuts showed no consistent differences in Aspergillus spp. incidence or aflatoxin levels measured by ELISA when compared to chlorothalonil or nontreated controls. However, terbutrazole-treated peanuts had 56.4% less kernel infection by Aspergillus spp. than peanuts receiving standard foliar sprays for leafspot control with chlorothalonil. Measured aflatoxin concentrations in terbutrazole treated peanuts were 25% lower than in chlorothalonil treated peanuts.

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TRANSMISSION STUDIES OF MAIZE WHITE LINE MOSAIC VIRUS. R. Louie, J. J. Abt, and J. K. Knoke, USDA/ARS and Ohio State Univ., Wooster, OH 44691.

Maize white line mosaic virus (MWLMV) was transmitted from MWLMV-infected roots to roots of Seneca Chief sweetcorn seedlings in a modified slant-board hydroponic system. Transmission was accomplished by direct placement of root inoculum onto seedling roots. ELISA was used to determine root infection and it consistently detected MWLMV in roots of inoculated seedlings treated with Tilt, Benlate, Diazinon, Ridomil, gentamycin, chloramphenicol, vancomycin, streptomycin, or lincomycin-spectinomycin. Similar frequencies of transmission (90-100%) occurred in roots inoculated with inoculum placed 1-2 cm distance from test seedlings, inoculum contained in polycarbonate bag filters (pbf) of 0.2-8.0 µm pore sizes, 0.1-0.3 gm of inoculum, and inoculum heated at 30-60 C. MWLMV transmission was reduced to 30% when inoculum was contained by a pbf of 0.05 µm pore size.

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BEAN MILD MOSAIC VIRUS AS A CONTAMINANT IN GREENHOUSE VIRUS STUDIES. P. Sepulveda and A.W. Saettler. ARS, USDA, Dept. of Botany and Plant Pathology, Michigan State University, East Lansing, MI 48824.

During host range studies of several strains of bean yellow mosaic virus (BYMV), we observed virus symptoms in several plant species reported to be resistant to BYMV. We also observed mild mosaic symptoms on a number of the noninoculated control bean plants. Mild mosaic symptoms developed in plants of the Domino bean cultivar ten days after inoculation with sap from the control plants exhibiting symptoms. Large numbers of spherical virus particles, 28 nm diameter, were observed with TEM in leaf dip preparations negatively stained with 2% ammonium molybdate. Identity of the spherical particles as bean mild mosaic virus (BMMV) was determined using agar gel double diffusion and immunosorbent electron microscopy; antisera was provided by Dr. F. Morales, CIAT. Further studies revealed the presence of BMMV as a contaminant in several of our strains of BYMV.

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A NEW VIRAL DISEASE OF CYAMOPSIS TETRAGONOLOBA (L) TAUB. M.E.C. Rey and H Ben-Moshe. Department of Microbiology, University of the Witwatersrand, Johannesburg, P O Wits, 2050.

Cyamopsis tetragonoloba (L) Taub. (guar) was found to exhibit symtoms of reduced leaf size and inflorescence number (pods exhibited 50% sterility) and often the stems elongated and remained green after the plant had set seed. Investigations revealed a flexuous rod (750 x 15nm) which is transmissible to several Phaseolus vulgaris cultivars, Vigna unguiculata and Lycopersicum esculentum L. by mechanical inoculation. Symptoms included systemic red veins and chlorosis. Nonpersistent transmission with the aphid, Myzus persicae (Sulzer) was also successful in some cases. ELISA tests, and immunoblots of polypeptide bands separated by PAGE gave positive results with several potyvirus (BCMV, BYMV, SMV and PVY) antisera. These symptoms have been named "green sterile" and "little leaf" and are unlike any other viral diseases reported in guar. These symptoms appear to be associated with a potyvirus but confirmation of this virus being the sole causal agent is required.

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A GENE FOR RESISTANCE TO WHEAT STREAK MOSAIC VIRUS ON CHROMOSOME 6 OF MAIZE. M. D. McMullen[1] and R. Louie[2], USDA-ARS, Depts. of Agronomy[1] and Plant Pathology[2], OARDC, The Ohio State University, Wooster, Ohio 44691

Wheat streak mosaic virus (WSMV) induces generalized mosaic symptoms in selected maize inbreds. During 1988, WSMV was detected in many lines in our maize nursery. WSMV symptoms were associated with the expression of the polymitotic (po) marker in a B73 genetic background. The polymitotic locus is on the short arm of maize chromosome 6. An isolate of WSMV from these naturally-infected plants was used to mechanically inoculate maize plants segregating po/po or po/+. After inoculation, all po/po plants (10) exhibited generalized mosaic symptoms, all po/+ plants exhibited either symtomless (14) or delayed, limited symptom (5) responses. All B73 plants (+/+) were symptomless. These results indicate that there is a gene on chromosome 6 affecting resistance to WSMV. RFLP analysis places this gene either on the short arm of chromosome 6 or on the long arm proximal to the RFLP marker locus UMC-59.

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A VIRUS DISEASE COMPLEX OF SWEET POTATO FROM PUERTO RICO.

M. Laakso and J. W. Moyer, Dept. of Plant Pathology, North Carolina State University, Box 7616, Raleigh, NC 27695-7616.

We report the etiology of a disease of sweet potato which occurs in Puerto Rico. This disease is the result of a synergistic interaction between sweet potato feathery mottle virus (SPFMV) and a nonmechanically transmitted virus-like agent. The predominant symptoms of the disease complex consist of severe stunting and general chlorosis of sweet potato. This is in contrast to the typical symptoms associated with SPFMV. Symptoms in sweet potato and I. setosa caused by SPFMV alone were consistent with those caused by other isolates of SPFMV. The virus-like agent causes small diffuse chlorotic spots and ringspots in sweet potato and a distinct mosaic in I. setosa. Antiserum which reacts with a 29 kd protein has been prepared from partially purified preparations and a cDNA cloned which are both specific for plants infected with the nonmechanically transmitted agent.

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IDENTIFICATION AND INCIDENCE OF CUCURBIT VIRUSES IN SOUTH LOUISIANA. F. J. Fernandes, 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, LA 70803.

A survey for viral diseases was conducted during 1988 in the cucurbit producing areas of south Louisiana. Samples from plants showing virus-like symptoms were collected from different locations and tested for the presence of five cucurbit viruses using ELISA and host reaction. Identification of the viruses was confirmed by dsRNA analysis and electron microscopy. Out of 178 samples analyzed, 104 (58%) were found to be infected with papaya ringspot virus (PRSV), 69 (39%) with watermelon mosaic virus (WMV), 68 (38%) with zucchini yellow mosaic virus (ZYMV), 60 (34%) with cucumber mosaic virus (CMV), and 13 (7%) with tobacco ringspot virus (TRSV). Mixed infections were common. Crops tested included squash, watermelon, cantaloupe, and cucumber.

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SEVERE MAIZE CHLOROTIC DWARF DISEASE CAUSED BY DOUBLE INFECTION WITH MILD VIRUS ISOLATES. R. E. Gingery[1] and L. R. Nault[2], USDA-ARS and Department of Plant Pathology[1], and Department of Entomology[2], The Ohio State University-Ohio Agricultural Research and Development Center, Wooster, OH 44691.

Two maize chlorotic dwarf virus (MCDV) isolates that caused only mild symptoms in maize were isolated from johnsongrass and independently maintained in maize by serial transfer with leafhopper vectors. Isolate #1 differed from isolate #2 by producing milder vein-clearing, less stunting, and delayed anthesis. Double infection with isolates #1 and #2 in the greenhouse produced severe stunting and leaf-tearing symptoms similar to those attributed to MCDV in the field. Both isolates reacted with anti-MCDV serum in ELISA tests. The molecular weights of two of the three capsid proteins of isolate #1 were different from those of the corresponding proteins of isolate #2. Because of these differences, plants could be scored for infection with isolate #1 by SDS-polyacrylamide gel electrophoresis of isolated virions.

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USE OF MONOCLONAL ANTIBODIES IN THE STUDY OF CLOSTEROVIRUSES ASSOCIATED WITH GRAPE LEAFROLL DISEASE. J. S. Hu, D. Boscia, and D. Gonsalves, Dept. of Plant Pathology, Cornell University, NYSAES, Geneva, NY, 14456, USA.

Stable hybridoma cell lines secreting monoclonal antibodies (mAB) to the NY-1 isolate of closteroviruses associated with grape leafroll disease (GLRaV) were produced. The mAB reacted with the NY-1 isolate and several serologically related isolates, but not with isolates in other serotypes. The reactions were the same in 5 different kinds of ELISA, ISEM, dot-immunoblotting, and Western blotting assays. The serological reactivities of the mAB were found to be as good as those of rabbit polyclonal antibodies for the detection of the virus in grape leaf tissue in double antibody sandwich direct ELISA. With the double gold labelling electron microscopy technique, we were able to detect mixed infections of grapevines with different serotypes of GLRaV. A very sensitive Western blotting assay was used to estimate the molecular weight of virus coat protein of the GLRaV from partially concentrated samples using the mAB.

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THE ULTRASTRUCTURAL ASPECTS OF INFECTION OF *ZEA MAYS* CV. SILVER QUEEN BY SORGHUM YELLOW BANDING VIRUS (SYBV). C.E. McClelen and R.W. Toler, Texas A&M University, College Station, TX 77843

Rapidly expanding leaves from *Zea mays* cv. 'Silver Queen' inoculated 30 days prior with SYBV and healthy controls, were ultrastructurally viewed and compared to determine cytological effects and cellular location of the virus. Specific infection by SYBV was confirmed by Ouchterlony tests. SYBV was found throughout the infected leaves in all cell types. The highest concentrations were observed in the epidermal and parenchyma cells. The virus was packed into large vesicles which had expanded into the vacuolar space and cytoplasmic areas adjacent to the chloroplasts and nuclei. A large reduction in starch accumulation was noted in the chloroplasts of infected leaves. Mitochondria appeared swollen and contained reduced and irregular cristae. SYBV was associated with and seen in the endoplasmic reticulum. No viral particles were detected in the nuclei.

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A NEW SOURCE OF WHEAT STREAK MOSAIC VIRUS RESISTANCE. N. A. Tuleen and R. W. Toler, Department of Soil and Crop Sciences and Department of Plant Pathology and Microbiology, respectively, Texas A&M University, College Station 77843.

Eight accessions of *Agropyron intermedium* from Iran and Russia were crossed to *Triticum aestivum* cv Chinese Spring. The 42 chromosome F_1 hybrids displayed a high level of resistance to wheat streak mosaic virus (WSMV). These F_1 plants were pollinated by Chinese Spring and plants with 61-63 chromosomes saved and then backcrossed to Chinese Spring for two or three generations. Resistant progeny with the lowest chromosome number were selected for additional backcrossing. Resistant monosomic chromosome additions, ie. plants with 21" of wheat chromosomes and a univalent chromosome from *Agropyron* were obtained from crosses of three different accessions; two from Iran and one from Russia. Disomic additions were selected in the self progeny. Two of the additions were morphologically similar and may involve the same Agropyron chromosome, while the third had a different head type. In crosses involving the remaining accessions, plants with lower chromosome numbers were associated with a lower level of resistance to WSMV.

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RELATIVE IMPORTANCE OF TRANSIENT VERSUS RESIDENT APHIDS IN THE SPREAD OF MAIZE DWARF MOSAIC VIRUS IN SORGHUM. J. D. Alexander and R. W. Toler, Dept. of Plant Path. and Microbiol., TAES, TAMU, College Sta., TX. 77843.

Spread of maize dwarf mosaic virus, strain A (MDMV-A), in sorghum by aphids was investigated. Two types of sorghum, one susceptible to infection by MDMV-A via aphid vectoring (Pioneer 8199) and one relatively resistant to infection by MDMV-A (Tx2786) were planted in pure stands as well as mixed stands of approximately 25:75 and 60:40 %, respectively. Half of the plots were treated with systemic insecticide (Aldicarb). Disease incidence was recorded 10 weeks after emergence. Regression analyses showed the relationship between plant mixture proportions and the infection levels to be highly linear, indicating no dilution of the rate of virus spread among the susceptible plants. This suggests that the mode of any secondary spread typically spanned, and possibly included, several plants at a time since intervening resistant plants did not affect the virus spread. The insecticide treatment resulted in a 3% average increase in disease. These results are consistent with virus spread mainly due to the series probing activities of transient aphids whose activity may be aggravated by insecticides, rather than by aphids colonizing infected plants and then spreading the virus.

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ENDOGENOUS ANTIVIRAL PROTEINS: IMPLICATIONS FOR SELF-RECOGNITION. Meyer Chessin and Allan Zipf, Division of Biological Sciences, University of Montana, Missoula, Montana 59812.

Many higher plants contain endogenous proteinaceous inhibitors of virus infection which are typically ineffective when assayed on the source host. An inhibitor from Datura stramonium has been tested on a wide host range, and its specificity is manifested at the intrageneric level using TMV as test virus. The mechanism of specificity of the pokeweed inhibitor may involve ribosome function since it reduces cell-free protein synthesis using wheat ribosomes but not with pokeweed ribosomes. Recent work with the Datura protein suggests that it acts as a competitive inhibitor of virus establishment. Can differences in cellular receptors for virus therefore account for some of the specificity, as well?

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CHARACTERIZATION OF EXTRACELLULAR PECTIC ENZYMES PRODUCED BY STREPTOMYCES SPECIES. F.R. Spooner Jr. and R. Hammerschmidt. Department of Botany and Plant Pathology, Michigan State University, East Lansing, MI 48824.

Isolates of Streptomyces pathogenic on potato (Solanum tuberosum L.) and nonpathogenic isolates of Streptomyces were observed to produce extracellular pectinases when grown in medium containing 1% polygalacturonic acid. Enzyme activity was detected for all isolates by thiobarbituric acid, reducing sugar and viscometric assays. Pectolytic activity was optimal at pH 8.4 and was stimulated by millimolar concentration of calcium. Pectolytic activity was minimal or nonexistent at pH 4.8 for most isolates. This is indicative of pectate lyase activity with minimal polygalacturonase. The production of extracellular pectinases did not correlate with pathogenicity on potato for Streptomyces species.

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BACTERIAL STUNT DISEASES OF BLUE GRASS AND BERMUDA IN CALIFORNIA AND THEIR CONTROL BY HARRY FERTILIZER AND PLANT PROTECTANT. M.J.Thirumalachar, Jeersannidhi Anderson Institute,POB-506, Locust st.Walnut Creek, CA 94596 and Marvin D.Whitehead, 817 Clifton Rd.,N.E.,Atlanta, GA 30307.
Xylem limited bacterium Clavibacter (=Burkholderella)xyli subsp. cynodontis causing Bermuda grass stunt disease is sparsely distributed in Contra Costa ,but a similar stunt disease of Poa pratense L.disfiguring lawns with linear rows of dead fuzzy stolons and brown patches is widespread.The identity of the causal organism which is close to the Bermuda stunt organism is being determined.Lawn mowing machines and sprinklers spread the infection. Harry Fertilizer and Plant Protectant (HF-PP) developed at Jeersannidhi Anderson Institute is a phytobactericide and fungicide containing organic and inorganic nitrogen sources as plant food.Spraying HF-PP at 40 cc per gallon of water after lawn mowing,makes cut plant parts and roots absorb HF-PP and lawn healthy and uniformly green again.

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HYPOXIC RESISTANCE IN POTATO TUBERS TO THE SOFT-ROT BACTERIUM Erwinia carotovora subsp. carotovora.L. S. Antonov, M. E. Vayda, and G. H. Lacy, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061-0330.

Potato tubers placed on wire mesh above water in closed containers were incubated aerobically or hypoxically by bubbling air or argon, respectively, into the water at about 60 cc/min. Under hypoxic conditions, the 0_2 concentration reached 2.2%. Inoculated tubers rotted much more in hypoxic than in aerobic conditions. Incubation in hypoxic conditions prior to inoculation with bacteria and rotting in hypoxic atmosphere made the tubers resistant to soft rot to a degree comparable with aerobic resistance. This hypoxic resistance increased with increasing times of preincubation. Bacteria grown in hypoxic conditions were able to overcome hypoxic resistance. Cycloheximide applied before hypoxic preincubation eliminated hypoxic resistance but had no effect when applied at inoculation. We conclude that during hypoxic preincubation some proteins crucial for the hypoxic resistance accumulate in potato tubers.

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RESISTANCE OF PELARGONIUM SEEDLINGS TO BACTERIAL BLIGHT OF GERANIUM. K. B. Dunbar, and C. T. Stephens, Dept. of Botany and Plant Pathology, Michigan State University, East Lansing, MI 48824.

Five-week-old seedlings of Pelargonium species growing in culture tubes containing 15 ml of Hoagland's solution solidified with 0.7% agar were inoculated with a suspension of Xanthomonas campestris pv. pelargonii cells. Eight weeks after inoculation all seedlings from susceptible P. x hortorum cultivars were dead or severely blighted. Seedlings of P. capitatum, P. frutetorum, P. fulgidum, P. fruticosum, P. inquinans and P. zonale were not significantly different from P. x hortorum in reaction to X. c. pv. pelargonii. However, Pelargonium reniforme seedlings were significantly more resistant than P. x hortorum, and P. cordifolium seedlings were significantly more resistant than P. reniforme. This is the first reported screening of P. cordifolium for resistance to bacterial blight. This species may be useful for breeding resistance into horticulturally important cultivars.

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SPREAD OF BACTERIAL SPOT DURING THINNING OF DIRECT-SEEDED TOMATOES AND MANAGEMENT WITH BACTERICIDE HAND WASHES. Ken Pohronezny, Michael A. Moss, Wilbur Dankers, and James Schenk, University of Florida, IFAS, Tropical Research and Education Center (TREC), Homestead, FL 33031.

The spread of bacterial spot during thinning of direct-seeded tomatoes was studied in two experiments at TREC. In late spring 1988 (warm and humid), bacterial spot incidence was less when plants were thinned in the afternoon after foliage was dry (44%) vs. those thinned in the morning when laden with dew (87%). In non-thinned rows, disease incidence was as low as 5%. Ethanol (70%) and 10% povidone-iodine, applied as hand washes between exposure to diseased foliage and thinning of hills, reduced disease incidence 65% and 81%, respectively. Ethanol or povidone-iodine hand washes reduced populations of the pathogen (10^6 cfu/ml) by 97% or more in most cases. In late fall 1988 (cool and dry), disease incidence as reduced from 55% to zero by simply waiting until plants were dry before thinning.

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COMPARISON OF RESISTANT AND SUSCEPTIBLE LETTUCE CULTIVARS TO CORKY ROOT IN FLORIDA. L. E. Datnoff and R. T. Nagata, University of Florida, Everglades Research and Education Center, Belle Glade, 33430.

Corky root of lettuce (Lactuca sativa L.)is a disease caused by a gram negative bacterium. Little information is available on yield losses due to this disease in commercial lettuce production, especially on organic soils. Susceptible ('Ithaca' and 'Shawnee') and resistant ('Raleigh' and 'Southbay') crisphead lettuce cultivars were planted in a randomized complete block design in fields with various cropping histories. Root disease severity was significantly lower ($P \leq 0.05$) on the resistant cultivars (4.6 and 4.5) in comparison to the susceptible cultivars (9.9 and 9.8) based on a rating scale of one to ten. Total fresh and marketable trim weights of the susceptible cultivars were reduced 37% and 46%, respectively, when compared to the resistant types.

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MONITORING THE EPIPHYTIC SURVIVAL OF THE HALO BLIGHT ORGANISM ON BEANS WITH A PHASEOLOTOXIN GENE HYBRIDIZATION PROBE. A. J. Olson[1], D. K. Arora[1], and N. W. Schaad[2]. [1]Div. Plant Path./PSES, Univ. of Idaho, Moscow, ID 83843; [2]Harris Moran Seed Co., San Juan Bautista, CA 95045.

A phaseolotoxin gene DNA hybridization probe was used to monitor survival of Pseudomonas syringae pv phaseolicola (Psp) inoculated on beans grown under furrow irrigation. Leaves were periodically collected and washings plated on MSP semiselective agar. Suspect colonies were purified, tested for pathogenicity, spotted on membranes and probed. The number of Psp cfu/cm^2 leaf area after 0, 7, 21, 42 and 63 days were 120, 1600, 18, .65 and .99 x 10^3 in inoculated plots and 0, 17, 5, 1 and 2 in uninoculated plots, respectively. No symptoms of halo blight were observed. Almost all pathogenic isolates tested were probe positive while all nonpathogenic isolates were probe negative.

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SELECTIVE CHEMICALS FOR ISOLATION OF XANTHOMONAS CAMPESTRIS PV. ORYZAE (ISHIYAMA) DYE. Wuqiao Yuan, Department of Plant Pathology & Physiology, Clemson University, Clemson, SC 29634

Cephradine (40 ug/ml), cephaloridine (1 ug/ml), crystal violet (4 ug/ml), methyl violet (3 ug/ml) and nalidixic acid (4 ug/ml) all permitted growth of Xanthomonas campestris pv. oryzae in liquid media and were tested separately by replica plating with 5 chinese and two IRRI (PSC-79 & PXO-86) strains of X. c. oryzae and 34 bacteria from rice seeds. All chemicals permitted growth of all X. c. oryzae strains. Crystal violet inhibited all 7 $Gram^+$, 7 of 17 non-yellow, $Gram^-$ and 1 of 6 yellow, oxidative, $Gram^-$ bacteria while methyl violet inhibited 4 of the same $Gram^+$ and 4 of the same non-yellow, $Gram^-$ bacteria. Nalidixic acid (1 ug/ml) inhibited all 3 yellow, fermentative, $Gram^-$ bacteria. Cephradine inhibited 7 non-yellow, $Gram^-$ and 5 $Gram^+$ bacteria while cephaloridine inhibited 4 of the same non-yellow, $Gram^-$ and 3 of the same Gram+ bacteria. Five yellow, oxidative, $Gram^-$ bacteria were not suppressed by the chemicals. Cycloheximide (>100 ug/ml) inhibited X. c. oryzae. Combinations of chemicals should be tested for isolation of X. c. oryzae.

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CONTROL OF WATERMELON FRUIT BLOTCH BY SEED HEAT-TREATMENT. G. C. Wall, Agricultural Experiment Station, College of Agriculture and Life Sciences, University of Guam, Mangilao, GU, 96923.

Pseudomonas pseudoalcaligenes subsp. citrulli is the causal agent of fruit blotch, foliar lesions, and seedling blight on watermelon. Seed infested with this pathogen develops into infected seedlings. Symptoms are first apparent as watersoaked lesions on the cotyledons. Most infected seedlings die, as the infection spreads into the stem. Splashing water carries the inoculum to other nearby plants. Surviving plants, and those infected later, harbor the bacteria in foliar lesions. Under prolonged rainy weather and warm temperatures, the pathogen attacks the fruit. Infected fruit have a reduced shelf life. Other fruit in contact with these may also become infected. Heat treatment of infested seed at 50° C for 20 min effectively controlled disease development in seedlings in a completely randomized experiment with 4 replications, repeated 3 times. Seed were heat-treated by placing them in gauze sacks and immersing in a waterbath held at constant temperature, then growing in pots in the greenhouse.

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STRAINS OF XANTHOMONAS CAMPESTRIS ISOLATED FROM AMBARELLA (SPONDIAS CYTHEREA SONN.) IN THE FRENCH WEST INDIES BELONG TO PV. MANGIFERAEIDICAE. O. Pruvost, IRFA/CIRAD, Laboratoire de Phytopathologie, B.P. 180, 97455 SAINT PIERRE Cedex, Ile de la Reunion; and J. Luisetti, INRA, Station de Pathologie vegetale, Route de Saint Clement, Beaucouze, 49000 ANGERS, France. (O. Pruvost)

Based on pathogenicity on Mango (Mangifera indica L.) and Cashew (Anacardium occidentale L.), apigmented Xanthomonas campestris strains isolated from Ambarella (Spondias cytherea Sonn.) in the French West Indies were identified as pv. mangiferaeindicae. Mango isolates of that pathovar, however, are avirulent on Ambarella and on Mombin (Spondias mombin). Thus, these Ambarella strains are probably new pathogenic forms of Xanthomonas campestris pv. mangiferaeindicae.

The relationship of the Ambarella strains to Mango under natural conditions in the French West Indies needs to be determined.

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STABILITY OF ANTIGENIC DETERMINANTS OF XANTHOMONAS CAMPESTRIS PV. ORYZAE DETECTED BY MONOCLONAL ANTIBODIES. P. D. Roberts, A. M. Alvarez, and A. A. Benedict. University of Hawaii, Honolulu, HI 96822.

The stability of antigenic determinants recognized by three monoclonal antibodies (mAbs) specific to strains of Xanthomonas campestris pv. oryzae (Xco) was examined. Philippine and Texas strains were serially passed through rice plants over a period of 20 wks. Samples from the passages were evaluated with mAbs using ELISA. The reactivity of the mAbs to the Philippine strains did not change over the 20 wk period. The weakly virulent Texas strains could not be recovered for the full period. However, their reactivity to the mAbs did not change. Thus, the antigenic determinants recognized by the mAbs are considered stable for Xco. A dot blot using the mAbs was developed for testing field samples of Xco.

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XANTHOMONAS OFFICINARUM SP. NOV., A SECOND ORGANISM INVOLVED IN SUGARCANE GUMMING DISEASE. M. Qhobela and L. E. Claflin. Dept. of Plant Pathology, Kansas State Univ., Manhattan 66506

A critical examination of 22 strains of Xanthomonas campestris pv. vasculorum, the incitant of gumming disease of sugarcane, revealed two distinct groups of organisms. The first group (16 strains) consisted of X. campestris pv. vasculorum sensu stricto; whereas the other consisted of a yet undescribed Xanthomonas species. The two organisms differed from each other in serological reactivity, host range, DNA homology values, DNA restriction endonuclease patterns, and by membrane protein profiles on sodium dodecyl sulfate poly-acrylamide gel slabs. Strains of the undescribed Xanthomonas were pathogenic on sugarcane and tigergrass but not pathogenic on maize, sorghum, or pearl millet. Strains of this undescribed Xanthomonas clearly formed a distinct group and the name Xanthomonas officinarum sp. nov. is proposed for this organism.

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XANTHOMONAS CAMPESTRIS PV. ZINNIAE STRAINS RAPIDLY PRODUCE LARGE QUANTITIES OF EXTRACELLULAR PROTEASE. X. P. Sun and D.F. Ritchie. Dept. of Plant Pathology, N.C. State Univ., Raleigh, NC 27695.

The substrate azocasein and milk agar overlaid on sucrose peptone agar plates were used to detect and quantify protease. Larger quantities of extra-cellular protease were detected more rapidly in strains of X. campestris pv. zinniae than were detected in strains of pvs. pruni, campestris, vesicatoria, pelargonii, or oryzae. Protease was produced in a defined medium lacking casein or other proteins. A crude enzyme preparation of at least 10-fold concentration was obtained by precipitation of bacterial-cell-free supernatant with 60% ammonium sulfate.

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INDOLEACETIC ACID PRODUCTION BY EPIPHYTIC BACTERIA ASSOCIATED WITH PEAR FRUIT RUSSETTING. E. Clark and S. E. Lindow, Department of Plant Pathology, University of California, Berkeley, CA 94720.

Russetting of Bartlett pear fruit at harvest is significantly increased by application of bacterial producers of indole-3-acetic acid (IAA) to pear flowers at 50% bloom. IAA producers and non-producers were isolated from pear fruit and leaf surfaces; IAA producers consisted primarily of fluorescent Pseudomonas spp. and yellow-pigmented enteric spp. A cloned DNA fragment encoding the two enzymes in the IAA biosynthetic pathway of the gall-forming pathogen Pseudomonas savastanoi was radiolabelled and used as a probe to test for the presence of related sequences in IAA-producing epiphytes. Cross-hybridization to DNA isolated from several IAA-producing strains was detected by Southern blot analysis at low stringency but not at high stringency; hybridization was not observed with DNA from the non-producers tested. This result indicates that at least part of the IAA biosynthetic pathway of some epiphytic IAA producers may resemble that of characterized pathogenic species.

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COMPUTER-AIDED ANALYSIS OF RADIOLABELED PROTEIN PROFILES OF BACTERIAL ISOLATES FROM CORN ROOTS. C.Hendrick, D.Haefele, J.Marlow. Microbial Genetics Division, Pioneer Hi-Bred International, Inc., Johnston, IA 50131.

We are evaluating methods for fingerprinting bacteria selected as potential biocontrol agents for seedling diseases of corn. To assess the variability in total radiolabeled protein profiles between closely related bacteria of diverse geographic origin, we analyzed 58 isolates of Pseudomonas fluorescens and 12 isolates of Serratia plymuthica from corn roots. Proteins from each isolate were labeled by incorporation of ^{35}S-methionine, separated by SDS-PAGE, and visualized by auto-radiography or by scanning dried gels with a 2-dimensional beta-scanner. Cluster analysis of scan data showed that the P.

fluorescens isolates fell into at least 7 groups and the S. plymuthica isolates fell into 4 groups. There was no correlation between the geographic source of an isolate and its electrophoretic group. The method is rapid and reproducible and is useful for subgrouping closely related bacterial isolates.

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CELL WALL-MACERATING-ENZYME ACTIVITY IN MYCENA CITRICOLOR. J.P. Tewari, and D.V. Rao, Department of Plant Science, University of Alberta, Edmonton, Alberta, Canada, T6G 2P5.

Mycena citricolor, the causal agent of the American leaf spot of coffee produces oxalic acid in culture and in infected tissue. As a result of this, the pH of coffee cell wall medium was lowered to 4.4, a condition which is known to favor macerating-enzyme activity. However, the enzyme pectin methyl esterase was absent and significant levels of polygalacturonase (PG) and cellulase (Cx) were not produced. Furthermore, the fungus hydrolized cellulose (Cl) at a slow rate. The lesion tissue also showed low PG and Cx activities. This suggested that tissue deterioration during lesion development was not primarily caused by macerating enzymes. Extensive deposition of calcium oxalate crystals was associated with the cell wall material. These results suggested a primary and direct role of oxalic acid in tissue deterioration through the formation of calcium oxalate.

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SMALL, CONJUGATABLE PLASMID IN COPPER-RESISTANT STRAINS OF XANTHOMONAS CAMPESTRIS PV. VESICATORIA. V. Dittapongpitch, D.F. Ritchie, and R.G. Upchurch. Department of Plant Pathology, N.C. State Univ., Raleigh, NC 27695.

Thirty-two strains of Xanthomonas campestris pv. vesicatoria isolated from pepper and tomato were tested for sensitivity to 200 µg/ml copper sulfate in sucrose peptone agar. Sixty percent were copper resistant. Plasmid profiles indicated the presence of at least two plasmids in all strains. All copper-resistant strains contained an approximately 3 kbp plasmid. This plasmid was transferred via conjugation to copper-sensitive strains. Transconjugates contained the 3 kbp plasmid and were copper resistant. Preliminary anaylsis indicated the plasmid was digested by restriction enzymes PstI, Sau3A, AluI, and TaqI, but not by EcoRI, BamHI, HindIII, or XhoI.

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EXISTENCE OF CHITINASE ACTIVITY IN MATURE CORN KERNELS (ZEA MAYS). J. N. Neucere and T. E. Cleveland, USDA, ARS, Southern Regional Research Center, P.O. Box 19687, New Orleans, LA 70179

Reducing infection by Aspergilli species that produce aflatoxins in corn is an area of intense interest. One speculation is that chitinase is a resistance factor involved in inhibiting fungal growth. Mature kernels of yellow corn and white corn were assayed for chitinase activity. Results showed activity primarily in germ tissues with disparity between the two varieties. The highest activity per quantity of protein was observed in the germ of white corn. Immunochemical assays showed different profiles; two precipitin bands present in the endosperson of white corn were not detected in either the germ of white corn or whole kernels of yellow corn. Strategies for purification and characterization of active components will be discussed.

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OCCURRENCE OF PEANUT POD ROT IN OKLAHOMA: 1983-85. A. B. Filonow and C. C. Russell. Department of Plant Pathology, Oklahoma State University, Stillwater, OK. 74078.

Peanut fields in Bryan, Caddo, Hughes, and Okfuskee counties were surveyed for pod rot from early September to mid October. Thirty plants per acre from 2-8 acres per field were sampled. In 1983, 41.7% of 36 fields had pod rot as diagnosed by symptoms and isolations of Pythium myriotylum or Rhizoctonia solani from diseased pods. In 1984 and 1985, 73.1% of 26 fields and 43.5% of 46 fields, respectively had pod rot. Mean disease incidence for all fields in 1983, 1984, and 1985 was 6.1%, 21.3%, and 5.8%, respectively. In 1984 no linear correlation (r=-0.09) was found between % calcium in hull pieces of pods from individual fields and pod rot incidence. Over 3 years P. myriotylum or R. solani were isolated from symptomatic pods in 42-60% or 30-35% of the fields, respectively. Sclerotium rolfsii was found in rotted pods in 52-79% of the fields, suggesting a possible role in pod rot. Meloidogyne hapla was the most common plant-parasitic nematode found in roots or soil. Others included Pratylenchus, Criconemella, and Tylenchorhynchus spp.

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STROMATIC HYPHAL INOCULUM FOR KABATIELLA ZEAE. C. A. Martinson. Department of Plant Pathology, Iowa State University, Ames, Iowa 50011

Kabatiella zeae, the causal agent of eyespot of maize, was grown in reciprocal shake culture on potato dextrose broth amended with 0.1% yeast extract (PDB) for 5 to 7 days, until black stromatic hyphae formed. About 250 ml stromatic hyphal culture was seeded into PDB in a 10 liter fermentor; yields of 122 and 138g (dry wt. equiv.) of stromatic hyphae were harvested after 3 and 4 days respectively. Stromatic hyphae were washed, ground, coated onto #37 white quartz sand, air dried overnight and could be stored dry for one year. About $4x10^5$ lesions developed/g of dried stromatic hyphae on a susceptible variety following whorl inoculation. With routine resistance screening, about 1 mg stromatic hyphae was used per g dry sand; about 1 g sand was directed into the plant whorl with a pipet modified into an hour-glass type applicator. Sand inoculum sporulated in the whorl and continued to fall back into the whorl and resporulated several times. Inoculation were made any time of day.

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PATHOGENICITY OF ROTYLENCHULUS RENIFORMIS ON COTTON. Julio C. Borbon, William E. Batson, Jr., and Gary W. Lawrence, Dept. of Plant Pathology and Weed Science, Mississippi State University, Miss. State, MS 39762.

The effect of the reniform nematode (Rotylenchulus reniformis Linford & Oliveira) on the growth of cotton seedlings (Gossypium hirsutum L.) "Stoneville 112" was determined in a greenhouse study. Soil was infested with 0, 1,000, 5,000, 10,000, 15,000, and 20,000 young adults/500 g of soil. Plant height was measured weekly for 10 weeks. At harvest, fresh and dry weights of shoots and roots were recorded. Plant height was significantly reduced by an initial population density of 10,000 nematodes after seven weeks. Initial nematode population densities of 1,000 or higher suppressed shoot growth. Initial nematode population densities of 15,000 and 20,000 were required to reduce root growth.

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EFFECTS OF CYST NEMATODE RACE 5 ON SUSCEPTIBLE SOYBEANS AT FOUR PLANTING DATES. R. P. Pacumbaba and W. Tadesse. Dept. of Plant and Soil Science, Alabama A&M University, Normal 35762.

Effects of soybean cyst nematode race 5 (SCN) on susceptible Essex and Lee were investigated in 1, 15, 30 April and 14 May planting dates for 3 seasons. The means of the two soybean cultivars for the three-year period at different planting dates were significantly different for the size and number of nodules, soil temperatures, plant heights, number of cysts, SCN rate and % SCN field infestation, and yield. For 14 May planting date, significantly larger size of nodules, increased number of nodules and cysts, higher SCN rate and % SCN field infestation were noted as well as significantly lower yield and shorter plant height. Planting susceptible soybean cultivars in cyst nematode race 5 infested soil earlier than the regular planting dates in Northern Alabama (14 May-14 June) increases plant height and yield but decreases the number of cysts, SCN rate and % SCN field infestation. These indicated that the soybean cultivars tested escaped SCN infestation in the field.

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INTERACTIVE EFFECTS OF POTASSIUM, BENOMYL, AND CULTIVAR ON INCIDENCE OF PHOMOPSIS SEED DECAY IN SOYBEAN IN MISSISSIPPI. K. W. Roy, N. H. Buehring, W. F. Jones, and K. S. McLean. Mississippi State University, Drawer PG, Mississippi State, MS 39762.

In a 6-yr (1980-1985) field study the soybean cultivars Tracy and Centennial were sown in soil treated with 0, 37.5, 75.0, or 150 kg/ha muriate of potash (K). At growth stages R2 and R4 foliage was treated with benomyl (0.56 kg a.i./ha) or left untreated. Mature seeds were assayed for Phomopsis longicolla. Phomopsis seed decay was reduced by K in 1982, 1983, and 1985. Significant K by cultivar interactions occurred in 1982 and 1983. In 1982, 37.5, 75.0, and 150 kg K reduced seed decay in Centennial by 52, 73, and 86%, respectively, whereas only 75 kg K reduced (30%) seed decay in Tracy. In 1983, 75 and 150 kg K reduced seed decay in Centennial by 46 and 71%, respectively, whereas K had no effect on Tracy. In 1985, 150 kg K reduced

seed decay by 10%. Phomopsis seed decay was reduced by benomyl alone in 1984 (27%) and 1985 (8%) but was increased in 1981 (64%) and 1983 (65%). In 1980, benomyl increased seed decay by 68% in the absence of K but had no effect in its presence.

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EFFECTS OF SOIL INOCULUM DENSITIES OF FUSARIUM MONILIFORME ON GRAIN SORGHUM GROWTH AND ROOT DEVELOPMENT. D. K. Tuopay, L. E. Trevathan, and G. W. Lawrence. Dept. Plant Pathology and Weed Science, P. O. Drawer PG, Mississippi State, MS 39762.

The relationship of inoculum concentration of Fusarium moniliforme and growth of grain sorghum (Sorghum bicolor) variety DeKalb 59 was determined under greenhouse conditions. Microconidial inoculum was incorporated in sterile soil mix at concentrations of 0, 2.0, 17.0, 1.7×10^2, 1.7×10^3 and 1.7×10^4 conidia per gram. Growth of leaves and shoots was measured weekly from the base of the plant to the tip of the uppermost leaf for eight weeks. At the end of this period, plants were lifted from soil and shoot fresh and dry weights and root fresh and dry weights were recorded. F. moniliforme was recovered from 69% of stem and root sections of plants growing in soil infested with the fungus. Plant height, shoot fresh and dry weights and root fresh and dry weights were significantly reduced at inoculum levels of 1.7×10^2 conidia per gram of soil and greater.

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SEEDLING BLIGHT OF GRAIN SORGHUM CAUSED BY GIBBERELLA THAPSINA. Douglas J. Jardine, Department of Plant Pathology, Kansas State University, Manhattan, KS 66506.

A new species of Gibberella, G. thapsina was consistently isolated from mature sorghum (Sorghum bicolor) plants. Laboratory inoculations of three-day-old sorghum seedlings (cv. 'Pioneer 8515') were made by dipping the roots into a spore suspension (10^7 cfu/ml) and incubating for three days at 27 C. Seedling roots dipped in sterile distilled water served as the control. Three Kansas isolates, FKMF 1321, FKMF 1337 and FKMF 1427, induced symptoms typical of seedling blight on grain sorghum. Symptoms consisted primarily of the formation of reddish-brown lesions at the base of root laterals, and after 3 days, death of the infected lateral. On some plants, the entire root system was necrotic. No symptoms developed on the roots of the control plants and G. thapsina was reisolated from inoculated roots but not from the controls.

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IN VITRO EFFICACY OF FUNGICIDES AGAINST CERCOSPORELLA RUBI. Barbara J. Smith, USDA-ARS, Small Fruit Research Station, P. O. Box 287, Poplarville, MS 39470.

Rosette of blackberry, caused by Cercosporella rubi, is severe in the southeastern U.S. and is not adequately controlled by currently registered fungicides. Twenty-two fungicides were screened in vitro for efficacy against C. rubi at 3 to 5 rates ranging from 0.05 to 500 µg a.i./ml incorporated into potato dextrose agar prior to pouring into petri plates. Mycelial plugs of C. rubi were transferred to each plate, and colony diameter was measured after 7 days. The fungicides were placed into 5 efficacy groups based on the lowest level of each fungicide causing a significant growth reduction of C. rubi compared to its growth on unamended PDA: 1) 0.05 µg/ml: flusilazole, propiconazole, captafol, benomyl, ferbam, diniconazole, bitertanol, myclobutanil; 2) 0.5 µg/ml: DCNA, fenarimol, maneb, iprodione, mancozeb; 3) 5 µg/ml: dodine triadimefon, triforine, metalaxyl, folpet; 4) 50 µg/ml: anilazine, cupric hydroxide, captan; 5) 500 µg/ml: sulfur.

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PRUNUS SELECTIONS UNSUITABLE AS HOSTS FOR *CRICONEMELLA XENOPLAX*. S. W. Westcott, III and E. I. Zehr, Department of Plant Pathology and Physiology, Clemson University, Clemson, SC 29634.

Selections of *Prunus* are being screened for suitability as hosts to *Criconemella xenoplax* and ultimately for use as rootstocks. Seeds were collected from trees maintained in the *Prunus* Germplasm Collection at Clemson University. Seedlings were grown in steamed sand infested with *C. xenoplax* (100-300/plant) for periods of 9-15 wk in a greenhouse. Seedlings that supported little or no reproduction by the nematode were tested in a similar manner one or more additional times. Those that consistently were poor hosts for the nematode were propagated by rooting cuttings and were tested again for host suitability to *C. xenoplax*. From a collection of 18 seeds from *P. besseyi* seven seedlings were identified as poor hosts for *C. xenoplax* (no detectable reproduction by the nematode). Eleven seedlings from *P. besseyi* supported about a ten-fold increase in nematodes in 10 weeks, that was similar to the reproduction seen on *P. persica* 'Nemaguard' seedlings. Similarly, four seedlings from *P. pumila* 'Mando' were poor hosts, while ten others supported substantial population increases. The phenotype for host suitability appears to be segregating in the seedling populations from these two sources. Other selections may be identified as unsuitable hosts for *C. xenoplax* in the near future.

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PHYTOPHTHORA SHUCK AND KERNEL ROT OF PECAN FRUIT. C. C. Reilly[1], F. F. Hendrix, Jr.[2] and M. W. Hotchkiss[1], [1]USDA-ARS, P.O. Box 87, Byron, GA 31008, [2]Dept. of Plant Pathology, University of Georgia, Athens, GA 30602.

Phytophthora shuck and kernel rot of pecan Carya illinoensis (Wang.) K. Koch. is a new disease of pecan fruit. The causal agent is Phytophthora cactorum (Leb & Cohn) Schroet. The disease was first observed on maturing fruits in central GA in early September 1988. Yield and quality of the kernels was reduced by >50% in some central GA orchards and to a lesser extent in south GA. Symptoms developed within 4-6 da, usually starting at the attachment end of the fruit with a distinct margin between the necrotic and healthy tissue. The shucks turned brown, dried over a 2-3 wk period and stuck tightly to the shell. The kernel had a dark brown seed coat with gray rotted endosperm. Koch's postulates were satisfied by laboratory and field innoculations of nut clusters. Symptoms appeared in 4 da for laboratory and 6-8 da for field innoculations.

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T. A. Frisina, R. D. Milholland, and R. A. Fong. Sporangial and oospore production by Phytophthora fragariae in roots of strawberry plants. North Carolina State University, Box 7616 Raleigh, North Carolina 27695-7616.

Sporangial production by P. fragariae was greatest when roots of the susceptible strawberry cultivar Tennessee Beauty were incubated for 8 days at 15 C following inoculation with a highly virulent isolate (NC-1) of race Pf-2. Duration of root incubation in unsterile soil leachate (2-6 days) for the total 8 day incubation period was not a significant factor in sporangial production. Sporangial production decreased after 8 days incubation. In contrast, oospore production increased between days 6 and 12. Temperature optima for maximum sporangial production by race Pf-2 were 15-20 C, whereas, temperature optima for sporangial production of a weakly virulent isolate of race Pf-7 (ATCC 18638) were 20-24 C. Race Pf-7 produced less sporangia/root than Pf-2 at all temperatures tested.

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Biological and cultural factors associated with citrus replant problems in Texas. M.Skaria and C.Farrald, Texas A&I University Citrus Center, P.O. Box 1150, Weslaco, TX 78596.

Three-year-old 'Marrs early orange' (Citrus sinensis) trees on sour orange (C. aurantium) rootstock show strikingly heterogeneous growth characteristics. Trunk diameter at an inch above the budunion and plant height range between 0.9 to 7.0 cm and 60 to 180 cm, respectively. Thirty percent of the total trees are stunted. Two fungi, Phytophthora parasitica and Ganoderma lucidum, and a nematode, Tylenchulus semipenetrans, have been found associated with the severely stunted trees. Star ruby grapefruit (C. paradisi), which occupied this 10 acre block, had a high incidence of P. parasitica infection. These trees were freeze-killed in 1983. The land was prepared for replanting in 1984 by cutting the trees at the soil surface, leaving the stump and the root system in the soil. Association of cultural as well as biological factors including the above mentioned organisms as causal agents of citrus replant problems will be discussed.

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HARRY FERTILIZER AND PLANT PROTECTANT IN TOMATO CROPPING. Marvin D.Whitehead and M.J.Thirumalachar.817 Clifton Road, N.E. Atlanta, GA 30307, and Jeersannidhi Anderson Institute,POB.506 Locust St.Walnut Creek, CA 94596.

Harry Fertlizer and Plant Protectant (HF-PP) is a systemic, non-phytotoxic bactericide and fungicide containing organic and inorganic nitrogen fertilizers. In experimental plots of BURPEE SUPER-STEAK tomato variety in Atlanta,pre-fertilized with farm yard manure and commercial booster fertilizers, foliar sprays on 30th and 40th.days after planting were given as follows: In treated plots, Hf-PP at concentration of four tablespoonfuls in a gallon of water, and in control plots Dithane Z-78 one tablespoon per gallon were used.The leaf blights by Alternaria solani and Cladosporium fulvum were completely controlled by HF-PP, and moderate in Dithane Z-78 plots.

HF-PP plots gave 30% more yield, and the fruits were uniformly 2 to 2.5 lbs in size, while in controls it was 1.75 lbs mostly. Disease control, plant growth stimulation and foliar nutrition by HF-PP was responsible for this improved cropping.

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EVALUATION OF SOIL SOLARIZATION FOR CONTROL OF VERTICILLIUM WILT IN CHERRY TOMATO. J. A. Liebman, D. P. Morgan, L. Epstein, and M. J. Jimenez. University of California, Berkeley, 94720.

Solarization controls many soil-borne plant diseases, but is limited by the need to apply plastic mulches to soil during the summer. If the field is planted there is too much shade; if the field is fallow the grower loses the season's production. We investigated the use of solarization to control Verticillium wilt of drip-irrigated cherry tomato, a crop which normally is staked, so the soil surface is not shaded. Compared to non-solarized controls, solarization significantly reduced soil inoculum density of the pathogen and led to significantly increased yields the following season. The effect was most dramatic when mulches were applied at time of transplanting (17 April) rather than later in the season (25 June or 30 July). Solarizing cropped ground was slightly more effective than solarizing fallow ground, and did not unduly interfere with normal agronomic practices.

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GROWTH OF PINK ROOT RESISTANT AND SUSCEPTIBLE ONION CULTIVARS IN ORGANIC SOIL INFESTED WITH PYRENOCHAETA TERRESTRIS. P. M. Coleman, L. A. Ellerbrock, and J. W. Lorbeer, Departments of Vegetable Crops and Plant Pathology, Cornell University, Ithaca, NY 14853.

Onion cultivars Kodiak and Paragon, reported to be resistant and susceptible to pink root, respectively, were grown in organic soil, not previously cropped to onion, in the greenhouse in pots and outdoors in flats. Blended mycelium of P. terrestris was mixed with soil in half the treatments; the control soil was unamended. Green leaf weight and bulb weight at harvest were not decreased in the presence of P. terrestris for either cultivar. Plants in amended soil had more pink roots at harvest than plants in unamended soil, but overall pink root levels were low. P. terrestris was isolated only from plants grown in the amended soil. Pink color of onion roots from control soil appeared to be due to naturally occurring fungi with pink mycelium which were isolated from such roots, but were not identified.

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INCIDENCE AND DISTRIBUTION OF ESCAROLE NECROSIS IN CICHORIUM ENDIVIA IN FLORIDA. R. N. Raid, R. T. Nagata, U. of Florida, Everglades Research and Education Center, Belle Glade, 33430 and L. L. McDaniel, ATCC, Rockville, MD 20852.

Commercial fields of Cichorium endivia L. were surveyed for escarole necrosis, caused by an uncharacterized virus (ENV), from Nov 1988 to Mar 1989. Six geographical locations in the Everglades Agricultural Area were sampled. Plants were visually observed for symptom development and indirect-ELISA was used to verify diagnoses. Escarole necrosis was detected in 5 of 6 locations and in 11 of 12 fields sampled. Disease incidence ranged from 0 to 894 plants per one hectare block (0 to 1.26%). Incidence and spatial distribution within the field appear to be related to the proximity and orientation of neighboring sugarcane fields or canals, suggesting the possibility that the virus is insect-vectored. Sequential observations suggest that there is little or no secondary infection. Results indicate that although escarole necrosis is widespread in south Florida, it is of little importance on C. endivia at the present time.

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IMPORTANCE OF SOIL AND TUBER-BORNE DISEASES OF POTATO IN SOUTH DAKOTA. D. J. Gallenberg, SDSU, Plant Science Department, Box 2109, Brookings, SD 57007.

Observations in commercial potato production fields as well as direct contacts with growers were made during 1985 - 1988 to determine the importance of soil and tuber-borne disease problems in northeastern South Dakota. In randomly selected fields, visual observations were made to determine presence or absence of diseases, and where necessary, laboratory diagnosis was used as confirmation. Estimates of disease severity were made where possible. Numerous diseases, including common scab, Fusarium tuber decay, and soft rot/blackleg, were observed, however Verticillium wilt and to a lesser extent Rhizoctonia canker seem to be the major disease problems. With Verticillium wilt there is an apparent association with rotation practices and use of susceptible cultivars. These two diseases will be the focus of continuing studies.

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ROOT AND CROWN DECLINE OF GLOBE ARTICHOKES WITH AN UNKNOWN ETIOLOGY. J. C. Correll and S. F. Colbert. Department of Plant Pathology, University of California, Berkeley, CA 94720

Artichokes *(Cynara scolymus)* have been grown extensively in the Monterey County region of California for approximately 80 years; it is not unusual to find fields which have been cropped exclusively to artichokes for 10-15 years. Recently, a root and crown decline problem of unknown etiology has been observed in numerous fields. Symptoms include a dark brown to black root discoloration in the cortex as well as the stele. Larger roots (2-4 cm) have been observed with severe cortical discoloration and necrosis of the stele. Advanced root decline symptoms include a complete collapse of the stele where the tissue develops soft rot type symptoms. This soft rot appearance can progress into the above ground crown. Work has been initiated to examine the cause and possible control of this problem. Several fungi, including *Fusarium solani*, Cylindrocarpon sp., and *Rhizoctonia solani* have been frequently isolated from necrotic root tissue. Also the stunt nematode, Merlinius sp. has been isolated from soils with the decline syndrome. Grubs of the cribrate weevil *(Otiorhynchus cribricollis)* also have been observed to cause substantial feeding injury to roots. A working hypothesis is that the root decline problem is the result of a disease complex.

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VIRAL DISEASES ASSOCIATED WITH CUCURBITS CROPS IN HAWAII. J. J. Cho, D. E. Ullman, T. L. German, and D. Custer. P. O. Box 269, University of Hawaii, Kula, HI 96790.

Farm surveys were conducted on three Hawaiian islands including Maui, Molokai and Oahu to determine the major mosaic viruses infecting cucurbit crops. High disease incidences (greater than 30%) were associated with all zucchini plantings sampled. Disease incidences in cucumber and watermelon plantings were lower at less than 10%. Zucchini yellow mosaic virus (ZYMV) was the only virus found on Molokai farms, the predominant virus on Maui crops, and also found on Oahu. Watermelon mosaic virus 1 (WMV1) was the predominant virus on Oahu; and accounted for 3%, 29% and 49% of infected plants on Maui. Cucumber mosaic virus (CMV) and watermelon mosaic virus 2 (WMV2) were not detected on any of the surveyed farms. Forty-two different aphid species have been identified on Maui. Three known vectors predominate including Aphis gossypii, A. craccivora, and Myzus persicae. JMS stylet oil delayed the onset of disease and disease incidence.

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POLYMERASE EXPRESSION MODULATION BY RIBOSOMAL FRAMESHIFTING IN RED CLOVER NECROTIC MOSAIC VIRUS. Z. Xiong, S. A. Lommel, Department of Plant Pathology, North Carolina State University, Raleigh, NC., 27695.

Nucleotide sequence analysis of the red clover necrotic mosaic virus (RCNMV) genomic RNA-1 revealed that the polymerase gene appears to be regulated by a frameshift mechanism. To definitively establish the capability of ribosomal frameshifting at the identified sequence, reporter gene fusions where constructed and assayed for the presence and quantity of fusion protein product. A portion of the β-galactosidase gene was fused downstream (in a translational context) to the frameshift region. Bacterial colonies transformed with the construct turned blue in the presence of x-gal. However, the blue color was much less intense when compared to a construct where the frameshift region was eliminated and the β-galactosidase gene was fused inframe before the frameshift region. The differential blue reactions suggest that ribosomal frameshifting acts as a down regulatory mechanism for polymerase expression. The detailed expression of these constructs *in vitro* and *in vivo* will be reported.

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SEQUENCE ANALYSIS OF THE ZUCCHINI YELLOWS MOSAIC VIRUS COAT PROTEIN GENE. Guowei Fang and Rebecca Grumet, Horticulture Department, Michigan State University, East Lansing, MI 48824.

Zucchini yellows mosaic virus (ZYMV) is a highly aggressive member of the potyvirus group that infects cucurbit crops (cucumbers, squashes, melons). We seek to genetically-engineer resistance to this disease. To this end we have cloned and sequenced the ZYMV coat protein gene. A ca. 1.5 Kb clone from the 3' end of the ZYMV genome was cloned into a Bluescript vector and digested to form a set of nested deletions for use in Sanger dideoxy sequencing reactions. Predicted amino acid sequence shows many features in common with other potyviral coat proteins. These include the conserved motifs MVWCIENGTSP,

LARYAFD, QMKAA, FGLDG and TERH, in the central portion and 3' end of the gene. The sequence data, putative 5' and 3' ends of the gene, and comparisons to other potyviral coat protein sequences will be presented.

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OCCURRENCE OF A SMALL RNA IN MAIZE CHLOROTIC DWARF VIRUS (MCDV)-LIKE PARTICLES. X. Ge, D. T. Gordon and R. E. Gingery (USDA), Dept of Plant Pathology, and M. D. McMullen (USDA), Dept of Agronomy, Ohio State Univ., Wooster, OH 44691.

A slower sedimenting virus-like particle (SSVLP) was detected along with MCDV virions in rate-zonal centrifuged preparations from MCDV-infected maize. SSVLP morphology was identical to that of MCDV (isometric, ca. 30 nm dia.) except that cores of SSVLPs were penetrated with uranyl-acetate. The buoyant densities of SSVLP and MCDV were 1.295 and 1.442 g(CS_2SO_4)/ml, respectively. SSVLP was positive in ISEM and immuno-precipitated with MCDV-specific antiserum. SDS-PAGE revealed three capsid proteins of corresponding mol. wts. for SSVLP and MCDV virions. ssRNA of about 1 kb was purified from SSVLPs and 10-kb ssRNA from MCDV virions. The 10-kb RNA hybridized with randomly primed cDNA probes synthesized from the 1-kb RNA, whereas the 1-kb RNA hybridized only weakly with cDNA from the 10-kb RNA. We hypothesize that MCDV capsid proteins encapsidate a RNA related to MCDV-genomic RNA to form the SSVLP.

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EFFECT OF GENOMIC POSITION ON THE EXPRESSION OF THE TOBACCO MOSAIC VIRUS MOVEMENT PROTEIN. J. N. Culver, K. M. Lehto, and W. O. Dawson. Dept. of Plant Pathology, University of California, Riverside, CA 92521.

Tobacco mosaic virus (TMV) mutants with various deletions in the coat protein (CP) gene were utilized to study the effect of genomic position on the expression of the viral 30 K movement protein. Viral proteins purified from inoculated leaves were analyzed using a Western blot technique to quantify the ratio of viral 126 K to 30 K proteins for each mutant. The ratio of 126 K/30 K allowed a comparison of viral infection to 30 K levels for each sample. TMV mutants having partial deletions in the CP gene showed a 3 to 4 fold increase in 30 K levels when compared to wild type TMV, while mutants having the entire CP gene deleted showed a 10 fold increase in 30 K levels. A mutant having a full-length but non-translatable CP gene produced 30 K levels equal to that of wild type TMV. This study demonstrates that positioning of the 30 K gene nearer the 3' terminus of TMV yields higher levels of protein.

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INFECTIVITY OF MUTATED cDNAs OF THE POTATO SPINDLE TUBER VIROID (PSTV). D.K. Lakshman and S.M. Tavantzis, Dept. of Botany & Plant Pathology, Univ. of Maine, Orono, ME 04469.

Five mutants were constructed from a 358-base cDNA clone (pAV401) of PSTV and subcloned at the BamH1 site of "Riboprobe" plasmids pSP64 and pSP65. Mutant LT1 has an A addition after base 53. Mutants APM4 and APM6 have short deletions (bases 93-97 and 286-288, respectively) at the two AvaI sites of LT1. In addition, APM6 has an insert of 24 bases next to the deletions. Mutant ST4 carries a two-base deletion (339 and 340) at the StyI site of LT1 whereas E9 is a four-base deletion (146-149) at the EagI site of LT1. Seedlings of the tomato cv. Rutgers were inoculated with BamH1-digested plasmids carrying monomers of the above PSTV cDNA mutants. Seedlings inoculated with pLT1, and pST4 exhibited symptoms and contained PSTV RNA. Experiments are underway to determine the infectivity of dimeric or trimeric cDNA and the respective (+) RNA transcripts of the above mutants.

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A TRANSGENIC PLANT APPROACH TO STUDYING VIROID PATHOGENESIS. S.M. Tavantzis, D.K. Lakshman, L.C. Burian, and B.P. Bandy. Dept. of Botany & Plant Path., Univ. of Maine, Orono, ME 04469.

Site-specific, short deletions or insertions were introduced into the sequence of a PSTV cDNA clone and subsequently verified by sequencing. Full-length or portions of the mutated PSTV cDNA were subcloned, in a plus or minus orientation and under the control of the CaMV 35S promoter, in the plant expression vector pBI121 and mobilized to the Agrobacterium tumefaciens strain LBA4404. Leaf discs of potato, tobacco, and tomato were inoculated with Agrobacterium cultures carrying the PSTV sequences. Regenerated potato and tobacco shoots that rooted on selective media were analyzed by Southern blot hybridization with PSTV-specific probes. The data showed that the PSTV cDNA sequences are integrated into potato and tobacco genomic DNA. Expression of the chimeric PSTV "genes" will be examined by Northern blot hybridization. Regenerated tomato shoots currently growing on selective media will be analyzed as described above.

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cDNA CLONING OF THE WHEAT STREAK MOSAIC VIRUS GENOME. K. R. Zagula and S. A. Lommel, Department of Plant Pathology, Raleigh, NC.

Wheat streak mosaic virus (WSMV), a putative member of the potyvirus group, is composed of a single-stranded RNA genome of approximately 8.5 kb and a capsid protein of 42kDa. A 2.1 kb 3' terminal cDNA clone was synthesized by oligo-dT priming. This clone has been sequenced and the capsid protein domain identified. Primer extension cloning was performed using an oligonucleotide primer homologous to the 5' terminus of the existing clone. A virus specific 3.2 kb clone not homologous to the existing clone was identified. Together these clones represent over 75% of the genome. Nucleic acid sequence, in vitro translation products, and a partial genome map are being determined.

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The Effects of Chemical Fixatives on Wheat Soil-borne Mosaic Viruses, Dr. R. L. Grayson and Mary Sue Mayes, Dept. of Plant Path., Phys. and Weed Sci., VPI&SU, Blacksburg, VA 24061-0331.

Viral suspensions of wheat soil-borne mosaic virus (WSBMV) were examined to determine the effects of fixation on particle morphology. Serum sensitive electron microscopy (SSEM) in combination with primary antibodies and secondary gold label antibodies were the techniques utilized. Gluteraldehyde and osmium tetroxide (OSO_4) were both shown to have a deterimental effect on WSBMV morphology. Results indicate that these chemicals alter the condition of the protein coat and cause a loss in viral integrity. Gluteraldehyde had the most severe effect on WSBMV. Disassociation of the viral coat was observed after one hour. OSO_4 had an effect on the viruses after exposure of 8+ hours. When viruses were exposed to more than one fixative, the viral distortion was much more severe. WSBMV exposed to gluteraldehyde and OSO_4 for time periods in excess of three hours showed the most severe effects. These results may indicate why little success has been achieved in observing WSBMV in thin sections.

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SEQUENCE ANALYSIS OF DEFECTIVE INTERFERING RNAS OF TOMATO BUSHY STUNT VIRUS. D A Knorr, T J Morris. Plant Pathology Dept., University of California, Berkeley, 94720.

Defective interfering (DI) RNA B10, derived through ten serial passages of tomato bushy stunt virus in Nicotiana benthamiana, was analyzed by cDNA cloning and sequencing. DI B10 is a population of TBSV deletion mutants, ≈600 nt in length, that is efficiently replicated and encapsidated, and also protects the host from lethal necrosis associated with helper virus infection. Six independent cDNA clones of DI B10 each contain the 5' viral leader sequence followed by an internal block of ≈250 nt from the polymerase domain. 3' termini include stop codons for the viral P19 and/or P21 ORFs, and a deletion of 179 nt of non-translated sequence. Size differences between the B10 cDNAs is due primarily to variation of the junction site between the polymerase and 3' domains.

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CONTROL OF CROWN ROT OF BANANA IN THE WINDWARD ISLANDS BY A THIABENDAZOLE-COATED PAD. A. Johanson, F.J. Proctor, J.R. Cox, and M.J. Jeger, Overseas Development Natural Resources Institute, Central Avenue, Chatham Maritime, Kent ME4 4TB, U.K.

In the Windward Islands bananas are cut from the stem and packed in the field. An absorbent cellulose pad coated with the fungicide thiabendazole (TBZ) is applied to the cut surface of the banana hand to absorb latex and to control crown rot. Crown rot, the most commercially important post-harvest disease of bananas, is caused by several fungi including Colletotrichum musae, Fusarium moniliforme, and F. pallidoroseum. Analysis of banana crowns for TBZ residues indicated limited penetration of TBZ into the tissues from the pad. The mean level of TBZ in the surface 2mm was 8.9 mg/kg; in the next 5mm, 0.7 mg/kg. Below this, levels were undetectable by HPLC. Studies showed that some insensitivity to TBZ is present in field populations of the causal fungi.

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RELATIVE RESISTANCE OF WHEAT CLASSES AND CULTIVARS TO INVASION BY STORAGE FUNGI. D. B. Sauer, USDA-ARS, U.S. Grain Marketing Research Laboratory, Manhattan, KS 66502.

Wheat samples representing the major classes and cultivars were obtained from throughout the U.S. The 256 samples were inoculated with spores of Aspergillus glaucus, stored 3 weeks at 25 C and 83% relative humidity, and evaluated for visible mold, internal mold invasion, and equilibrium moisture content. The three red wheat classes, hard red winter, soft red winter, and hard red spring were similar in their average mold susceptibility. Durums were slightly more susceptible than the red wheats, and their equilibrium moisture contents were lower. Western white wheats, including club wheats, were consistently among the most susceptible, but eastern whites averaged among the most resistant. Resistance was affected by both cultivar and growing location. However, one hard red spring cultivar, Stoa, was very resistant, regardless of where grown.

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RED ROT OF MUSKMELON: A POSTHARVEST DECAY. B. D. Bruton, USDA/ ARS, Lane, OK 74555; S. C. Redlin, Contract Scientist, USDA/ ARS, Beltsville MD 20705; J. K. Collins, Okla. State Univ., Lane, OK; P. Perkins-Veazie, USDA/ARS, Lane, OK.

A decay exhibiting red discoloration in muskmelon (Cucumis melo L.) fruits has been observed in postharvest storage studies and on occasion been involved in load rejection of melons grown in Southeastern Oklahoma. Epicoccum nigrum was consistently isolated from decay areas exhibiting the red discoloration. Koch's postulates were fulfilled in controlled inoculation studies of muskmelon fruit. This is the first report of E. nigrum as a pathogen of muskmelon. Inoculations of cucumber, tomato, yellow apple, and pear proved the fungus to be pathogenic to these fruit; whereas, eggplant displayed no decay. Fungal sporulation occurred only on tomato. Glucose, fructose, and sucrose, representing the three primary sugars found in muskmelon fruit, were incorporated into separate culture media. Mycelial extension was greatest on potato glucose agar at 20°C and very limited at 1,5, and 30°C. A proposed common name for the disease is red rot.

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RESISTANCE OF CITRUS FRUIT EXOCARP TO *PENCILLIUM DIGITATUM*. J. W. Eckert, M. Ratnayake, and J. Sievert. Dept. of Plant Pathology, University of California, Riverside, CA 92521.

Wounds in the exocarp developed resistance to infection by *P. digitatum* within 24 hrs at 25°C. The degree of resistance correlated with the amount of lignin, determined spectrophotometrically as lignin thioglycolate, associated with the walls of cells surrounding the wound site. Lignin formation was increased at high relative humidity. The thioglycollate derivative was extracted and digested with CuO. A TLC of the digest revealed three UV-absorbing spots with Rf values corresponding to p-hydroxybenzaldehyde, vanillin, and syringaldehyde, characteristic components of lignin.

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VARIABILITY IN THE EQUILIBRIUM MOISTURE CONTENT OF THREE CULTIVARS OF SOYBEANS. F. A. Lazzari and R. A. Meronuck, Department of Plant Pathology, University of Minnesota, St. Paul, MN 55108.

Equilibrium Moisture Content (EMC) data are necessary to design drying and storage regimes for hygroscopic products such as grain and seeds. Soybean seeds are subjected to spoilage by storage fungi when stored with excess moisture.

The average EMC as determined by oven drying single seeds using copper cups showed that after 120 days of storage at the same relative humidity (RH) and temperature, different cultivars of soybeans reach different moisture contents.

Three lots of Hardin, Corsoy 79, and Elgin 87 soybeans stored at 75% RH resulted in an EMC of 13.50%, 14.20%, and 14.61% respectively. The same cultivars kept at 80% RH reached an EMC of 16.06%, 16.81%, and 16.60%, respectively; and when kept at 85% RH they reached an EMC of 17.58%, 17.95%, and 19.01%, respectively.

This study reveals that different cultivars of soybeans reach different levels of moisture content under the same conditions.

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MOISTURE VARIABILITY IN SEEDS OF THREE SOYBEAN CULTIVARS BEFORE HARVEST. Flavio A. Lazzari and R. A. Meronuck, Department of Plant Pathology, University of Minnesota, St. Paul, MN 55108.

The variability in moisture content (MC) of a lot of grain or seeds may predispose the entire lot to the spoilage by storage fungi. There is little uniformity in the MC among single seeds from the same lot of soybeans.

The moisture content of individual seeds collected from maturing soybean plants in the field was determined by oven-drying single seeds at 103 C for 72 hours in copper cups.

The averages MC and variation between the lowest and highest moisture content value were found for three cultivars of soybean at harvest. Elgin 87 15.24% (11.15%-25.42%) range 14.30%, Hardin 13.20% (10.00%-19.10%) range 9.15%, and Corsoy 79 12.20% (11.30%-14.32%) range 3.00%.

The greater the MC at harvest the greater the variability in the MC of soybean seeds. The effect of cultivar on moisture content variability was found to be significant.

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DIACETOXYSCIRPENOL, NIVALENOL, FUSARIN C AND ZEARALENONE FORMATION BY GEOGRAPHIC ISOLATES OF FUSARIUM CROOKWELLENSE FROM POTATO, GRAIN, AND PASTURE HERBAGE. Ronald F. Vesonder, Northern Regional Research Center, Agricultural Research Service, USDA, 1815 N. University St., Peoria, IL 61604.

Eighteen Fusarium crookwellense isolates from the continents of Australia, Europe, and North America were compared for their ability to elaborate mycotoxins on corn at 25°C for 2 weeks. Extracts from corn fermented with each Fusarium isolate was analyzed by thin layer chromatography (TLC) and gas chromatography/mass spectroscopy (GC/MS) for mycotoxins. Toxins detected were zearalenone (13 isolates), fusarin C (11 isolates), nivalenol (4 isolates), and diacetoxyscirpenol (2 isolates).

Zearalenone and fusarin C were found to be expressed by isolates from each continent, while nivalenol was detected in the Fusarium isolates originating from Australia and one isolate from the United States. These studies suggest this species is a producer of zearalenone and other mycotoxins which could cause problems with reproduction and well-being of livestock.

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RAPID-CYCLING BRASSICAS FOR HANDS-ON TEACHING OF PLANT PATHOLOGY. P. H. Williams, Dept. of Plant Pathology, 1630 Linden Drive, University of Wisconsin, Madison, WI 53706.

Rapid-cycling stocks of Brassica rapa and five other Brassica species have been developed together with inexpensive self-contained growing systems that are suitable for exploratory learning in the classroom. The materials are suitable for investigations of growth and development (plants flower in 14 days and cycle in 35 days); reproduction (flowering, pollination, fertilization, embryogeny, cell and molecular biology); genetics (mendelian, cytoplasmic, molecular, quantitative, population, breeding, selection and evolution); physiology (hormones, GA, auxin, cytokinins, photosynthesis, respiration, nutrition, water relations, tropisms and photoresponses); and ecology (chemicals and symbionts, pests, pathogens and microbes). A number of explorations in host-pathogen relations using Albugo, Plasmodiophora, Leptosphaeria, Fusarium, Xanthomonas, TuMV and CaMV have been developed. Instructional materials and genetic stocks are available through the Crucifer Genetics Cooperative at the above address or at 608/262-8638.

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VIRUS STATUS OF SEVERAL UNITED STATES SMALL FRUIT GERMPLASM COLLECTIONS. Joseph D. Postman, USDA National Clonal Germplasm Repository, 33447 Peoria Road, Corvallis, Oregon 97333.

The U.S.D.A. maintains collections of strawberry (Fragaria), currant and gooseberry (Ribes), blackberry and raspberry (Rubus), blueberry and cranberry (Vaccinium), at the National Clonal Germplasm Repository - Corvallis. Cultivated varieties and wild relatives of these crops are preserved as clones or as seed. Clonal accessions are tested for latent virus infection by ELISA, inoculation of sensitive indicator plants, and visual inspection of clones. Plants found to be virus infected are subjected to heat-therapy and in vitro meristem culture. Resulting plants are retested for viruses, and replace infected accessions when tests are negative and plant identity has been verified. The percent of virus negative clonal accessions available to researchers is as follows: 69% of 400 Fragaria clones, 49% of 168 Ribes clones, 63% of 431 Rubus clones, and 78% of 323 Vaccinium clones.

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INCIDENCE OF TOMATO RINGSPOT VIRUS IN GRAPE IN VIRGINIA. D.C Bays and S.A. Tolin, Department of Plant Pathology, Physiology and Weed Science, VPI&SU, Blacksburg, VA 24061.

In an initial survey of ten commercial vineyards in Virginia to determine the incidence of tomato ringspot virus (TmRSV), the cultivars Chardonnay, Cabernet Sauvignon, Cabernet Franc, Sauvignon Blanc, White Riesling, Vidal 256 and Merlot, and the rootstocks SO4 and Kober 5BB, were sampled. Weed species dandelion, broad-leaf and narrow-leaf plantain, and white clover were also sampled. ELISA and indicator plants were used to detect virus and confirm its identity. Only one commercial cultivar, Vidal 256, at two locations, was positive for the TmRSV. Eight vines each of SO4 and Kober 5BB rootstocks were positive at a single location, and isolates of virus from these plants are being studied further. Infected dandelions were found at the two locations which had the infected Vidal, but the virus was slightly different from that in grape. No other weed species were infected, suggesting that wild hosts do not play a role in the epidemiology of TmRSV in grape in Virginia.

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DETECTION OF VIRUSES IN FLORIST GERANIUM USING A SIMPLIFIED METHOD OF dsRNA ANALYSIS. S. T. Adkins and S. T. Nameth, Dept. of Plant Pathology, The Ohio State University, Ohio Agricultural Research and Development Center, Columbus, OH 43210.

A simplified method for analysis of viral-associated dsRNA detected the presence of a number of viruses in infected geranium plants. Greenhouse-grown florist geraniums inoculated with known viruses such as CMV, TMV, TRSV, PFBV and commercially-produced geraniums showing symptoms of virus infection were extracted and analyzed. One to 7 gr. of geranium tissue was sampled. Samples were phenol extracted and subjected to cellulose column chromotography. The purified dsRNA was analyzed with a mini electrophoresis unit (125 V for 1 hr.) on 5% polyacrylamide vertical gels. The complete extraction and analysis process took 4 hrs. DsRNA profiles and molecular weights of known viruses were compared with dsRNA profiles from plants infected with unknown viruses. The precision and the accuracy of the assay was dependent on the size of the tissue sampled and the type of virus present. This method provides a rapid and reliable means of detecting viruses in geraniums.

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EFFECTS OF PMV-SAD ON ST. AUGUSTINEGRASS. G. B. Heidel and R. W. Toler, Dept. of Plant Pathology and Microbiology, Texas A&M University, College Station, TX 77843.

Texas Common St. Augustinegrass, Stenotaphrum secundatum (Walt.) Kuntze, was mechanically inoculated with individual isolates of N and W of panicum mosaic virus-St. Augustine decline strain (PMV-SAD). Infected and uninfected St. Augustinegrass was transplanted in late May to one meter square field plots established on a modified sand root zone. Plants were observed through the growing season and into November to determine effects on growth and development due to PMV-SAD. Percent coverage was reduced significantly (a=0.05) in virus infected grass. Stolon number, internode length, leaf length and width, root length and dry and fresh weights of leaves, stolons and roots were not significantly affected over a six month period.

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DESMODIUM YELLOW MOTTLE VIRUS AND CUCUMBER MOSAIC VIRUS INFECTING WILD GROUNDNUT (Apios americana). R. A. Valverde, R. Provvidenti, and C. A. Clark, Dept. of Plant Pathology and Crop Physiology, Louisiana Agricultural Experiment Station, Louisiana State University, Agricultural Center, Baton Rouge, LA 70803 and Cornell University, Geneva, NY 14456.

Wild groundnut (Apios americana), a native legume of eastern North America is being investigated for potential domestication in Louisiana. Two virus isolates: desmodium yellow mottle virus (DYMV) and cucumber mosaic virus (CMV) were obtained from different accessions in experimental plots in Baton Rouge. Symptoms in wild groundnut induced by both viruses were indistinguishable and consisted of a mild yellow mottle. Both viruses were identified by host reaction, serology electron microscopy and dsRNA analysis. Cucumber mosaic virus was isolated from 12 out of 20 plants showing virus-like symptoms. Desmodium yellow mottle virus was found in 3 out of 20 plants tested.

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H. M. Fouly and C. J. D'Arcy. Titers of barley yellow dwarf virus-RPV-IL in tolerant and susceptible sister oat lines. Dept. of Plant Pathology, Univ. of Illinois, Urbana, IL 61801-4709.

The titers of barley yellow dwarf virus-RPV-IL (BYDV-RPV-IL) in two pairs of sister oat lines tolerant and susceptible to BYDV-PAV were measured by enzyme-linked immunosorbent assay (ELISA) and by purification. Oats were inoculated with viruliferous Rhopalosiphum padi 7, 15, or 21 days after planting. Symptoms of BYDV-RPV-IL infection were mild in all lines. Root and shoot samples were collected from 8 to 33 days after inoculation. The time of peak virus concentration, as measured by ELISA, varied with the age of plants at inoculation. No consistent pattern of virus titer was found in tolerant and susceptible sister oat pairs. BYDV-RPV-IL concentrations were higher in susceptible plants at some sampling times and in some tissues, whereas at other times or in other tissues the titer was higher in the tolerant sister oats. Yields of purified BYDV-RPV-IL were similar from tolerant and susceptible sister oat lines.

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VIRIONS OF A MISSISSIPPI ISOLATE OF SUBTERRANEAN CLOVER RED LEAF (SOYBEAN DWARF)-LIKE LUTEOVIRUS OBSERVED IN PHLOEM OF INFECTED SUBTERRANEAN CLOVER. M. R. McLaughlin, USDA, ARS, Crop Science Research Laboratory, Forage Research Unit, P. O. Box 5367, Mississippi State, MS 39762-5367.

Excised petioles from symptomatic leaves of Trifolium subterraneum L. cv. Geraldton, experimentally infected with the 'Meteora' isolate of subterranean clover red leaf (soybean dwarf)-like luteovirus (Phytopathology 78:1584), were fixed in Karnovsky's fixative, treated with RNase to remove ribosomes, postfixed in osmium, stained in uranyl acetate, and embedded in Spurr's resin. Thin sections were stained in uranyl acetate and lead citrate and examined by electron microscopy. Electron-dense spherical virus particles about 22nm in diameter were observed in the cytoplasm, nucleus, vacuoles, and plasmodesmata of some phloem cells. Vesicles containing electron-dense fibrils, but no virus particles, were observed in the cytoplasm of some infected cells. Consistent with luteovirus cytopathology, only phloem cells contained virions.

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INFECTION OF SWEET CORN AND WHEAT WITH MAIZE WHITE LINE MOSAIC VIRUS BY ARTIFICIAL INOCULATION. L. Zhang and T. A. Zitter, Department of Plant Pathology, Cornell University, Ithaca, NY 14853

Maize white line mosaic virus (MWLMV) was transmitted by wounding healthy corn or wheat seed and adding purified virus to the wound site. The MWLMV-infected corn or wheat seedlings exhibited typical MWLM symptoms. Of the major cereal crops (corn, rice, barley, sorghum, oat, wheat, etc.) tested with this technique, only corn and wheat were found to be infected. Results were confirmed using ELISA and cDNA techniques. No significant difference in susceptibility to MWLMV was found among 11 sweet corn varieties with three different genes (sh2, su, se) encoding sugary phenotypes. This technique can easily provide a continuous supply of diseased material.

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DETECTION OF TOMATO SPOTTED WILT VIRUS IN IMPATIENS USING BIOTINYLATED MOUSE MONOCLONAL ANTIBODIES. H. T. Hsu and R. H. Lawson, USDA-ARS, Beltsville, Maryland 20705

Virus-specific biotinylated monoclonal antibodies were utilized in ELISA for detection of tomato spotted wilt virus (TSWV) in the Impatiens cultivar, Mojave. Rooted cuttings from naturally infected plants were grown in a greenhouse and leaf samples removed from the apex, mid-portion and base of the stem were tested for TSWV. Leaves from the apex tested positively for TSWV while those lower on the same stem were negative. Cuttings were made from TSWV-positive and TSWV-negative portions of the same plants and grown for about 3 months. Plants from all cuttings tested positively for TSWV. Plants from cuttings of infected Impatiens grown in high temperature (27 C day; 24 C night) or low temperature (21 C day; 18 C night), with 16 and 8 hr light and dark periods, all tested positively for TSWV. At the lower temperatures, leaves were symptomless while necrosis and some leaf distortion was present at the higher temperatures.

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MAIZE DWARF MOSAIC VIRUS-VENEZUELAN STRAIN INFECTION IN Sorghum bicolor AND Zea mays BUNDLE SHEATH CELLS. F. Mayobre and M. L. Mayoral. Instituto Venezolano de Investigaciones Científicas, C.M.B.C., Apdo. 21827, Caracas 1020 A, Venezuela.

The purpose of this work was to determine MDMV-V infection effects on the ultrastructure of bundle sheath cells (BSC) and compare them to those of mesophyll cells (MC) of Sorghum bicolor

(L) var. Wray and Zea mays (L) var. Ohio 28. Seedlings were inoculated with MDMV-V. After 20 days, leaf samples were processed for electron microscopy study. Bundle, rod, laminate and pinwheel virus inclusions were observed within BSC and MC cytoplasm but not associated with organelles. Chloroplasts and mitochondria degenerating membranes were observed in both cell types. Increased starch accumulation was observed in BSC chloroplasts but not in MCchl. Cytoplasmic, nuclear, and tonoplast membranes were disrupted and, ingrowth and outgrowth were seen. Virus effect was more evident in MC than in BSC. The results indicate that BSC can provide the necessary energy for virus multiplication, functioning not only as a passive transporter of the virus particles but as active agent in the infection process.

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MOLECULAR CLONING OF A TOMATO HMG CoA REDUCTASE GENE AND ITS DEFENSE-RELATED EXPRESSION. H. S. Park, C. J. Denbow, and C. L. Cramer, Department of Plant Pathology, Physiology and Weed Science, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061-0330.

3-Hydroxy-3-methylglutaryl CoA reductase (HMGR) mediates a key rate-limiting step in isoprenoid biosynthesis leading to production of phytoalexins, rubber, electron transport components, sterols, carotenoids, gibberellins, and abscisic acid in plants. We have isolated a full-length tomato genomic clone encoding HMGR based on cross-hybridization with yeast HMG1 (Basson, et al. 1986, PNAS 83:5563). Partial sequence analysis reveals regions exceeding 65% nucleic acid and 70% derived amino acid identity with yeast HMGR. Southern analyses of tomato DNA probed with this clone reveal multiple HMGR genes. In Northern blots, HMGR sequences hybridize to mRNA of 2.4 to 2.7 kb. HMGR mRNA levels are greatly increased in early-log phase cells treated with Verticillium albo-atrum or Fusarium oxysporum cell-wall components suggesting defense-related induction.

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STRAIN-SELECTIVE RESPONSE OF LEAF DISCS OF CHRYSANTHEMUM MORIFOLIUM TO AGROBACTERIUM TUMEFACIENS IS AFFECTED BY HORMONE PRECONDITIONING. A.L. Bush and S.G. Pueppke, Department of Plant Pathology, University of Missouri, Columbia, MO 65211.

The tumorogenic response by leaf discs of two cultivars (Gem and Puritan) of Chrysanthemum morifolium to two strains (B6 and Chry 5) of Agrobacterium tumefaciens reflects the response of whole plant stem inoculations. Hormones had a preconditioning effect on the discs, enhancing tumor production, but not changing the strain X cultivar response. Leaf discs were cultured on water agar, or MS medium plus or minus hormones for 3 days before submersion for 5 minutes in A. tumefaciens (10^8 cells/ml). The leaf discs were cultured on water agar for 3-5 days, then moved to MS medium minus hormones. Tumors were visible within 1 week of co-cultivation. Tumor number was greatest when the discs were pretreated with hormones. Gem, for example, produced tumors in response to Chry 5 but not B6 in stem inoculations. Leaf discs on water agar produced an average of 1 tumor/disc in response to either strain. On MS + 0.1 mg 2,4-D/l the values were 2 and 14 tumors/disc in response to B6 and Chry 5, respectively.

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STIMULATION OF GERMINATION OF TELIOSPORES OF PUCCINIA PUNCTIFORMIS BY SEVERAL ALKYL ISOTHIOCYANATE DERIVATIVES. R. C. French, USDA-ARS, Frederick, MD 21701

Volatiles from onion and garlic tissues stimulated germination of teliospores of P. punctiformis. Known components of these aromas, and related compounds, including various allyl, sulfide, thiocyanate and isothiocyanate compounds, were tested for stimulatory activity. Most active was dodecyl isothiocyanate (C12-NCS), followed by decyl-NCS and nonyl-NCS. Octadecyl-NCS was slightly active. With dodecyl-NCS at concentrations of 1 to 100 µl/L, germination ranged between 65 and 85% at 21 days, 18 C. With decyl-NCS, germination ranged between 70 and 30% at concentrations of 1 to 50 µl/L. With nonyl-NCS, germination ranged from 55 to 15% at concentrations of 1 to 25 µl/L. These isothiocyanate derivatives are much slower acting than our previously reported hexane extract of thistle roots. However, they are the first compounds of known structure found to stimulate teliospores of P. punctiformis.

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MUTANTS OF CERCOSPORA KIKUCHII ALTERED IN CERCOSPORIN SYNTHESIS AND PATHOGENICITY. Robert G. Upchurch, D. Carey Walker, and Margaret E. Daub. Department of Plant Pathology, N.C. State University, Raleigh, NC 27695-7616.

Five mutants altered in the synthesis of the photosensitizing phytotoxin, cercosporin, were isolated following UV mutagenesis of conidia of the soybean fungal pathogen Cercospora kikuchii. The toxin mutants were found to be of two types: type 1 synthesizes no toxin on all laboratory media tested and type 2 has reduced toxin synthesis on rich (PDA) medium only. Parental isolate PR synthesized cercosporin on all media tested. Cercosporin synthesized by the mutants ranged from 0 to 36% of the parental isolate. Soybean cultivars Lee and Centennial were inoculated by three methods in the glasshouse: mycelial plug inoculation and by atomizing conidia and mycelial fragments onto leaf, pod and stem surfaces. In addition, detached pods were inoculated by conidial and mycelial suspensions. Mutants lacking the ability to synthesize toxin on all laboratory media tested did not incite leaf, pod or stem lesions.

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SILICON ENHANCES RESISTANCE OF BARLEY TO POWDERY MILDEW (Erysiphe graminis f. sp. hordei). D. Jiang, R. J. Zeyen and V. Russo, Department of Plant Pathology, University of Minnesota, St. Paul, MN 55108.

Mildew susceptible barley cvs. (Atlas and Proctor) were grown in low mineral content peat, uniformly fertilized and watered with distilled water. Silicon supplied plants (Si+) were given 10 g of $CaSiO_3$ or Na_2SiO_3 per kg of peat; whereas, silicon deficient plants (Si-) were untreated. Si+ plants grew more quickly and had significantly higher dry weights and Si contents than Si- plants. When inoculated, Si+ plant leaves had far fewer and smaller mildew colonies than did Si- leaves, and mildew sporulation was greatly reduced. Microscopic examination showed mildew germling penetration efficiency (# of germlings producing haustoria ÷ # of encounter sites) was greatly reduced on Si+ leaves, and was associated with unpenetrated host cell papillae. The decrease of fungal germling penetration efficiency was positively correlated with total Si concentrations in Si+ leaves. Si may be a structural component of epidermal cell walls, making fungal penetration difficult.

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DEGRADATION OF CELLS AND TISSUES OF SELECTED DICOTS AND GRAMINEOUS MONOCOTS BY PECTATE LYASE AND XYLANASE. C. A. Rodrigues and E. J. Braun, Dept. of Plant Pathology, Iowa State Univ., Ames, IA 50011.

The primary cell walls of grasses contain far less pectic material and far more xylan than the walls of dicots. Our goal is to determine which enzymes are necessary for the degradation of grass tissues. Endopectate lyase (PL) and endoxylanase isolated from a corn stalk rot strain of Erwinia chrysanthemi (SR120A) were tested, both alone and together, for cell-killing and tissue macerating ability. Levels of PL which killed 50-60% of root cap cells isolated from beans and cucumber killed only 10-15% of the cells isolated from oats and corn. Addition of xylanase to PL preparations had no effect on bean cells but had an additive effect on oat cell death and a synergistic effect on corn cell death. In tissue maceration studies, the number of leaf cells released by PL treatment was over 230% greater than the control for tobacco, 170% for cowpea, 16% for oats and 16% for corn. Maceration experiments using xylanase are currently in progress.

508

EXTRACELLULAR PROTEASES OF *XANTHOMONAS CAMPESTRIS* PV. *MALVACEARUM*. R. K. Gholson, C. Rodgers, and M. Pierce. Dept. of Biochemistry, Oklahoma State University, Oklahoma Agricultural Experiment Station, Stillwater, OK 74078.

Strain 3-1 of *X. campestris* pv. *malvacearum* (Xcm) produces at least three extracellular proteases, which showed different patterns of peptide bond cleavage. The protease-deficient mutant PM2 produced barely detectable levels of protease in culture under optimal inducing conditions, amounting to about 6% of that produced by the parental strain per bacterium at the same stage of culture. Purification of polypeptides from culture supernatants and assays for protease activity showed the presence in PM2 cultures of the major neutral protease P1 of Xcm. Whether the other two proteases are also present at reduced levels is being investigated. In pathogenicity tests in susceptible Acala 44, PM2 grew less well than strain 3-1, and the growth yields, although only differing by a factor of 2 or 3 at an inoculum concentration of 5×10^6 cfu/ml, differed by more than a factor of 10 when inoculation was with 10^8 cfu/ml. We have demonstrated homology between the Xcm genome and a genomic clone of the protease structural gene from *X. campestris* pv. *campestris* given to us by M. Daniels. We will report on cloning of this homologous DNA.

509

ACTIVITY, ISOZYME PATTERNS AND CELLULAR LOCALIZATION OF PEROXIDASE AS RELATED TO SYSTEMIC RESISTANCE OF TOBACCO TO BLUE MOLD INDUCED BY PERONOSPORA TABACINA AND TOBACCO MOSAIC VIRUS. X. S. Ye, S. Q. Pan and J. Kuc, Department of Plant Pathology, University of Kentucky, Lexington, KY 40546-0091

Stem injection with P. tabacina or leaf inoculation with TMV in tobacco cultivar Ky 14 induced systemic resistance to blue mold and TMV. The treatments also elicited a systemic increase in peroxidase activity which was positively correlated with induced resistance. Upon challenge with P. tabacina, peroxidase activity was increased earlier and more rapidly in the induced plants than in controls. The greatest increases in peroxidase activity were found in intercellular fluids and cell wall fractions. Isozyme patterns of peroxidase were detected on isoelectric focusing gels and showed an increase of some anionic peroxidases as a function of induced resistance. Some salt-soluble cell wall proteins also were higher in the induced plants as compared to control plants.

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CONSTITUTIVE CONJUGATES OF DAIDZEIN AND GENISTEIN MAY PLAY MULTIPLE ROLES IN EARLY RACE SPECIFIC ANTIBIOTIC RESISTANCE IN SOYBEAN. T. L. Graham, Dept. of Plant Pathology, The Ohio State University, Columbus, OH 43210.

In earlier work we demonstrated that constitutive pools of conjugates of the glyceollin precursor, daidzein, are utilized in a race specific manner for glyceollin synthesis in Phytophthora megasperma f. sp. glycinea (PMG) infected soybean cotyledon tissues. Both the rate of hydrolysis of the conjugates and the subsequent utilization of daidzein for glyceollin are race specific events. Although the rate of conjugate hydrolysis is not affected by light, the rate of glyceollin biosynthesis from free daidzein is. In the light, glyceollin synthesis is well underway within 24 hr with only moderate accumulation of free daidzein. In the dark, however, free daidzein (and closely related genistein) accumulate to high levels within 24 hr followed only later (24-48 hr) by glyceollin accumulation. We hypothesize that under those conditions where glyceollin elicitation is inefficient, the isoflavones themselves may play an additional role as direct antibiotics.

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FUNCTIONAL ANALYSIS OF CHALCONE SYNTHASE PROMOTER SEQUENCES FROM BEAN IN TRANSGENIC TOBACCO. B.A. Stermer[1], J. Schmid[2], C.J. Lamb[2] and R.A. Dixon[1]. [1]Plant Biology Division, The Noble Foundation, P.O. Box 2180, Ardmore, Oklahoma 73402. [2]Plant Biology Laboratory, Salk Institute, P.O. Box 85800, San Diego, California 92138.

Chalcone synthase (CHS) catalyzes a key step in isoflavonoid phytoalexin biosynthesis. The cis-acting regulatory sequences of CHS were examined using chimeric genes consisting of the 5'-flanking regions of bean CHS fused with the coding sequence of a "promoter-less" β-glucuronidase (GUS) gene. Transgenic tobacco plants containing these constructs were assayed for GUS activity after various inducing treatments. UV irradiation or application of $HgCl_2$ caused a 4-fold increase in GUS activity within 6 or 50 h, respectively. Infiltration of a nonpathogenic isolate of Pseudomonas syringae into transgenic tobacco leaves caused a several-fold increase in the GUS activity surrounding the hypersensitive lesions. These studies will help define the factors which coordinate defense gene expression.

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BIOTINYLATION OF TOBACCO SUSPENSION-CULTURED CELLS AND EFFECTS ON PLANT-BACTERIA INTERACTIONS. G.H. Harmon and C.J. Baker. USDA-ARS, Microbiol. and Plant Pathology Lab., Beltsville, MD 20705

Two biotinylating reagents, sulfosuccinimidobiotin and sulfosuccinimidyl 6-(biotinamido)hexanoate were used to label surface plasma membrane proteins of N. tabacum suspension cells. Biotinylation of cells at pH 6.0 for one hour prior to inoculation with Pseudomonas syringae pv syringae did not affect the H+ uptake/K+ efflux response observed in controls. Protoplasts isolated from biotinylated cells were viable and not subject to easy rupture. However, biotin labeling of protoplasts after cell wall removal resulted in an increased number of nonviable and/or fragile cells. Protoplast cell surface plasma membrane proteins labeled with either biotin reagent could be detected on western blots. These results show that biotinylation of intact cells may be a useful tool for studying plasma membrane involvement in plant-bacterial interactions.

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ORGANIZATION OF PECTINS IN POTATO TUBER CELL WALLS AND THEIR HYDROLYSIS BY PECTATE LYASE FROM PSEUDOMONAS VIRIDIFLAVA. K. Sasaki, G. Nagahashi & C.-H. Liao, USDA/ARS, Eastern Regional Research Center, Philadelphia, PA 19118

To understand the mechanism of tissue maceration by pectate lyase from P. viridiflava (SF 312), we first studied the pectin organization in potato tuber cell walls as a natural substrate. Chemical and ultrastructural analyses revealed that nearly half of pectic polymers were held in the region nearer the inner surface of the cell wall by Ca^{2+}-bridges and the rest in the middle lamella by covalent linkages. Next, potato tuber cell walls were treated with purified pectate lyase to study how this enzyme attacks these different groups of pectic polymers. After 3 h at 30 °C, 70% of the galacturonic acid was solubilized from cell walls and after 24 h, 85%. The results indicate that this enzyme can hydrolyze most pectic polymers including the middle lamella of the potato tuber cell walls. Further studies of the degradation of cell wall structure by pectate lyase will also be presented.

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CORRELATION BETWEEN IN VITRO SYNTHESIS OF PHENYLACETIC ACID AND VIRULENCE IN RHIZOCTONIA SOLANI. [1]S.M. Tavantzis, [2]B.L.Perkins, [2]R.J. Bushway, and [1]B.P. Bandy. Depts. of [1]Botany and Plant Path. and [2]Food Science, Univ. of Maine, Orono, ME 04469.

A study conducted by our group and data reported by other research teams have shown that hypovirulent isolates of R. solani (AG 3) promote an increased rate of growth in potato. Earlier work has suggested that in vitro cultures of R. solani produce phenylacetic acid (PAA) which caused disease symptoms on potato attributed to this pathogen (Frank & Francis, Can. J. Bot. 54, 2536-2540). We carried out a study to determine the amount of PAA and derivatives produced by AG 3 isolates representing a wide spectrum of virulence. PAA and derivatives were partitioned in an organic solvent and quantitated by HPLC. The amount of PAA present in the culture filtrates was proportional to the virulence of the corresponding R. solani isolate, and ranged from 45 to 595 ug of PAA per gram dry weight of mycelium. There was no correlation between the presence or amount of PAA derivatives (m-OH, p-OH, and o-OH) and virulence.

515 Withdrawn

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DIRECT DETECTION OF β-1,3-GLUCANASE ISOZYMES ON POLYACRYLAMIDE ELECTROPHORESIS AND ISOELECTROFOCUSING GELS. S. Q. Pan, X. S. Ye and J. Kuc, Department of Plant Pathology, University of Kentucky, Lexington, KY 40546.

A procedure is described to assay isozymes of β-1,3-glucanase directly on polyacrylamide electrophoresis (PAGE) and isoelectrofocusing (IEF) gels by using 2,3,5-triphenyltetrazolium chloride. The reagent reacts with reducing sugars released by β-1,3-glucanases from the substrate laminarin. Acidic and neutral isozymes of β-1,3-glucanase were detected and quantified on 17.5% native PAGE gels run with an anodic discontinuous buffer system. A significant linear relationship ($\alpha = < 0.01$, $R = 0.991$) was observed between amounts of β-1,3-glucanase loaded and intensities of bands stained with the reagent on native PAGE gels. A fuller isozyme pattern was obtained on 7.5% IEF gels with a pH range of 3.5-9.5. The IEF gels were heated in a microwave oven during the staining process to minimize diffusion.

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EFFECTS OF SOIL PHOSPHORUS (P) AND GLOMUS INTRARADICES ON GROWTH, CARBOHYDRATES, AND PHOTOSYNTHETIC ACTIVITY OF CITRUS AURANTIUM L. S. Nemec and J. C. V. Vu, USDA, ARS, Orlando, FL 32803.

Sour orange grown in low-P (9-12 ppm) and high-P (450 ppm) soil inoculated with or without Glomus intraradices (G.i.) were evaluated for biomass and various photosynthetic parameters. Growth of the low-P, no G.i. plants was lowest, with total dry biomass depressed up to 50% of the low-P, G.i. treatment. Leaf nonstructural carbohydrates (NSC) were 40% lower in low-P, no G.i. plants, compared to the other treatments. G.i. in low-P soil enhanced leaf $^{14}CO_2$ uptake by 81%, chlorophyll content by 28%, and RuBPCase activity by 34%, compared to the low-P, no G.i. treatment. Increased P-use efficiency by G.i. in low-P soil was equally effective as high-P nutrition in improving $^{14}CO_2$ uptake and NSC. PEPCase activity in low-P, no G.i. leaves, however, was at least threefold higher than the other treatments, suggesting a possible alteration in organic acid metabolism due to P deficiency.

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CHARACTERIZATION OF AN EXTRACELLULAR PROTEASE OF ERWINIA CAROTOVORA. S. Kyostio and G.H. Lacy, Plant Molecular Biology, VPI, Blacksburg, VA 24061.

Erwinia carotovora subsp. carotovora (EC14) causes soft-rot on many plants by secreting several plant cell wall degrading enzymes. To clarifiy the role of protease in potato soft-rot we have characterized a proteolytic enzyme. The intra- and extracellular fractions of EC14 were precipitated with 95% NH_4SO_4. Protease activity was detected only in the extracellular fraction. A protease inhibitory factor was detected in the intracellular fraction. The molecular weight (35 kd) and pI (4.8) of protease were determined. Protease activity was inhibited by EDTA, but not by PMSF or pepstatin suggesting that it is a metalloprotease. Protease activity was detected in EC14 cultures grown in mineral media supplemented either with polygalacturonic acid, gelatin, casamino acids, or tryptone. No protease activity was observed with glycerol as a carbon source. For further characterization a protease has been subcloned from cosmid pCA7 into plasmid pSK- and is being sequenced.

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CONIDIAL DEVELOPMENT IN COLLETOTRICHUM GRAMINICOLA. D.G. Panaccione, L.J. Vaillancourt, Z. Yang, and R.M. Hanau. Dept. of Botany and Plant Pathology, Purdue University, West Lafayette, IN 47907

Colletotrichum graminicola produced two types of conidia in culture and during lesion development on corn leaves. One was falcate and was produced from morphologically distinct conidiogenous cells. The second type was oval, variable but smaller in size than falcate conidia, and was from hyphae lacking distinct conidiogenous cells. Oval conidia were the only type produced by cultures grown in the dark whereas development of falcate conidia was light dependent. In vitro translation of poly(A)-RNA from vegetative and conidiating hyphae showed state-specific differences in mRNA at early stages in falcate conidial development.

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EVIDENCE FOR A COMPONENT OF RESISTANCE LIMITING NUMBER OF BACTERIAL BLIGHT INFECTIONS IN RICE. M.F. Koch, J. Parlevliet. International Rice Research Institute, Los Banos, Philippines and Agricultural University Wageningen, P.O. Box 386, 6700 AJ Wageningen, Netherlands

Lesion size and lesion number were measured on cultivars of rice inoculated by clipping or by spraying with virulent isolates of Xanthomonas campestris pv. oryzae, the causal organism of bacterial blight. Correlations between the methods were high (r=0.82) but specific cultivars consistently deviated from this relationship. These cultivars had lower numbers of lesions following spray inoculation and slow disease progress during the first nine weeks after transplanting in a screenhouse bed. An increased resistance component inhibiting bacterial entry appears to be present in such cultivars. In order to select rice entries with high quantitative resistance to bacterial blight, based on both resistance to bacterial entry and to bacterial spread, two screenings, one for lesion length after clipping, and one for lesion number after spraying, are advised.

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RESPONSES OF WHEAT SEEDLINGS TO LEAF RUST AT TWO TEMPERATURE REGIMES. Beatriz A. Perez and A. P. Roelfs, Department of Plant Pathology, University of Minnesota and Cereal Rust Laboratory, USDA/ARS, St. Paul, MN 55108.

TcLr34 is a near-isogenic wheat (Triticum aestivum) for this important adult plant resistance to leaf rust (Puccinia recondita f. sp. tritici). Seedlings of TcLr34, the near-isogenic lines used in race identification, selected cultivars, and Thatcher were tested against isolates of wheat leaf rust at 5 C, and 20 C, with 8 h dark/16 h light cycle (15,000 lux). At 20 C, seedlings of TcLr34 and cultivars postulated to have Lr34 were susceptible to isolates with a wide range of virulence. At 5 C, TcLr34 and the cultivars Chris, Era, Fletcher, Frontana, Klein Cometa, Marcos Juarez Inta, Polk, Rio Negro, Sonalika, Surpresa, Veranopolis, and Wheaton had low infection types to some of the isolates tested. The period required for full pustule development on the susceptible check varied from 23-25 days and 12-14 days at 5 C and 20 C respectively. The ability to detect Lr34 in the seedling stage would enable a more rapid and extensive testing program.

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AVIRULENCE OF WHEAT LEAF RUST TO BUCK MANANTIAL. Beatriz A. Perez and A. P. Roelfs, Department of Plant Pathology, University of Minnesota and Cereal Rust Laboratory, USDA/ARS, St. Paul, MN 55108.

Buck Manantial is an Argentinian spring wheat (Triticum aestivum) derivative of Americano 25c through Buck Quequen, General Urquiza, and Klein San Martin. Buck Manantial has been resistant to leaf rust in Argentina since it was released in 1965. The Canadian wheat Kenyon, postulated to have Lr13 and Lr16, was derived from Buck Manantial. Seedlings and adult plants of selected near-isogenic lines, wheat cultivars, and the susceptible check Thatcher were tested with isolates of Puccinia recondita f. sp. tritici with a wide range of virulence. Seedlings of Americano 25c, a land cultivar from Uruguay, and Kenyon were susceptible when tested against isolates virulent on Lr16. Seedlings of Buck Manantial were immune to all isolates evaluated; therefore, resistance beyond Lr16 is present. Adult plants of Buck Manantial were resistant to all cultures to which they have been evaluated. It is currently unknown if Lr13 is the major source of this resistance.

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GENES FOR POWDERY MILDEW RESISTANCE IN CULTIVARS OF SOFT RED WINTER WHEAT, S. Leath and M. Heun, USDA-ARS and Dept. of Plant Pathology, North Carolina State University, Raleigh 27695, and Lehrstuhl fur Pflanzenbau und Pflanzenzuchtung, D-8050 Freising-Weihenstephan, FRG, respectively.

Twenty-two soft red winter wheat cultivars were inoculated with isolates of Erysiphe graminis f. sp. tritici to determine genes for resistance to the fungus. Cultivars were tested with isolates that had been characterized from reactions on differential host lines. Determinations were completed independently in two laboratories with different isolates and results combined. Inoculations were done on intact 10-day-old seedlings or on detached leaves on benzimidazole amended agar (50 ppm). Evaulations were done 10-14 days later and based on pustule number and type. Some cultivars were not fully characterized while results also indicated other cultivars had no genes for powdery mildew resistance. Gene Pm3a was identified as occurring most often in the cultivars tested.

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Screening methods to evaluate resistance to net and spot blotch diseases of barley. T. G. Fetch, Jr., B. J. Steffenson, J. D. Frankiowiak and V. D. Pederson. Dept. of Plant Pathology, North Dakota State University, Fargo, ND 58105.

Net (Pyrenophora teres) and spot blotch (Cochliobolus sativus) are the most prevalent barley diseases in North Dakota. Disease control is best obtained by use of resistant cultivars. Establishment of quick and reliable techniques to evaluate barley lines is vital to the breeding program. Several screening methods were tested for assessing resistance in barley. The best seedling test was the ragdoll germination procedure using a leaf-dip inoculation and the best adult plant (early heading) testing method was a spray-gun inoculation technique. Plants were rated 7 days after inoculation using a 9 point scale, and results were compared to field ratings. Correlation coefficients between seedling and field (0.43, 0.71), and adult and field ratings (0.64, 0.76) were highly significant for net and spot blotch, respectively. These methods have proven successful for early detection of resistance in barley breeding material.

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RESPONSE TO S_1 RECURRENT SELECTION FOR MAIZE GRAIN YIELD IN A DISEASE-STRESS ENVIRONMENT. M.L. Carson and Z.W. Wicks, III, SDSU, Plant Science Department, Brookings, SD 57007.

Selection of genotypes that yield well in a disease-stress environment has been proposed as a way to increase disease resistance and yield potential in the absence of disease simultaneously. S_1 recurrent selection was conducted for two cycles in the BS-19 maize synthetic for: 1) grain yield in the presence of northern leaf blight (NLB) caused by Exserohilum turcicum, and Diplodia stalk rot (DSR) caused by D. maydis, 2) grain yield in the absence of disease, and 3) resistance to both NLB and DSR. The original synthetic and the two advanced cycles from the three selection schemes were evaluated in 1988 in separate trials for yield in a NLB and DSR stress environment, yield in the absence of disease, and for NLB and DSR resistance. Selection for grain yield in the disease stress environment was effective in increasing NLB resistance and yield potential in the absence of disease. Direct selection of NLB and DSR resistance was also effective.

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VARIATION IN FUSARIUM RESISTANCE IN SOMACLONES REGENERATED FROM ASPARAGUS OFFICINALIS CV. LUCULLUS 234. M.L. Smither, T.L. Wacker and C.T. Stephens. Dept. of Botany and Plant Pathology, Michigan State University, East Lansing, MI 48824

Asparagus cv. Lucullus 234 is tolerant of Fusarium crown and root rot. Callus formed when seedlings were germinated in the dark at 27 C on MS medium supplemented with 3% sucrose, 1.5 mg/L 2,4-D, 1. mg/L NAA, and 0.5 mg/L kinetin. Callus was subcultured on the same medium without 2,4-D. Shoots regenerated on MS medium supplemented with 1.5% sucrose and 1 mg/L kinetin at 27 C with a 16 hr. daylength, then rooted on MS medium containing 3% sucrose, 0.3 mg/L NAA, and 0.7 mg/L kinetin. Somaclones were transferred to soil for 2 wks. under plastic, then to Fusarium-infested soil. Disease development was least in the resistant control A. sprengeri, and greatest in susceptible UC 157. Disease expression in Lucullus somaclones ranged from resistant to susceptible.

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INHERITANCE OF RESISTANCE TO RACES 0, 1, AND 2 OF FUSARIUM WILT IN MUSKMELON LINE MR-1. F. W. Zink and C. E. Thomas. Department of Vegetable Crops, Univ. of California, Davis, CA 95616 and USDA, ARS, U. S. Vegetable Laboratory, Charleston, SC 29414

In artificial inoculation studies, muskmelon (Cucumis melo L.) breeding line MR-1 was resistant to races 0, 1, and 2; but not 1,2y or 1,2w of Fusarium wilt incited by Fusarium oxysporum f. sp. melonis. Segregation of F_1, F_2, and BC_1 populations of crosses between resistant MR-1 and susceptible Top Mark indicated that resistance to races 1 and 2 is conferred by a single dominant gene. Linkage tests indicated that the genes for resistance to races 1 and 2 are not linked. Allelism tests showed that the single dominant genes in MR-1 that confer resistance to races 0 and 2 and races 0 and 1 are the same genes or alleles of Fom-1 in differential cultivar Doublon and Fom-2 in differential line CM 17-187, respectively.

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EFFECTS OF HOST GENOTYPE ON INCIDENCE OF CERCOSPORA ARACHIDICOLA AND CERCOSPORIDIUM PERSONATUM ON PEANUT IN FIELD ISOLATION PLOTS. B. B. Shew and M. K. Beute, North Carolina State University, Box 7629, Raleigh, NC 27695.

Six peanut genotypes, having various levels of resistance to Cercospora arachidicola (CA), Cercosporidium personatum (CP), or both pathogens, were planted in 9.8 m x 3.7 m plots in May 1988. Each of the total 120 plots was separated by at least 7.3 m of corn. A potted plant infected with CA, CP, or CA + CP was placed in the center of each plot on 2 August as a point source of inoculum. No infected plants were placed in control plots. AUDPCs of CA, CP, or both leaf spots were calculated from % incidence on 9 rating dates. CA and CP were detected in all plots. AUDPCs for CP were greatest in plots with a CP or CA + CP inoculum source, but genotype rankings varied depending on the source of inoculum. Inoculum source did not affect

AUDPCs for CA or rankings of genotypes by AUDPC-CA. AUDPCs were smallest on NC 3033 for CA, on Southern runner for CP, and on GP-NC 343 for both leaf spots.

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REGENERATION OF TOLERANT SOYBEAN PLANTS TO SEPTORIA GLYCINES FROM ORGANOGENIC CALLI. H. S. Song, S. M. Lim, and J. M. Widholm. Dept. of Plant Pathology, University of Illinois, Urbana, IL 61801.

Organogenic calli obtained from 225 immature embryos of soybean cv. BSR 201 were transferred to MSR medium ammended with pathotoxic culture filtrates of Septoria glycines (2:1, v/v). More than 90% of the calli died within 48 hrs. The surviving calli were transferred again 4 times onto the ammended MSR medium and which survived were selected. Calli which developed shoots were transferred to MSR medium and evaluated for growth. After two months, the shoots were transferred to MSS basal medium ammended with the pathotoxin. Surviving shoots developed roots. Three regenerated plants were grown to maturity in the greenhouse and seeds were harvested. A detached leaf assay on R_2 plants (second selfed generation) and control BSR 201 plants indicated that development of brown spot symptoms and pycnidia were significantly delayed on the leaves of th R_2 plants when inoculated with spore suspensions of S. glycines.

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SUSCEPTIBILITY OF FLORIDA SUGARCANE CULTIVARS TO PUCCINIA MELANOCEPHALA. R. N. Raid, University of Florida, Everglades Research and Education Center, Belle Glade, 33430 and B. R. Eiland, Okeelanta Corp., South Bay, 33493.

Twelve commercial sugarcane cultivars were assessed for sugarcane rust severity resulting from natural infection during 1988. A total of 36 top visible dewlap leaves from 3 replications were examined per cultivar for disease severity and host response. Significant differences ($p \leq 0.05$) were detected among cultivars in both disease severity and response. Cultivars were classified as being resistant, and moderately or highly susceptible, exhibiting uredia with profuse sporulation and a mean disease severity of 39%. CP72-1210, CL73-239, CP70-1257, and CP74-2005 were classified as moderately susceptible with spore-producing uredia frequently being observed. Resistant cultivars exhibited only necrotic or chlorotic flecking. Based upon these results, over 64% of Florida's sugarcane acreage is currently planted to cultivars of moderate or high susceptibility.

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Use of phytotoxic components from Septoria nodorum and wheat callus cultures for in vitro germplasm screening. S. Leath and K. E. Papke, USDA-ARS, Department of Plant Pathology, North Carolina State University, Raleigh 27695-7616.

In an attempt to exploit possible somaclonal variation for resistance to Septoria leaf and glume blotch of wheat, a tissue culture based screening procedure was developed. Crude extract (CE) was obtained from 3-wk-old fungal broth cultures of two Septoria nodorum isolates with three successive ethyl acetate extractions; activity was similar to that of purified mellein. Immature embryo callus of six wheat cultivars ranging in resistance to S. nodorum was cultured on a modified Murashige and Skoog (MMS) medium with 1.0 mg 2,4-D/l. Cultures that did not show leaf or root differentiation after approximately two weeks were transferred to media amended with CE. Single challenges at 1000, 750, and 500 ppm and repeated challenges at 250 and 500 ppm were utilized and then cultures were transferred to MMS without CE. Evaluation of germplasm obtained with both selection techniques using progeny from regenerated plants is currently underway.

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AN IN-VITRO TECHNIQUE FOR SCREENING ALFALFA SEEDLINGS FOR RESISTANCE TO PHYTOPHTHORA ROOT ROT. M. J. Horstman and R. B. Carroll, University of Delaware, Newark, Delaware, 19717-1303 and E. R. Jones, Delaware State College, Dover, Delaware 19901.

An in-vitro technique for screening alfalfa seedlings for resistance to root rot caused by Phytophthora megasperma f. sp. medicaginis (Pmm) was evaluated. Microbe-free germlings of Agate, WL-318, Iroquois and Saranac were grown aseptically in test tubes containing an inverted filter paper cone moistened with Hoagland solution. Tubes were placed in growth chambers in a randomized complete block design with 10 replications. After 4 weeks seedlings were inoculated with zoospore suspensions of pathogenic Pmm isolates. Disease ratings made 3 weeks later were compared to greenhouse experiments. For both methods ratings of resistant cultivars were significantly lower than susceptible (P=0.05). Results indicate that the in-vitro technique is reliable and effective.

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DISTRIBUTION OF GLOEOTINIA TEMULENTA, CLAVICEPS PURPUREA, AND ANGUINA AGROSTIS AMONG GRASSES IN THE WILLAMETTE VALLEY OF OREGON IN 1988. S. C. Alderman, USDA/ARS, 3450 S.W. Campus Way, Corvallis, OR. 97331

During the summer of 1988 a survey of commercial grass seed production fields in the Willamette Valley of Oregon was initiated to determine the incidence of the grass seed diseases, ergot, blind seed, and seed gall nematode. A total of 492 fields of various preselected cultivars were examined. Ergot was found in 52, 13, 1, 2, and 2% of the bluegrass, bentgrass, tall fescue, Italian ryegrass, and perennial ryegrass fields, respectively. Blind seed was detected in 26-30% of the tall fescue, annual ryegrass and perennial ryegrass fields. Seed gall nematode was found in 9% of the bentgrass fields. A survey of weed grasses indicated that ergot was widespread throughout the valley on tall fescue, annual ryegrass, and quackgrass.

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VERTICILLIUM WILT OF ALFALFA IN SOUTHERN CALIFORNIA. D. C. Erwin, R. A. Khan and Amy Howell. Dept. of Plant Pathology, Univ. of California, Riverside, CA 92521.

Verticillium albo-atrum (*Vaa*), alfalfa strain, which causes a vascular wilt of alfalfa, has been found in San Bernardino, Los Angeles and Riverside counties and in the extreme south side of Kern county, but not in the Central Valleys. Optimal temperatures for growth were 21 C and 24 C for different isolates. Although maximum temperature for growth was 30 C, *V. albo-atrum* was isolated from infected plants in August when air temperatures were over 38 C. Pathogenicity of several isolates was tested on alfalfa by root dip inoculation (8×10^6 spores/ml); all isolates reproduced wilt symptoms and *Vaa* was reisolated. Germ plasms from southern California and the nondormant cultivars CUF101, UC Cibola and Moapa 69 were susceptible; the semidormant cultivar Vernema (Peaden, USDA-ARS) was moderately resistant and Vertus (France) was resistant. Cowpea (a new host) and cantaloup were highly susceptible. Beans, garbanzo beans, and peas were symptomless hosts.

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AN EFFICIENT METHOD FOR INOCULATION OF SUBTERRANEAN CLOVER WITH CERCOSPORA ZEBRINA IN THE GREENHOUSE AND FIELD. R. G. Pratt, USDA, ARS, P.O. Box 5367, Miss. State, MS 39762

A mixture of wheat and oat grains in flasks was inoculated with hyphal fragments of Cercospora zebrina and incubated for 2 wk at 25-30 C. The infested substrate (WO) was removed, air-dried, fragmented by blending while dry, and sieved. Particles that passed through a 40-mesh screen were dusted onto leaves of subterranean clover (Trifolium subterraneum) sprayed with a sticker. Plants were incubated in a saturated atmosphere for 4 days and subsequently in ambient air for 4-8 days at 25-30 C. Necrotic lesions that developed on leaves were identical to those obtained by inoculations with spore suspensions, and C. zebrina was reisolated. Numbers of lesions and percentage necrosis of leaf tissue increased with inoculum concentration except at high levels. Resistant and susceptible reactions of cultivars were expressed with WO inoculum as with spore suspensions. Whole infested WO applied to field plots induced symptoms as efficiently as naturally infested plant debris.

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INFECTION AND DEVELOPMENT OF THE SMUT PATHOGEN USTILAGO SYNTHERISMAE IN LARGE CRABGRASS. D. A. Johnson and A. B. A. M. Baudoin, Dept. of Plant Pathology, Physiology, and Weed Science, VPI&SU, Blacksburg, VA 24061.

Ustilago syntherismae, which causes loose smut of large and smooth crabgrass, infects its host systemically and prevents seed production. In the field, diseased plants were usually not observed until late in the growing season, well after crabgrass had commenced flowering. Greenhouse experiments were carried out to determine the mode of infection, the disease incidence that can be obtained by artificial inoculation, and

the reasons for the late observance of the disease. U. syntherismae infected large crabgrass (Digitaria sanguinalis) by both inoculation of seed (vacuum-infiltration with a suspension of teliospores or dusting with dry teliospores) and application of teliospores to the surface of potting mix containing germinating seeds. Disease incidence was high (80-100%) in some treatments. Emergence of the inflorescences of infected plants was delayed by 2-5 weeks compared to those of healthy plants.

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YIELD RESPONSES OF SOYBEANS TO FUNGICIDES APPLIED DURING VEGETATIVE GROWTH STAGES. P. A. Backman and J. C. Jacobi, Dept. of Plant Pathology, Auburn University, AL 36849-5409.

Disease severity of anthracnose and Septoria brown spot and yield response of soybeans to benomyl applications were evaluated in replicated field trials during 1987 and 1988. Each treatment received a single benomyl application during a different week of the vegetative period. Responses were compared to treatments receiving benomyl during the currently recommended reproductive growth stages. Yields were significantly increased in each of the tests, though disease control did not always correlate with yield. Other diseases such as the Diaporthe-Phomopsis complex may also have been affected, but were not rated. These results offer the possibility of altered timings to control midseason diseases, and additionally indicate that suppression of latent infections caused by the D-P complex or anthracnose during vegetative growth stages may result in yield improvements similar to those seen from recommended reproductive stage sprays.

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IMPACT OF PECAN SCAB AND PECAN LEAF BLOTCH ON LEAF GAS EXCHANGE OF PECAN. A. B. Gould, J. H. Aldrich, and P. C. Andersen. University of Florida, AREC, Rt. 4 Box 63, Monticello, FL 32344.

The influence of pecan scab (Cladosporium caryigenum) and pecan leaf blotch (Mycosphaerella dendroides) was assessed on leaf gas exchange and chlorophyll concentration of pecan (Carya illinoensis) cvs. 'Cape Fear' and 'Choctaw'. Scab lesions occupying ca. 5% of the surface of expanded summer-flush leaves reduced net CO_2 assimilation (A) and transpiration rates (E), leaf conductance to water vapor (gl) and chlorophyll concentration (Chl) by 20 to 55% for both cultivars. Leaf blotch lesions covering 50% of the expanded summer- and spring-flush leaf surfaces decreased A by 65 to 85%, but had a lesser impact on E, gl, and Chl. Intercellular CO_2 concentration increased and Chl decreased in leaves affected by either disease. These data indicate that pecan scab and leaf blotch have a direct impact on the photosynthetic apparatus.

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DETECTION OF ISOLATE DIFFERENCES IN *PERONOSPORA TABACINA* UTILIZING A CORESTA LEAF DISK BIOASSAY. M. D. Wiglesworth, W.C. Nesmith, C.E. Main, M.R. Bonde, G.L. Peterson, and M.R. Siegel. University of Kentucky, Lexington, Kentucky 40546; North Carolina State University, Raleigh, N.C.; and USDA, Ft.Detrick, Md.

Isolates of the blue mold pathogen, *Peronospora tabacina*, were maintained in liquid nitrogen at the University of Kentucky and the U.S.D.A. laboratory at Ft.Detrick, Maryland. This collection included domestic (Texas 1983-1988, Kentucky 1985-1987, and western North Carolina 1986-1987) and international (Mexico 1987 and Bulgaria 1988) isolates of *P. tabacina*. To detect whether differences existed between these isolates, a leaf bioassay was developed utilizing cultivars of the CORESTA trap collection, acetone-dipped leaf disks (4mm in diameter), and flourescent microscopy. Measurements of sporangiospore germination and penetration on inoculated disks indicated that isolates of domestic origin were similar within a particular year of collection. Comparison between years indicated a significant difference (PR>0.0001)among domestic isolates. Within a particular year, comparisons of locations indicated the isolates collected in Texas and Kentucky were similar in the years 1985-1987. Isolates collected from North Carolina were similar to the Texas and Kentucky isolates in 1987. Mexican and Bulgarian isolates were significantly different (PR>0.0001) from all other isolates. For each of the isolates, significant (PR>0.0001) cultivar/ isolate interaction was observed with the nine cultivars of the CORESTA collection. These data are consistent with the hypothesis that the Texas isolates may be the source of inoculum for epidemics in Kentucky and North Carolina. Differences noted in the foreign isolates may indicate the presence of different fungal strains.

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TEMPERATURE, PH AND FREE WATER EFFECTS ON IN VITRO GERMINATION OF CONIDIA OF A DISCULA SP. ISOLATED FROM DOGWOOD ANTHRACNOSE LESIONS. Kerry O. Britton, USDA Forest Service, SEFES Carlton St., Athens, GA, 30602

In vitro germination of a Discula sp. isolated from dogwood anthracnose lesions from north Georgia was tested. Conidia were sprayed on pH 5.6 water agar (WA), and incubated 24 hr at 5, 10, 15, 20, 25, 30, and 35C. Mean germination was 0, 3, 48, 80, 68, 65, and 3 percent, respectively. The optimum temperature for germination was 20C in two experiments, but a previous study with a different isolate gave equally good germination at 24C. Acid rain solutions with pH 2.0, 3.0, 4.0, 5.0, and 5.6 were incorporated in 2% agar plates, seeded with conidia and incubated 24 hr at 24C. Spore germination was 0 at pH 2.0, but did not differ significantly over the range of pH 3.0 to 5.6 (mean = 53%). Conidia in sterile water (pH 5.6) failed to germinate after 5 d, but when transferred to WA, 49% of these germinated within 24 hr.

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VARIATION IN MUTANTS OF SEPTORIA GLYCINES INDUCED BY ULTRA-VIOLET IRRADIATION. H. S. Song and S. M. Lim. Dept. of Plant Pathology, University of Illinois, Urbana, IL 61801.

A single spore isolate of Septoria glycines was grown on potato-dextrose agar plates and irradiated with ultra-violet light (260 nm) for 7 mins at 24 C. Approximately 0.4% of the irradiated spores survived and exhibited morphological, physiological and pathogenic variation. Examples of variation included hyaline spores, a leucine auxotroph, and loss of virulence on soybean cv. Williams. Some avirulent mutants were identical to the wild type isolate except for pathogenicity. Culture filtrates of the avirulent mutants did not exhibit pathotoxicity when cotyledons of Williams were bioassayed whereas culture filtrates of the wild type isolate exhibited pathotoxicity. Also, soybean callus growth, which was inhibited by culture filtrates of the wild type isolate, was not inhibited by culture filtrates of the avirulent mutants. Thus, pathogenicity of S. glycines appears to be related to pathotoxin production.

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ASCOKEY - A COMPUTER SOFTWARE PROGRAM FOR IDENTIFYING GENERA OF ASCOMYCETES. R.T. Hanlin and D.D. Pope, Department of Plant Pathology, University of Georgia, Athens, GA, 30602.

ASCOKEY was developed to assist mycologists at all levels to identify important genera of ascomycetes. The software is coded in PASCAL and is completely menu driven; it can be run on any IBM-compatible computer. It currently contains keys and descriptions to 100 genera, but it can be readily expanded. The program contains three main modules. The first module consists of a dichotomous tree structure containing appropriate couplets which the user traverses to reach a particular genus. This key is also reversible. The second module provides a synoptic key for identifying genera on the basis of morphological characteristics. The third module contains descriptions of each genus; these descriptions can be accessed from either key or directly from the opening menu. Illustrations of each of the 100 genera are available in the published version. The key will be a useful aid both for training mycology students and for field mycologists. The key is under copyright to APS Press, which is also publishing the book on which the key is based.

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RHIZOCTONIA SPECIES ASSOCIATED WITH BEDDING PLANTS, NURSERY PLANTS AND SOME VEGETABLE CROPS IN SOUTH AUSTRALIA:IDENTITY AND PATHOGENICITY. D.A. Schisler, S.M. Neate, and G. Masuhara, CSIRO Division of Soils, Adelaide.

Diseased plants and soil were collected from 30 nurseries and four vegetable crops in South Australia. Forty-nine isolates of *Rhizoctonia* were obtained from diseased plants, soil and soil baited with seedlings of wheat, radish, brussel sprout, ornamental lupin, zinnia and bell pepper. Twelve isolates were multinucleate and 37 binucleate. When the multinucleate isolates were tested for anastomosis with isolates from AG-1 to AG-8, four were AG-2-1, seven were AG-4 and one could not be identified. All isolates were then grouped according to origin and morphology and representatives were fruited by the soil over agar method. Five isolates formed the *Thanatephorus cucumeris* teleomorph, six were *Ceratobasidium cornigerum*, one was *Ceratobasidium pseudocornigerum* and one was a *Ceratobasidium* sp. Electrophoretic patterns of pectic enzymes produced by the isolates showed differences between genera and species. Isolates were tested in growth cabinets for their pathogenicity on a representative from the Gramineae, Cruciferae, Leguminosae, Compositae and Solanaceae. Host plant and temperature significantly influenced the pathogenicity of isolates.

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RELATIONSHIP OF CELL WALL COMPONENTS TO QUIESCENT INFECTION OF STRAWBERRY. W. S. Conway, USDA, ARS, Hort. Crops Quality Lab, Beltsville, MD 20705, B. D. Bruton, USDA, ARS, SCARL, Lane, OK 74555, K. C. Gross, USDA, ARS, Hort. Crops Quality Lab, and J. L. Maas, USDA, ARS, Fruit Lab, Beltsville, MD 20705.

In order to determine the possible effect that various cell

wall components have on quiescent infection of strawberry caused by B. cinerea, cell walls were extracted from fruit harvested at different stages of maturity from several strawberry cultivars. Cell wall neutral sugars rhamnose, arabinose, galactose, xylose, mannose, and glucose all decreased by varying amounts on a g fresh wt basis as the fruit matured. Both total calcium and cell wall bound calcium decreased with maturity on a g fresh wt basis as well. Since both sugar and calcium content of the host tissue have been shown to affect fungal growth and pectolytic enzyme production and activity, the possible relationship that these cell wall components have to quiescent infection will be discussed.

547 Withdrawn

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CLONING AND SEQUENCE ANALYSIS OF GENE(S) ENCODING β-TUBULIN IN THE HEMIASCOMYCETE PLANT PATHOGEN *GEOTRICHUM CANDIDUM*. S. E. Gold and N. T. Keen. Dept. of Plant Pathology, University of California, Riverside, CA 92521.

Sequences homologous to β-tubulin were cloned with the goal of obtaining taxonomic data and developing a transformation system for the hemiascomycete *Geotrichum candidum* (*G.c.*). Additionally, wild type *G.c.* is resistant to benomyl adding to the interest in understanding the structure of these genes. Using the cloned *Neurospora crassa* Tub (Bn^r) from plasmid pSV50, two groups of clones were isolated from an EMBL3 *G.c.* genomic library. The two groups differed in intensity of hybridization signal and restriction pattern. A 2.8 kb fragment including 550 base pair (bp) 5' and 600 bp 3' to the coding region has now been sequenced from one of these clones. Salient features relevent to taxonomy will be presented. The second group of clones has yet to be characterized. Transcriptional fusions using noncoding regions of the sequenced β-tubulin gene to flank antibiotic resistance gene(s) are being tested to develop a transformation system for the fungus.

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PROTOPLAST FORMATION AND TRANSFORMATION OF *HYPOXYLON MAMMATUM*. D. H. Griffin, M. DeVit, and R. Tuori. SUNY, College of Environmental Science and Forestry, Syracuse, NY 13210.

A transformation system for *Hypoxylon mammatum* has been accomplished using protoplasts obtained from mycelium of 40 hr cultures in defined medium inoculated with mycelial fragments. Protoplasts were released using Novozym® 234 in 1.2M $MgSO_4$. Addition of β-mercaptoethanol increased the yield of protoplasts. Protoplasts were purified from hyphal fragments with a sorbitol step gradient, but sorbitol in the regeneration medium was inhibitory, as were the osmoticants mannitol and KCl. Optimal viability of 20-25% was obtained with sucrose or glucose at 0.8M with bovine serum albumin treated protoplasts. Transformation efficiency was optimal at 50 mM $CaCl_2$ in the presence of aurantricarboxylic acid. Stable transformants were obtained using pBT6 carrying the *Neurospora crassa* $benA^r$ gene. Characterization of the transformants will be discussed.

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CLONING AND CHARACTERIZATION OF THE EIN GENE OF FUSARIUM SOLANI f.sp. PHASEOLI. G.H. Choi, E.T. Marek, C.L. Schardl and D.A. Smith, Department of Plant Pathology, University of Kentucky, Lexington, KY 40546-0091.

The EIN gene of Fusarium solani f.sp. phaseoli and Fusarium.oxysporum f.sp. cucumerinum is induced by various stress factors, including ethanol. Previously, a cDNA clone of EIN mRNA from F. oxysporum was sequenced. The EIN gene was isolated from a cosmid genomic library of F. solani f.sp. phaseoli by homology with the heterologous cDNA. The 3' region of the F.solani gene has been sequenced. It revealed an intron of 48 bases. The 5' and 3' splice sites as well as internal consensus sequences of the intron matched well with those of Neurospora crassa. The 3' region of the gene showed a very high degree of homology (86%) to the EIN cDNA of F.oxysporum. The promoter of this highly expressed gene will be used to construct a transformation vector for Fusarium spp.

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IMPROVED TRANSFROMATION OF COLLETOTRICHUM GRAMINICOLA PROTOPLASTS AND ITS USE IN CLONING THE PYR1 GENE. J.B. Rasmussen, D.G. Panaccione, and R.M. Hanau. Dept. of Botany and Plant Pathology, Purdue University, West Lafayette, IN 47907

Transformation of C. graminicola was improved by adding spermidine (8 mM) and heparin (1 µg/µl) to DNA prior to transformation and by osmotically stabilizing the polyethylene glycol with KCl (600 mM). With these modifications, over 1000 benomyl-resistant transformants were recoverd per µg of pCG7 DNA which encodes a mutant ß-tubulin gene from the fungus. The procedure was used to screen a C. graminicola cosmid library for sequences that would complement C. graminicola strain M2001 which is a uracil auxotroph isolated by selection for resistance to 5-fluoroorotic acid. PYR1, the gene responsible for complementing the mutation in M2001, was rescued and subcloned as a 3 kb Hind III/Sal I fragment creating vector pJR70. Transformation with pJR70 yields in excess of 1000 PYR^+ transformants per µg of plasmid.

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EXPRESSION OF MBC (BENOMYL) RESISTANCE IN ASPERGILLUS FLAVUS TRANSFORMED WITH A HOMOLOGOUS B-TUBULIN GENE. E. R. Seip, C. P. Woloshuk, G. A. Payne and C. R. Adkins. Dept. Plant Pathology, North Carolina State University, Raleigh, NC 27695.

Aspergillus flavus was transformed with plasmid pBRG4 containing both the pyr4 gene of Neurospora crassa and a B-tubulin gene from A. flavus conferring resistance to MBC (the active component of benomyl). Transformants were selected for uracil prototrophy at a frequency of 15 per ug transforming DNA. Thirty transformants were tested for resistance to MBC. Six were as sensitive as the recipient strain (sensitive to 1 ug/ml MBC), 19 were intermediate (grew on 1 or 2.5 ug/ml MBC) and 5 were resistant to 5 ug/ml MBC. Vector DNA appeared to integrate at the homologous locus in all phenotypic classes. Selection of transformants on 0.75 ug/ml MBC resulted in an average frequency of 2.8 per ug transforming DNA. The lower frequency as compared to uracil selection was presumably caused by the failure to select transformants that were sensitive or intermediate in resistance to MBC.

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ISOLATION AND FUSION OF PROTOPLASTS FROM FUSARIUM OXYSPORUM f.sp CONGLUTINANS AND f.sp RAPHANI E.A. Momol, F. N. Martin, and H. C. Kistler. Plant Pathology Department, University of Florida, Gainesville, FL. 32611.

Protoplasts were obtained from strains of two formae speciales (f.sp) of the wilt pathogen Fusarium oxysporum using cell wall degrading preparation Novozyme 234. Protoplasts of isolates 722 (f.sp conglutinans, a cabbage pathogen) and 724 (f.sp raphani pathogenic of radish) were labelled with fluorescein isothiocyanate and hydroethidine, respectively. Labelled protoplasts were fused in the presence of PEG and sorted based on size and fluorescence using flow cytometry. Regeneration frequency of inter-forma specialis fusion products was approximately 0.5%. Mitochondrial and nuclear backgrounds and the presence of mitochondrial plasmids in fusion products were examined using DNA probes specific for each forma specialis. Fusion products were obtained having non-parental combinations of nuclear and mitochondrial markers; the influence of this on host specialization will be determined.

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ELECTROPHORETIC CONFIRMATION OF CHROMOSOME REARRANGEMENTS IN COCHLIOBOLUS HETEROSTROPHUS --- TRANSLOCATION AT TOX1. Tzeng, T., H. Chang, C. R. Bronson, Department of Plant Pathology, Iowa State University, Ames, IA 50011.

We are confirming linkage group assignments in our RFLP map of C. heterostrophus by hybridizing the probes used for constructing the map to electrophoretically separated chromosomes. To date, several differences in chromosome arrangement between the isolates used to make the map have been

verified. A translocation linked to Tox1 has also been detected. To determine the tightness of this linkage, probes around Tox1 were hybridized to chromosomes of near-isogenic race T (TOX1) and race O (tox1) strains. Results support our hypothesis that race T and race O differ by a reciprocal translocation with its breakpoint at or near Tox1.

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A TECHNIQUE FOR PRODUCING PROTOPLASTS AND RECOVERING INTACT CELLS FROM THE PATHOGENIC FUNGUS USTILAGO HORDEI. K.E. Duncan and D.D. Pope, Department of Plant Pathology, University of Georgia, Athens, GA 30602.

A technique was developed to obtain high yields of Ustilago hordei protoplasts and to recover intact cells from protoplasts as a prerequisite for transformation experiments. Protoplasts were obtained from log-phase, haploid, sporidial cells by degrading fungal cell walls with a mixture of enzymes (Novozyme-234) dissolved in a KCl buffer solution. After 24h treatment with the enzyme solution, no intact cells were found; only protoplasts remained. Healthy colonies with intact cell walls were recovered with an efficiency of 5.6% by incubating protoplasts in liquid complete medium amended with KCl prior to plating on nonamended complete medium. Efficiency of regeneration of cell walls was measured by comparing colony counts from KCl incubated protoplasts with counts from protoplasts incubated in water. Recovery of healthy colonies was fivefold greater for KCl incubated protoplasts versus the water treated control.

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OCCURRENCE OF DNA PLASMIDS IN ISOLATES FROM ANASTOMOSIS GROUPS (AG) 2,3,4, AND 5 OF RHIZOCTONIA SOLANI. T. Syminis and S.M. Tavantzis, Department of Botany and Plant Pathology, University of Maine, Orono, ME 04469.

Fourteen Rhizoctonia isolates members of AG-4 were examined for the presence of DNA plasmids. Five isolates were found to contain two plasmid bands which were associated with the mitochondrial fraction. The buoyant density of these molecules was lower than that of the nuclear DNA of R. solani. These plasmids were sensitive to pancreatic deoxyribonuclease I and resistant to pancreatic ribonuclease A, lambda exonuclease and exonuclease III. Southern blot hybridization analysis indicated the presence of homologous sequences between the two plasmids within an isolate and plasmids in the other AG-4 isolates. There was no homology between the plasmids and nuclear or mitochondrial DNA. The presence of the above plasmids in AG-4 isolates is not correlated with a certain phenotype. Furthermore, this study showed that a number of different DNA plasmids are present in R. solani isolates from AG-2,-3, and -5.

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OCCURRENCE OF MITOCHONDRIAL PLASMIDS IN ENDOPHYTIC FUNGI. K.L. Mogen, C.L. Schardl, and M.R. Siegel. Department of Plant Pathology, University of Kentucky, Lexington, Kentucky 40546-0091.

Acremonium spp., sect. *Albo-lanosa*, are fungal symbionts of various grasses. They are closely related to, and in some hosts the anamorph of, the choke pathogen *Epichloë typhina* (Clavicipitaceae). Grasses infected with the endophytes exhibit greater drought and insect resistance, as well as more vigorous growth, compared to non-infected plants. We are studying the genome structures and gene expression in the endophytes. Total and mitochondrial DNAs from several isolates were analyzed. The mitochondria of one *E. typhina* isolate (from *Lolium perenne*) contained at least three plasmids with sizes of 7.5 kb, 2.1 kb and 2.0 kb. These three plasmids are linear DNA species with no detectable homology to the mitochondrial chromosomes. Interestingly, all other isolates of *E. typhina* and the various *Acremonium* spp. contained one or both of the smaller plasmids. Exonuclease digests showed that they were double-stranded and had blocked 5'-termini. Plasmid sequence analysis is underway.

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INDUCTION OF MATING TYPE CHANGE IN PHYTOPHTHORA INFESTANS BY METALAXYL. T. T. Chang and W. H. Ko, Department of Plant Pathology. University of Hawaii, Hilo, Hawaii 96720

Isolates of P. infestans from various sources were grown on medium containing 0, 20, or 50 ug/ml metalaxyl to determine if this fungicide can cause mating type change. Among 70 A^1 and 53 A^2 tested, 1 A^1 and 1 A^2 formed oospores in sectors after growing on medium containing 20 or 50 ug/ml metalaxyl for 6 wk, indicating the appearance of the opposite mating type. The rest of the isolates tested did not form oospores after 3 months on the same medium. Single-zoospore cultures obtained from the oospore sector of isolate 902 (A^1) consisted of 108 A^1, 61 A^2 and 1 A^1 A^2, and those obtained from the oospore sector of isolate 920 (A^2) consisted of 47 A^1 and 113 A^2. Our results suggest that recent appearance of A^2 mating type of P. infestans in Europe may have resulted from the commercial application of metalaxyl for control of late blight of potato and tomato in that region.

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EVIDENCE FOR HORMONAL REGULATION OF SEXUAL REPRODUCTION IN PYTHIUM SPLENDENS. L. Y. Guo and W. H. Ko, Department of Plant Pathology, University of Hawaii, Hilo, Hawaii 96720

When + and - mating types of Pythium splendens were paired on opposite sides of a polycarbonate membrane (0.2 um, 90 mm diam.) for 6 days at 24 C in darkness, + but not - isolate produced oospores by selfing. When newly inoculated (0-hr) culture of - isolate was paired with 0-, 6-, 12- or 24-hr culture of + isolate, the 12-hr culture of + isolate produced the largest amount of oospores. The - isolate did not produce oospores in any of the experiments involving different age combinations. Our results show that sexual reproduction in P. splendens requires hormone produced by - isolate to initiate oospore formation by + isolate. This represents a new phenomenon of hormonal heterothallism in the biological world.

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MOLECULAR CHARACTERIZATION OF A ß-TUBULIN GENE FROM ERYSIPHE GRAMINIS. John E. Sherwood[1] and Shauna C. Sommerville[2]. [1]Dept. Plant Pathology, Montana State Univ., Bozeman 59717, and [2]DOE-Plant Research Lab, Michigan State Univ., E. Lansing 48824

A ß-tubulin gene from Erysiphe graminis f.sp. hordei, an obligate fungal pathogen of barley, was cloned from a genomic lambda library prepared from conidial DNA, using the Neurospora crassa tub-2 gene as a probe. The E. graminis structural gene, which encoded a polypeptide of 446 amino acids, had six introns with positional and splice sequence homologies to tub-2 from N. crassa. The predicted amino acid sequence also showed a high degree of homology with other fungal ß-tubulins. A major difference between the E. graminis gene and the majority of other fungal ß-tubulin genes was the lack of codon usage bias observed with the E. graminis gene, in which 60 of the 61 sense codons were used. While N. crassa tub-2 hybridized to only one sequence of the E. graminis genome, the E. graminis ß-tubulin clone hybridized to an additional genomic sequence. This result suggests that there is a second ß-tubulin gene whose sequence diverges considerably from that of the N. crassa gene.

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ISOZYME PHYLOGENETIC RELATIONSHIPS AMONG ANASTOMOSIS AND INTRA-SPECIFIC GROUPS OF RHIZOCTONIA SOLANI KUEHN. Z. Liu, D. L. Nickrent, and J. B. Sinclair, Departments of Plant Pathology and Plant Biology, University of Illinois, Urbana, IL 61801

Protein extracts of mycelium from 28 isolates of Rhizoctonia solani belonging to 12 anastomosis (AG) and intraspecific (ISG) groups (AG-1, AG-2-1, AG-2-2IIIB, AG-2-2IV, AG-3, AG-4, AG-5, AG-6, AG-7, AG-8, AG-9 and AG-BI) were analyzed electrophoretically for isozyme variation. Eleven enzyme systems were used: ACO, ACP, EST, GPI, GSR, HXK, IDH, LAP, MDH, PGM, and 6-PGD. For each enzyme, bands with the same relative mobility were treated as distinct characters, hence the matrix reflected the presence or absence of each enzyme variant. Relationships among all ISGs were inferred using PAUP. Each ISG occupied a separate position on the cladogram which provided an indication of their genetic distinctiveness. Within each ISG, representative isolates clustered together regardless of geographic origin. Isolates from AG-2-1 and AG-2-2 clustered separately. Within AG-2-2, AG-2-2IIIB and AG-2-2IV were distinguished by this isozyme analysis.

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^{125}I RADIOLABELING OF SPIROPLASMA CITRI SURFACE PROTEINS. J. Fletcher, Department of Plant Pathology, Oklahoma State University, Stillwater, OK 74078-9947.

Recently we used proteases and surface immunoprecipitation to identify five surface proteins of the wall-less mollicute S. citri (Phytopathology 77:1726). Only one of these, spiralin (29 KDa), was previously shown to have a surface location. In the present study, radiolabeling was used to enhance detection of surface proteins. Surface-exposed proteins of S. citri were iodinated using $Na^{125}I$ and Iodobeads. Autoradiograms of labeled proteins had fewer bands than did profiles of total cell protein. When surface-iodinated spiroplasmas were used

for surface immunoprecipitation, autoradiograms showed twelve bands, five of which correspond to those of our previous studies and seven additional polypeptides, which were interpreted as surface proteins.

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INFLUENCE OF PRUNUS ROOTSTOCKS ON FEEDING PREFERENCE OF PHONY PEACH DISEASE VECTOR (HOMALODISCA COAGULATA) AND AMINO ACID CONCENTRATIONS IN XYLEM FLUID. W. J. French, J. H. Aldrich, A. B. Gould, B. V. Brodbeck, R. F. Mizell, and P. C. Andersen. University of Florida, AREC, Rt. 4 Box 63, Monticello, FL 32344.

Phony peach disease (PPD) is caused by the xylem limited bacterium Xylella fastidiosa (Xf) and vectored by the leafhopper Homalodisca coagulata. Although prevalent in the southeastern U. S., PPD has not been observed in South America. Three rootstocks, domestic peach ('Nemaguard') and plum ('1-1'), and a peach rootstock from Brazil ('A-82'), were budded with 'Flordaking' peach. The effect of rootstock on vector feeding preference was assessed during 1985 to 1988. Xylem fluid amino acid levels were assessed in 1986 to 1988, and Xf concentrations were assessed in 1987 and 1988. Leafhopper counts and asparagine and arginine concentrations were rootstock dependent. Interactions among leafhoppers, amino acids and PPD are discussed.

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DETECTION AND DIFFERENTIATION OF MYCOPLASMA-LIKE ORGANISMS USING MLO-SPECIFIC RIBOSOMAL RNA OLIGONUCLEOTIDE SEQUENCES. B. C. Kirkpatrick and J. D. Fraser, Department of Plant Pathology, University of California, Davis, CA 95616.

Computer-assisted comparisons of 16S ribosomal RNA (rRNA) sequences of the X-disease MLO (X-MLO), plant chloroplasts, and culturable Mycoplasmas were used to identify several oligonucleotide sequences which could be used as specific or general probes to detect MLO but not plant nucleic acids. One oligonucleotide (WX1) hybridized with nucleic acids from plants infected with X-, aster yellows (AY), walnut/pecan bunch (W/PB) MLOs, and culturable Mollicutes. Another oligonucleotide (WX2) hybridized with nucleic acids from plants infected with the X- and WP/B-MLOs but not to culturable Mollicutes or AY-MLO-infected plants. ^{32}P-labelled WX1 and WX2 provided 500 to 800 times more sensitive detection of MLOs when used in rRNA:DNA as compared to DNA:DNA hybridizations. These results suggest that MLO-specific rRNA oligonucleotides can provide very sensitive and specific detection of plant pathogenic MLOs.

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IDENTIFICATION AND DIFFERENTIATION OF ASTER YELLOWS MLO STRAINS USING ASTER YELLOWS MLO-SPECIFIC DNA PROBES AND RFLP ANALYSIS. C. R. Kuske, B. C. Kirkpatrick, Department of Plant Pathology, University of California, Davis, CA 95616

DNA extracted from celery infected with the severe strain of aster yellows MLO (AY-MLO) was digested with EcoRI and BamHI, ligated into pUC18, and cloned in E. coli JM109. Two transformants, containing inserts of 2.0 kb (pAYC3) and 4.1 kb (pAYC4), were identified by DNA hybridization analyses as fragments of the MLO chromosome. In Southern blot analyses of EcoRI-digested DNA from plants infected with a number of Mollicutes, pAYC3 and pAYC4 did not hybridize with DNA from healthy plants; plants infected with elm yellows, western-X, or BLTVA MLOs; nor with Spiroplasma citri or S. kunkelli. The two probes hybridized with three type strains of Western AY-MLO, two AY-MLO field isolates from northern California, and AY-MLO strains from other geographic regions in the U.S. and Canada. Hybridization patterns of EcoRI or HindIII-digested DNA from many of the AY-MLO strains differed. Such RFLPs may be useful in characterization of geographical strains of AY-MLO.

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16S rRNA SEQUENCE SHOWS THAT PLANT PATHOGENIC "MYCOPLASMA-LIKE ORGANISMS" ARE EVOLUTIONARILY DISTINCT FROM ANIMAL MYCOPLASMAS. P.O. Lim[1] and B.B. Sears[1,2]. [1]Genetics Program and [2]Department of Botany and Plant Pathology, Michigan State University, East Lansing, MI 48824.

The plant pathogenic "mycoplasma-like organisms" (MLOs) are so-named because they lack cell walls. In order to establish a definitive phylogeny, the 16S rRNA gene from a representative of this group has been cloned and sequenced. Sequence comparisons indicate that this MLO is related to Mycoplasma capricolum and that these two bacteria share their phylogenetic origin with Bacillus subtilis. Furthermore, a low G+C content of this gene and its presumed secondary structure indicate that MLOs belong in the same class as the animal mycoplasmas, the Mollicutes. However, the presence of certain characteristic oligonucleotides indicates a closer relationship to another family of this order, the Acholeplasmataceae.

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EFFICACY OF TRIAZOLE FUNGICIDES ON FOLIAR DISEASES COMMON TO WHEAT IN OKLAHOMA. E. Williams Jr., K. E. Jackson, and P. W. Pratt. Plant Pathology Department, Oklahoma State University, Stillwater, OK 74078-9947.

Winter wheat plots were established in north central and east central OK from 1984-1987 to determine efficacy of propiconazole and tebuconazole to foliar fungal infections. Plots were 2.4 X 9.1 m, replicated 4 times in randomized complete block. Fungicides applied in water by ground sprayer (187 l/ha) at growth stage 9 (Feekes scale). Cited significance represents LSD tests ($P \leq 0.05$). In 1987-88, significant reductions in Puccinia recondita f. sp. tritici severity resulted from both fungicides; however, tebuconazole provided significantly lower severities than propiconazole. Both fungicides significantly reduced Erysiphe graminis f. sp. tritici, Septoria tritici, Septoria nordorum, and Pyrenophora tritici-repentis. Significant grain yield increases were obtained in 37.5% of tests for tebuconazole and 25% for propiconazole.

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ERADICANT AND PROTECTANT ACTIVITY OF FUNGICIDES AGAINST BOTRYOSPHAERIA OBTUSA ON APPLE FOLIAGE. L. F. Arauz and T. B. Sutton. Dept of Plant Pathology, North Carolina State University, Raleigh, 27695-7616.

Reduction of radial growth of Botryosphaeria obtusa was determined on fungicide-amended PDA. EC 50 values (ug/ml) were: benomyl (be), 0.032; bitertanol (bi), 0.043; flusilazole (fl), 0.045; mancozeb (ma), 10.26; myclobutanil (my), 0.426; penconazole (pe), 0.132; pyrifenox (py), 1.77; terbutrazole (te), 0.036. Six selected fungicides were tested on apple seedlings for protectant and eradicant activity. When plants were inoculated 7 days after fungicide application, mean disease severities (lesions/cm^2) were: be, 0.046; bi, 0.046; fl, 0.207; ma, 0.004; pe, 0.116; te, 0.009; control, 1.02. Percent leaf area diseased for fungicides applied 48 hr after inoculation was: be, 5.6; bi, 4.2; fl, 3.2; ma, 3.9; pe, 2.7; te, 2.9. The length of the period of eradicant activity to achieve a 2/3 reduction in disease severity as compared to the control was 30, 45 and 48 hr for fl, te and pe, respectively. This reduction was not obtained with the other fungicides.

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PERSISTENCE OF FUNGICIDES FOR CONTROL OF ALTERNARIA LEAF BLIGHT OF MUSKMELON. H. Suheri and R.X. Latin, Department of Botany and Plant Pathology, Purdue University, West Lafayette, IN 47907.

The objective of this research was to determine the relative persistence of two fungicides, chlorothalonil and mancozeb that are used against Alternaria cucumerina, the causal agent of Alternaria leaf blight of muskmelon. A cellophane bioassay with A. cucumerina as the test organism and inhibition of spore germination as the response variable was used to determine persistence. Subsequent to treatment, plants were exposed to both wet (12 h) and dry

regimes and were sampled at 1, 3, 5, 7, and 10 days after fungicide application. A linear relationship between fungicide loss and time resulted for all treatments. Under the wet regime, initial loss and the rate of fungicide loss were significantly greater for mancozeb than chlorothalonil. No significant differences occurred under the dry regime where the bioassay showed a relatively slow decrease in effectiveness of both fungicides for 9 days.

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EFFECTS OF FUNGICIDE SEEDPIECE TREATMENTS ON EMERGENCE AND YIELD IN POTATO. D. J. Gallenberg, SDSU, Plant Science Department, Box 2109, Brookings, SD 57007.

Several fungicide seedpiece treatments were used in field trials during 1986 - 1988 to determine the effects of these materials on plant emergence and tuber yield. Trials were conducted at Watertown, SD (early planting) and Brookings, SD (late planting), and utilized two cultivars each season. Stand counts were made approximately four weeks and six weeks after planting, and total tuber yield was determined at the end of the season. Yield differences between cultivars were evident at both locations in all three seasons. Within cultivars, there were no differences among treatments in stand count or yield in any season at Watertown. At Brookings, all fungicide treatments increased stand count and yield over the check for one variety only in 1987 and 1988.

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FUNGICIDE RESISTANCE IN BOTRYTIS CINEREA ISOLATES FROM PENNSYLVANIA GREENHOUSES. G. W. Moorman and R. J. Lease, Department of Plant Pathology, The Pennsylvania State University, University Park, PA 16802

Twenty Botrytis cinerea isolates from infected greenhouse floricultural crops in Pennsylvania were grown on a range of concentrations of benomyl, chlorothalonil, cupric hydroxide, mancozeb, thiophanate methyl + mancozeb, vinclozolin, and zineb in vitro. Five isolates were resistant to only benomyl and 14 were resistant to both benomyl and vinclozolin. Isolates with fungicide resistance infected and sporulated on excised geranium (Pelargonium) leaf disks that had been treated with the label rate of the fungicide to which they were resistant. Linear growth rates and sclerotium formation in vitro and sporulation in vivo, used as saprophytic and parasitic fitness parameters, were compared among isolates.

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Somoclonal variations in root development of Maricongo susceptible to "plantain decline." L. J. Liu, E. Rosa-Márquez and E. Lizardi, University of Puerto Rico, Río Piedras, PR 00927.

Drastic reduction in ratoon productivity on Maricongo, a major plantain cultivar, is of great concern to growers in Puerto Rico. Since such decline in ratoon crops has not occurred on Grand Naine (a banana cultivar) nor on Harton (a plantain cultivar) which seem to have a large root system, efforts were made to examine root development of somaclonal selections of Maricongo with the objective of screening for resistance to the "plantain decline." Results obtained indicate that some of the somaclonal selections (S_{23}) of Maricongo showed greater root development than others (S_2, S_{14}, S_{15}, and S_{24}) when cultured in a modified Murashige and Skoog's basal medium supplemented with 0.5 mg/l kinetin and 0.5 mg/l 6 benzyl amino purine and supported with 3 layers of filter paper. Such a phenomenon was also observed in greenhouse tests when plantlets derived from calli were planted in glass window boxes containing promix.

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TRANSFORMATION OF TOBACCO AND POTATO WITH PEA DISEASE RESISTANCE RESPONSE GENE (DRRG) PROMOTERS. C. C. Chiang, M. M. Chang and L. A. Hadwiger. Department of Plant Pathology, Washington State University, Pullman, WA 99164.

Pea tissue is able to inhibit many of the organisms that are pathogens of tobacco and potatoes. This resistance is associated with the enhanced activity of some specific pea genes. Transfer of the pea genes active in the pea's disease resistance response to tobacco or potatoes is potentially a new source of disease resistance. The promoter (5') region of the pea gene, DRRG-49, constructed in line with a CAT (chloramphenicol acetyl transferase) marker gene has been shown to be expressed in tobacco and potato. In the transformed tobacco tissue this promoter directed function is expressed following the challenge of tobacco leaves with Fusarium solani f. sp. pisi, a pathogen of peas.

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EXPRESSION OF STRIPE RUST RESISTANCE GENES IN DIFFERENTIAL WHEAT CULTIVARS. R.F. Line, and X.M. Chen, U.S. Dept. of Agriculture and Washington State Univ., Pullman, WA 99164-6430

Seedlings of parents and F_1, F_2, and BC_1 progeny from crosses among differential cultivars were inoculated with specific races of Puccinia striiformis. Chinese 166, Heines VII, Yamhill, T. spelta alba, Fielder, Heines Kolben, Heines Peko, Lee, Compair, Riebesel 47/51, Clement, and Moro have genes Yr_1, Yr_2, Yr_3, Yr_5, Yr_6, Yr_6, Yr_2 and Yr_6, Yr_7, Yr_8, Yr_9, Yr_9, and Yr_{10}, respectively. Druchamp, Stephens, and Yamhill have genes at the Yr_3 or Yr_4 locus. Lemhi, Tyee, Spalding Prolific, Moro, Druchamp, Lee, Fielder, and Compair each have a newly identified gene. Paha has three genes. Yr_1, Yr_5, Yr_7, Yr_8, Yr_9, and Yr_{10}, the Lemhi gene, and one Paha gene were dominant. Depending upon race that was used and/or parent in the cross, one Compair gene was either dominant or partially dominant, and Yr_2, Yr_3, Yr_4, Yr_6, the Tyee gene, one Lee gene, and two Paha genes were either recessive or dominant. Epistatic patterns also varied depending upon race and parent.

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INHERITANCE OF LATENT PERIOD OF PUCCINIA RECONDITA IN WHEAT. Greg Shaner and George Buechley, Purdue University, West Lafayette, IN.

Long latent period, a major component of slow leaf-rusting resistance in wheat, was studied in F_7 families derived from CI 13227 x Suwon 92 There were highly significant differences in latent period among families. The distribution of F_7 family means was skewed toward long latent period, and the population mean was significantly below the mid-parental value. These data, along with data from analyses of earlier generations from CI 13227 x Suwon 92, suggest that three loci with epistatic effects control latent period. Because approximately three-fourths of the F_7 families had values below the parental midpoint, we reasoned that a gene at one of the three loci exerted a major effect on latent period. If the three loci are symbolized as A, B, and C, with the capital letter indicating the allele for short latent period, then A can be considered the major gene. B is considered to have greater effect than C in the absence of A. C in the absence of A and B has only a small effect. Genotype aabbcc conditions the long latent period of CI 13227.

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MAPPING LEAF RUST RESISTANT GENES, Rph3 AND Rph7, ON BARLEY CHROMOSOME 3. Y. Jin, G. D. Statler, Department of Plant Pathology, J. D. Franckowiak, Department of Crop & Weed Science, North Dakota State University, Fargo, ND 58105.

Barley lines having the leaf rust resistant genes Rph3 (Pa3) and Rph7 (Pa7) were crossed with the lines having 1, 2 or 3 of the following recessive marker genes located on chromosome 3: al (Albino lemma), gs2 (glossy sheath 2), lnt (low number of tillers), msg5 (male sterile 5), sld (slender dwarf) and uz (semi-brachytic). Four to six hundred F_2 plants from each cross were tested for reaction to Puccinia hordei Otth. at the seedling stage and scored for genetic markers. Results indicated that Rph3 and Rph7 are not linked with any of these genetic markers (X^2 = 1.0 to 7.0). Also, no linkage was found between Rph3 and Rph7 (X^2 = .01). These two major resistant genes probably are not located on chromosome 3, as suggested by previous workers.

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PATHOGENICITY GROUPING OF LEPTOSPHAERIA MACULANS ISOLATES BASED ON THREE CULTIVARS OF BRASSICA NAPUS. A. Mengistu, S.R. Rimmer, E. Koch and P.H. Williams, 1630 Linden Dr., University of Wisconsin, Madison, WI 53706.

The pathogenicity of 39 isolates of Leptosphaeria maculans from N. America, Europe and Australia against a range of Brassica napus var oleifera (oilseed rape) cultivars was studied. Isolates were categorized into four groups based on differential pathogenicity on cotyledons of Westar, Quinta and Glacier. PG1 isolates can be distinguished by lack of virulence to Westar. PG2 isolates are virulent only on Westar but give slightly more susceptible interaction phenotypes on Quinta than on Glacier. PG3 isolates are virulent on Westar and Glacier and intermediate on Quinta. PG4 isolates are virulent on all three cultivars. Using these cultivars as differentials we have examined about 70 single ascospore isolates from rape seed debris from Saskatchewan and Manitoba, Canada and from Western Australia and New South Wales, Australia. All Canadian isolates tested were PG2 whereas isolates from Australia were characteristic of PG2, PG3 and PG4. Only Western Australian isolates produced pseudothecia.

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CHARACTERIZATION OF ADHESIONLESS MACROCONIDIAL MUTANTS OF NECTRIA HAEMATOCOCCA. M.J. Hickman, M. B. Buurlage, L. Epstein. Department of Plant Pathology, University of California, Berkeley, Berkeley, CA 94720

The macroconidia of N. haematococca attach to host and to nonhost tissues, and to inert hydrophilic and hydrophobic surfaces. To determine the role of adhesion in pathogenicity, we isolated adhesionless mutants. Uninucleate microconidia were mutagenized in 2.7 mM N-methyl-N'-nitro-N-nitrosoguanidine for 50 min (90-95% kill). Using an enrichment procedure, populations of macroconidia were repeatedly selected for the adhesionless phenotype. Six mutant strains were obtained that produced macroconidia with approximately a 50% reduction in wild-type adhesion. Mutant macroconidia germinated at a similar rate and obtained a maximum percentage of germination (approx. 80% in 4 h) as wild-type macroconidia. The mutants will be used in disease assays to assess the role of adhesion in pathogenesis.

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PATTERNS OF INTRAPOPULATION ISOZYME VARIATION IN THE BEAN RUST FUNGUS. J. W. McCain and J. V. Groth, Dept. of Plant Path., Univ. of Minnesota, St. Paul, MN 55108.

Mass field collections of Uromyces appendiculatus from Phaseolus vulgaris often are mixtures of races. We surveyed several single-uredinium isolates from each of 12 field collections, to seek similar intrapopulation variability of isozyme bands. Proteins extracted from urediniospores were assayed by polyacrylamide gel electrophoresis. Of 10 putative loci in five enzyme systems, seven loci varied in most collections. Among the 66 isolates, 25 isozyme phenotypes (races) were identified; 32% of the isolates belonged to the most common race, but no other race accounted for more than 9%. Two collections made 4 years apart from one location differed significantly in Shannon diversity index. The earlier collection was among the most variable, but the latter was the least, with mostly homozygous-like band patterns. This study confirms that multiple samples are necessary to determine the genetic heterogeneity of rust spore collections.

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HETEROKARYOSIS AMONG SUBSPECIES OF COLLETOTRICHUM GLOEOSPORIOIDES. R.J. Chacko, G.J. Weidemann and D.O. TeBeest. Department of Plant Pathology, University of Arkansas, Fayetteville, AR 72701.

Several subspecies of Colletotrichum gloeosporioides have potential as bioherbicides including C. gloeosporioides f. sp. aeschynomene (CGA) and C. gloeosporioides f. sp. jussiaeae (CGJ). Both subspecies lack a known teleomorph, therefore, genetic variability may arise through heterokaryosis and mitotic recombination. In vitro studies with nutritionally deficient mutants of GCA yielded heterokaryotic colonies at a rate of $1x10^{-5}$. Conidial isolations from several heterokaryotic colonies yielded both parental isolates but no putative diploids or recombinant genotypes. Crosses between compatible strains of CGA and CGJ mutants failed to produce heterokaryons. Studies of heterokaryosis may indicate the potential for genetic exchange between related subspecies of C. gloeosporioides following field release of bioherbicides.

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EARLY CELLULAR RESPONSES DURING BACTERIA-INDUCED HYPERSENSITIVITY IN ALFALFA AND TOBACCO. N.R. O'Neill, C.J. Baker, and L.D. Keppler. USDA, Agricultural Research Service, Beltsville, MD 20705.

The bacteria-induced hypersensitive response (HR) in alfalfa and tobacco is characterized as a rapid, localized necrotic response to pathogenic bacteria. We are investigating the nature of early interactions with compatible and incompatible bacteria and their association with defense gene activation in suspension cells. The addition of bacteria which induce the HR initiated a net uptake of extracellular H+ and net increase in extracellular K+. A transient and prolonged pH change was observed, and this response correlated with the evolution of active oxygen, determined by luminol-mediated chemiluminescense. Conductivity and bacterial cell death were also monitored. The physiological responses of various plant-bacteria combinations correlated with host-pathogen compatibility.

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HISTOCHEMISTRY AND ULTRASTRUCTURE OF INDUCED AND NONHOST RESISTANCE IN CUCURBITS TO COLLETOTRICHUM LAGENARIUM. B. D. Stein, K. L. Klomparens, and R. Hammerschmidt. Michigan State University, E. Lansing, MI 48824.

Cucumber plants (cv. SMR 58) were injected with Pseudomonas syringae pv syringae to induce systemic resistance against Colletotrichum lagenarium race 1. The third leaves of induced plants and controls were detached, inoculated with C. lagenarium, incubated in a moist chamber for 24-72 hours, and prepared for transmission and scanning electron microscopy. Seedlings of pumpkin (cv. Spookie), resistant to C. lagenarium, were inoculated with C. lagenarium and incubated for 48 hr. prior to sampling. Ultrastructural localization of lignin with $KMnO_4$ and Br_2 was carried out via TEM and X-ray analysis, respectively. Lignin was found to accumulate in the outer epidermal wall of resistant pumpkin and induced resistant cucumber. Peroxidase activity, as localized with diaminobenzidine, was also correlated with resistance. Appressoria on cucumbers with induced resistance were frequently found to be necrotic.

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GENOTYPE DEPENDENT RESISTANCE OR SUSCEPTABILITY TO ROOT-KNOT NEMATODE IN ROOTS GROWING FROM THIN CELL LAYER CULTURES OF TOMATO. D. N. Radin* and J. D. Eisenback**, Depts of Agronomy* and of Plant Pathology**, VPI & SU, Blacksburg, VA 24061.

A new aseptic root-culture system has been developed for the parasitic nematode, Meloidegyne incognita and tomato, Lycopersicon esculentum. Epidermal thin cell layer explants from floral stems of tomato produced up to 20 adventitious roots per culture in 7 to 14 days on MS medium plus 3% sucrose and the hormones kinetin and IAA. Rooted cultures were transferred to Gamborg's B-5 medium with 1% sucrose and inoculated with infective second-stage juvenile nematodes surface sterilized with hibitane. Gall initiation was apparent five days after inoculation in the susceptable (mi-/mi-) tomato cultivars, Rutgers and Red Alert. Resistant (Mi+/Mi+) genotypes, LA655, LA656, LA1022, exhibited a characteristic hypersensitive resistance response. This culture system has potential for use in nematode propagation or for experimental studies on the molecular basis of the plant-nematode relationship.

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REDUCED PECTINASE ACTIVITY OF ASPERGILLUS FLAVUS IS ASSOCIATED WITH REDUCED VIRULENCE ON COTTON. T. E. Cleveland and P. J. Cotty, USDA, ARS, Southern Regional Research Center, P.O. Box 19687, New Orleans, LA 70179.

An infective strain of Aspergillus flavus was shown to produce several pectolytic enzymes during invasion of cotton bolls (Cleveland and McCormick. 1987. Phytopathol. 77:1498-1503). Sixteen strains of A. flavus, varying in their ability to invade boll tissues (Cotty. 1989. Phytopathol. In press), were examined for their ability to produce pectinases. Aggressiveness of strains during the infection process was correlated with their ability to secrete pectinase(s) on sterilized cottonseed and in pectin-containing liquid media. When pectinases of the various strains were analyzed by isoelectric focusing, it was demonstrated that four fungal strains with the lowest ability to infect boll tissues lacked a major pectolytic activity which was always present in highly aggressive isolates. These results suggest that certain pectolytic enzymes produced by A. flavus during host infection are required for fungal virulence.

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IN PLANTA GROWTH OF PSEUDOMONAS SYRINGAE PV SYRINGAE HRP MUTANTS. I. Yucel and S. W. Hutcheson. Dept. of Botany, University of Maryland, College Park, MD 20742.

In an effort to study the role of nutrition in the growth behavior of hrp mutants, Pseudomonas syringae pv. syringae strains 61 (WT), B7 (hrp::Tn5) and 61-145 (a deletion mutant in a hrp regulatory determinant) were suspended in 10 mM NaPi buffer containing 10 mg/ml proteose peptone (PrP) or buffer alone and infiltrated into surface-sterilized, detached Nicotiana tobacum var. Samsun leaves. Leaves were incubated under high humidity and were reinfiltrated with fresh buffer three times during the initial 6 h of the interaction. Bacterial populations were monitored during the initial 48 h. Added PrP had little effect on WT growth but delayed onset of the hypersensitive response 6-12 h. Growth of B7 was only observed when PrP was present, but was <10% the WT level. No growth of 61-145 was observed under either condition in planta. P. fluorescens 55 grew to WT levels in the presence of PrP. These results suggest that the limited growth of hrp mutants in planta may not be due to nutritional limitations.

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MEASURING VIRULENCE ASSCIATIONS IN MASS UREDINIAL COLLECTIONS OF THE BEAN RUST FUNGUS. J. V. Groth, E. V. Ozmon, and D. C. Linde, Department of Plant Pathology, University of Minnesota. St. Paul, MN 55108.

A method was devised to directly measure departures from randomness of specific virulence traits in mass samples of urediniospores of Uromyces appendiculatus. Differential bean lines are used on which avirulent reactions are countable and virulence is polymorphic. Reciprocal serial inoculations are made on paired lines and on a universally susceptible host line. The random expectation is that the frequency of virulence on the differential is the same whether the inoculum has been screened through the other differential or taken from the susceptible line. Deviations from identity of frequency provide a measure of the degree of virulence coupling or repulsion association. On two lines tested with a single field rust collection, the two virulences were not random, with about half of the expected coupling associations being absent. This method should be useful for rapidly surveying or comparing multiple virulence associations in many collections.

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AN EFFICIENT AND RAPID IMMUNIZATION FOR GENERATION OF HYBRIDOMA ANTIBODIES SPECIFICALLY REACTING WITH PLANTS INFECTED BY AN UNCULTURED MYCOPLASMA-LIKE ORGANISM. H. T. Hsu, I. M. Lee*, and R. E. Davis*, USDA-ARS, Florist & Nursery Crops and *Microbiology & Plant Pathology Laboratories, Beltsville, Md.

A method to produce target-specific hybridomas for antigens which are difficult to purify is described. Mouse hybridomas secreting antibodies to a plant pathogenic mycoplasma-like organism (MLO) associated with tomato plants with symptoms of big bud disease were successfully produced. Neonatal mice were injected with extracts from normal host plants twice, once each on days 1 and 7. When they were 8 wk old, mice were immunized with an enriched MLO preparation in which about 10% were target antigen determined by dot-blot immunoassays. There were 589 hybrids produced from 20 96-well tissue culture plates seeded in 2 fusions. Tests by indirect ELISA selected 20 hybridomas that secreted antibodies specific to the plant pathogenic MLO. The dot-blot immunoassay was a sensitive method for detection of MLO infection.

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LONG-TERM LIQUID NITROGEN STORAGE OF CRONARTIUM QUERCUUM F. SP. FUSIFORME BASIDIOSPORES AND MYCELIUM. Pauline C. Spaine, USDA, Forest Service, SEFES, Carlton St., Athens, GA 30602.

Basidiospores and mycelium underwent slow chilling (1°C/min) in a Cryo-Med programmable freezer to -50°C, and were then plunged into liquid nitrogen. Several cyro-protectants were tried in initial studies including DMSO, glycerol, sucrose and milk. Germination of basidiospores from mass gall isolates frozen for seven months ranged from zero to 63%. A glycerol-milk combination favored spore viability, while 6% sucrose provided equally effective mycelial regeneration. Basidiospores derived from single aecial isolates frozen for 3 months had a mean germination of 83 and 90% for two isolates and 0 and 2.0% for two other isolates receiving the same treatment. Pine seedlings inoculated with spores derived from a single gall isolate in long-term storage showed 10% infection. Regeneration occurred in 96% of frozen mycelial plugs from single and mixed gall isolates.

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USE OF RESTRICTION FRAGMENT LENGTH POLYMORPHISMS TO DETECT PHENOTYPIC DIVERSITY IN THE BEAN RUST FUNGUS. D. C. Linde, L. J. Szabo, and J. V. Groth, Dept. of Plant Pathology, Univ. of Minn., and USDA/ARS Cereal Rust Laboratory, St. Paul, MN 55108.

Virulence and isozyme markers have been used previously to detect phenotypic diversity in the bean rust fungus, Uromyces appendiculatus. In a sample of 27 geographically diverse isolates, three fairly distinct clusters of isolates were identified with the virulence and isozyme markers. Restriction fragment length polymorphisms (RFLPs) are being developed as a new class of marker to 1) compare the overall phenotypic diversity detected by RFLPs with that detected by virulences and isozymes, and 2) confirm the presence of the three clusters in the same sample of 27 isolates. Random genomic clones derived from bean rust as well as clones of several known genes from other organisms will be used as probes in the RFLP analysis. If the presence of three clusters is confirmed with RFLP analysis, these clusters probably represent gene pools between which there is limited gene flow.

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SELECTIVE ISOLATION OF *GLIOCLADIUM VIRENS* AND *G. ROSEUM* FROM SOIL. Y-. H. Park and J. P. Stack. Department of Plant Pathology and Microbiology, Texas A&M University, College Station 77843.

Semi-selective media were developed for the isolation and enumeration of *Gliocladium virens* and *G. roseum* from soil. The basal medium consisted of 3.0 g glucose, 0.2 g $MgSO_4 \cdot 7H_2O$, 1.0 g K_2HPO_4, 0.5 g KCl, 1.0 g $NaNO_3$, 0.01 g $FeSO_4 \cdot 7H_2O$, 50 mg chloramphenicol, 50 mg rose bengal, 50 mg streptomycin sulfate, 500 µg benomyl, 500 mg sodium propionate, and 20 g Bacto agar in 1.0 L distilled water. The pH of the medium was adjusted to 6.0 with 25% phosphoric acid before autoclaving. By the dilution plate method, selective isolation (ca. 100% recovery) of *G. virens* from natural soil infested with *Rhizoctonia solani*, *Fusarium oxysporum* f. sp. *vasinfectum*, and *Pythium ultimum* was achieved by adding 20 mg gliotoxin to 1.0 L basal medium. At 3 days, no other fungi grew on this medium, and *G. virens* colony size was optimal for quantification. This medium is being used for population studies of *G. virens*. Selective isolation (ca. 35% recovery) of *G. roseum* from the same soil was achieved by adding 10 mg gliotoxin, 20 mg pentachloronitrobenzene, and 30 mg acriflavin to 1.0 L basal medium. The *G. roseum* selective medium is useful for isolation of the fungus but not for quantification, while that for *G. virens* is adequate for both.

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EFFECT OF AGAR BRAND ON THE EFFICACY OF A SELECTIVE MEDIUM. M.L. Courtney and J.C. Rupe, Department of Plant Pathology, University of Arkansas, Fayetteville, AR 72701.

The results of certain microbiological assays may depend upon the brand of agar used in the preparation of the medium. Five brands of agar were used in the preparation of modified Nash and Snyder's medium, selective for Fusarium solani. Samples of either F. solani conidia or soil infested with F. solani were diluted in 0.1% water agar and spread on plates of the selective medium made with each of the agars. Colony counts from media containing Sigma and Difco Bitek agars were significantly lower than from media containing Moorhead, Difco Bacto, and Northeast agars. Colony counts on Moorhead, Difco Bacto and Northeast agars were not significantly different.

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DETECTION OF OPHIOBOLIN IN CULTURE FILTRATES OF Bipolaris oryzae AND USE IN A WILD RICE ROOT ELONGATION ASSAY. D. R. Johnson and J. A. Percich, Department of Plant Pathology, University of Minnesota, St. Paul, MN 55108.

Ophiobolin was detected in cultures of Bipolaris oryzae, the causal organism of fungal brown spot of wild rice (Zizania palustris L.), and tested in a wild rice root elongation assay. Aqueous culture filtrates were extracted with chloroform 1:1 v/v for 24 h, the organic fraction was reduced to near dryness in a rotary evaporator and crystallized at 24 C. Ophiobolin was confirmed by 2-dimensional thin layer chromatography (2-D TLC) with an authentic ophiobolin standard. A rapid 1-D TLC detection method was devised for purified ophiobolin using plastic silica gel plates developed in toluene-ethyl acetate-formic acid (6:3:1). Developed chromatograms were sprayed with p-anisaldehyde or phosphomolybdic acid, and the limit of detection was 50 ng. Wild rice primary root elongation was significantly inhibited by ophiobolin concentrations of 10^{-4}, 10^{-5} and 10^{-6} M, but a 10^{-7} M solution was not different from H_2O controls.

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CLONING AND CHARACTERIZATION OF PATHOGENICITY GENES FROM XANTHOMONAS CAMPESTRIS PV. GLYCINES 8ra. I. Hwang, S. M. Lim, and P. D. Shaw. University of Illinois at Urbana-Champaign, Urbana, IL 61801.

N-methyl-N-nitro-N'-nitrosoguanidine was used to generate nonpathogenic mutants, and fifteen nonpathogenic and five reduced pathogenic mutants were isolated from two thousand colonies examined. A cosmid clone that complemented four nonpathogenic mutants was isolated from a genomic library, and it contained about 30 kb insert DNA. This suggests that the pathogenicity genes consisted of at least two complementation groups. A restriction map of the fragment was constructed, and a 10 kb HindIII fragment was subcloned into pLAFR3. That fragment, which also complemented the mutants, was mutagenized with lac fusion transposon, Tn3-HoHo1. Complementation tests indicated a minimum of three pathogenicity genes in the 10 kb fragment. The lac fusions also indicated that these genes may not be expressed in vitro.

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PLASMID-ASSOCIATED STREPTOMYCIN RESISTANCE OF *XANTHOMONAS CAMPESTRIS* PV. *VESICATORIA*. G.V. Minsavage, B.I. Canteros, and R.E. Stall. Plant Pathology Dept., Univ. of Florida, Gainesville, FL 32611.

Streptomycin-resistant strains of *Xanthomonas campestris* pv. *vesicatoria* (Xcv) from pepper and tomato grew variably on media containing streptomycin sulfate at concentrations of 50 to 1000 μg/ml. A DNA library of resistant strain BV 5-4a was constructed in the cosmid vector pLAFR-3. A clone of 17 kbp of Xcv-DNA conferred resistance to streptomycin after complementation in a sensitive strain of Xcv. A 5 kbp subclone encoding wild-type resistance to streptomycin was used as a probe in Southern hybridization analyses. The locus for the streptomycin-resistance gene(s) in strain BV 5-4a occurs on a plasmid of 45 MDa. The subclone hybridized with EcoR1-digested genomic DNA of three of twelve other resistant strains of Xcv as well as the plasmid DNA from streptomycin-resistant strains Psp 34 and Psp 36 of *Pseudomonas syringae* pv. *papulans*. No hybridization occurred with DNA from *Erwinia amylovora* strain UCBPP 829 or *P. cichorii* strain Pc 83-1, which are also resistant to streptomycin.

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Genetic analysis of a cloned DNA sequence required for coronatine production by Pseudomonas syringae pv. tomato. S-W. Ma[1], V.L. Morris[2], and D.A. Cuppels[1]. [1]Agriculture Canada Res. Centre and [2]Dept. of Microbiol. & Immunol., Univ. of Western Ontario, London, ON, N6G 2V4, CANADA.

A 30-kb EcoR1 fragment of P. syringae pv. tomato DC3000 DNA contains sequences that restore coronatine production to coronatine-negative Tn5-induced mutants of this pathovar. For further analysis, this cloned region was mutagenized with the transposon Tn3-Spice. Tn3-Spice carries the ice nucleation gene inaZ which acts as a "reporter" of the transcriptional activity of the genes into which it is inserted. Based on the map location of the Tn3-Spice mutations, the ice nucleation activity of these mutants, and the results of functional complementation tests with the Tn5 mutants and subclones of wild type DNA, the coronatine genes contained within this 30-kb region are organized into two large transcriptional units (operons) which are transcribed in opposite directions.

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Identification of a genetic locus required for the induction of leaf and fruit necrosis by Pseudomonas syringae pv. tomato. R. L. Schwartz, V. L. Morris, and D. A. Cuppels, Dept. of Microbiol. & Immunol., University of Western Ontario and Agriculture Canada, London, Ontario, N6A 5C1, CANADA.

Five P. syringae pv. tomato mutants able to induce leaf chlorosis but not necrosis (Nec$^-$) were generated using Tn5 mutagenesis. All grew poorly on leaves and did not infect fruit; four did not induce a hypersensitive response (HR) on tobacco. Southern blot analysis showed that Tn5 had inserted into a single unique site for each mutant. A pLAFR1 library of P. syringae pv. tomato wild type DNA was mated en masse into Nec$^-$ mutant DC3162 and the resulting transconjugants were screened for leaf necrosis. One Nec$^+$ clone, p007, which contained three EcoRI fragments (2.7, 6.2, and 12.5 kb) of wild type DNA, restored necrosis not only to 3162 but also to three of the other Nec$^-$ mutants. It also restored the HR, fruit necrosis, and a better growth rate on leaves.

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EVALUATION OF TN*4431*-INDUCED PROTEASE MUTANTS OF *XANTHOMONAS CAMPESTRIS* PV. *ORYZAE* FOR GROWTH *IN PLANTA* AND PATHOGENICITY. G. -W. Xu and C. F. Gonzalez. Department of Plant Pathology and Microbiology, Texas A&M University, College Station, TX 77843.

Studies to determine the role of extracellular enzymes in pathogenesis of *Xanthomonas campestris* pv. *oryzae* (*Xco*), the causal organism of bacterial leaf blight of rice were conducted. Transposon Tn*4431*, containing the promotorless luciferase (*lux*) operon and the tetracycline resistance gene (Tc^r) was introduced into *Xco* strain X1-5-R using the suicide vector pUCD623. Tc^r exconjugants were screened *in vitro* for production of extracellular enzymes. Southern blot analysis showed protease deficient mutants that were Tc^r had Tn*4431* inserted into their genome. The bioluminescence of the mutants was confirmed photographically. The protease mutants were reduced in pathogenicity and populations *in planta* were 10 to 100 fold lower than those observed for the parental strain. The results indicate that protease may play an active role in disease caused by *Xco*.

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CHARACTERIZATION OF A PATHOGENIC DETERMINANT FROM PSEUDOMONAS SYRINGAE PV. TOMATO. D.P. Jackson[1], D.A. Cuppels[2], and V.L. Morris[1]. [1]Univ. of Western Ontario, Dept. Microbiol. and Immunol., London, ON, N6A 5C1, Canada and [2] Agriculture Canada Res. Centre, 1400 Western Rd., London, ON, N6G 2V4, Canada.

The prototrophic Tn5-induced mutant P. syringae pv. tomato DC3481 is nonpathogenic on tomato plants yet is still able to incite a hypersensitive response on tobacco leaves. Although it is the result of a single Tn5 insertion, it also cannot use tartrate as a carbon source. Clone pDJ208, containing 30 kb of wild type DNA, restored the Path$^+$Tar$^+$ phenotype to 3481. Complete EcoR1 digestion of the insert DNA produced seven EcoR1 fragments: 13, 6.2, 4.2, 3.7, 2.5, 0.94, and 0.8 kb. A subclone containing the 4.2-kb EcoR1 fragment restored pathogenicity but at a reduced level; it did not restore the Tar$^+$ phenotype. Subsequent restriction enzyme mapping demonstrated that Tn5 had inserted into the 3.7-kb EcoR1 fragment and that it was in close proximity to the 4.2-kb fragment.

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CONSTRUCTION AND ANALYSIS OF *ERWINIA CHRYSANTHEMI* MUTANTS DEFICIENT IN MULTIPLE PECTIC ENZYMES. R. G. McGuire, S. Y. He, A. D. Brooks and A. Collmer, Department of Plant Pathology, Cornell University, Ithaca, NY 14853-5908.

E. chrysanthemi EC16 produces multiple pectic enzymes, including exo-poly-α-D-galacturonosidase (exoPG), exopolygalacturonate lyase (exoPL), pectin methylesterase (PME), and four isozymes of pectate lyase (PL). The bacterium can also utilize isolated plant cell walls or polygalacturonate as sole sources of carbon, cause maceration and cell killing in a wide variety of plants, and multiply in plant tissues. Mutations were introduced by marker exchange-eviction mutagenesis, using previously cloned genes, into *E. chrysanthemi* AC4150 (Nal^r derivative of EC16) or CU1006 (*ΔpelA,B,C,E* derivative of AC4150) to produce mutants with combinations of deficiencies in exoPG, exoPL, PME, and PL. Our results indicate that, although PL is responsible for most of the maceration, exoPG has an important role in the utilization of pectate, and factors other than these pectic enzymes can contribute to the ability of *E. chrysanthemi* to multiply and cause maceration in plant tissues.

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IDENTIFICATION AND REGULATION OF CELLULOSE SYNTHESIS GENES IN AGROBACTERIUM TUMEFACIENS. R.T. Lightfoot and A.G. Matthysse. Dept. of Biology, Univ. of North Carolina, Chapel Hill 27599

A subclone of a cosmid which complemented a transposon Tn5 induced cellulose synthesis deficient mutant of Agrobacterium tumefaciens has been identified. Multiple insertions of a Tn3 transposon containing a promoterless beta-galactosidase gene (Tn3HoHo1) into this clone have delineated the boundaries of a cellulose production region and indicate that several gene products are necessary for synthesis. Synthesis was increased by the addition of plant extract to the bacterial culture medium. A Tn5 insertion into a nearby gene resulted in cellulose overproduction, raising the possibility that this mutant was in a repressor gene. Tn3HoHo1 insertions into this gene indicate that its expression is insensitive to plant extract. Studies are underway to determine whether expression of these genes is regulated by the plant extract, and to explore the expression and regulation of cellulose synthesis in Agrobacterium tumefaciens.

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PHYSICAL CHARACTERIZATION OF MUTATIONS IN PLASMID pPT23A WHICH RESULT IN THE CORONATINE-DEFECTIVE PHENOTYPE. C. L. Bender and S. A. Young, Dept. of Plant Pathology, Oklahoma State Univ., Stillwater, OK 74078-9947.

We have recently demonstrated that plasmid pPT23A is involved in coronatine biosynthesis in Pseudomonas syringae pv. tomato PT23.2 (J. Bacteriol. 171:807-812). Nine coronatine-defective mutants of P. syringae pv. tomato PT23.2 were found to contain either Tn5 insertions or deletions in plasmid pPT23A. In the present study, the Tn5 insertions in five mutants were mapped to two EcoRI fragments, which were 7.9 and 18.5 kb in length. In four mutants which contained deletion derivatives of pPT23A, the extent of DNA deleted from the 101 kb plasmid pPT23A ranged from 8 to 30 kilobases. These four deletion derivatives of pPT23A were missing variable amounts of DNA from the two EcoRI fragments. These results strongly suggest that at least some of the genes for coronatine biosynthesis reside on two EcoRI fragments on pPT23A.

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CLONING OF A DNA REGION OF *PSEUDOMONAS SOLANACEARUM* STRAIN AW1 THAT AFFECTS BOTH THE HYPERSENSITIVE RESPONSE ON TOBACCO AND PATHOGENICITY ON TOMATO AND EGGPLANT. L. A. Macol and T. P. Denny, Dept. of Plant Pathology, UGA, Athens, GA 30602.

The suicide plasmid pSUP2021 was used to introduce Tn*5* into *Pseudomonas solanacearum* strain AW1. This strain elicits a hypersensitive response (HR) on tobacco and a disease response on tomato and eggplant. Four HR^- mutants were isolated by screening over 6,000 Km^r colonies for loss of phenotype on tobacco. All HR^- mutants had also lost pathogenicity on tomato and eggplant. This suggested that a *hrp* gene(s) had been inactivated. Southern blot analysis and genomic transformation confirmed that loss of phenotype was due to Tn*5* insertion. The Tn*5* element plus flanking DNA was subcloned from a HR^- mutant, designated AW1-101, and used to identify seven cosmid clones from a genomic library of AW1. Further characterization of this DNA region will involve complementation analysis, *in planta* growth assays, and physiological studies.

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GENETICS OF XANTHOMONADIN PRODUCTION IN XANTHOMONAS CAMPESTRIS PV CAMPESTRIS. A. R. Poplawsky, M. D. Kawalek[1], and N. W. Schaad[2]. PI. Path/PSES, University of Idaho Moscow, ID 83843. Current addresses: [1]USDA-ARS, Corvallis, OR 97330; [2]Harris Moran Seed Co., San Juan Bautista, CA 95045.

Fourteen white mutants of the yellow pigmented X.c.c. strain B-24 were shown by spectrophotometric methods to be greatly reduced in xanthomonadin pigments. Two cosmids from a pLAFR3 cosmid clone bank of strain B-24 were selected which complemented (restored) pigment production in the fourteen mutants and a naturally occurring X.c.c. white strain (B-122). These cosmids, pIG101 and pIG102, contained 32 and 27 kb DNA inserts, respectively, and restriction endonuclease digestion indicated that they contained a common 18 kb sequence. A five enzyme, restriction endonuclease map of the region and subclones were constructed. Complementation analysis of the fourteen mutants and strain B-122 with the subclones has identified five complementation groups within a contiguous region of about 25 kb.

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TRANSPOSON TN5 MUTAGENESIS OF XANTHOMONAS CAMPESTRIS PVS. CITRUMELO AND TRANSLUCENS. M.T. Kingsley, V.R. Waney and D.W. Gabriel. Plant Pathology Dept., University of Florida, Gainesville, FL 32611.

Suicide vector pSUP1011 was used to transfer Tn5 from E. coli strain SM10 to X. campestris pv. citrumelo strain 3048 (Sp^r); pRK600::Tn5uidA was used to introduce the transposon to X.c. pv. translucens strain 216.2 (Sp^r). Kanamycin resistant (25 μg/ml) exconjugants of both strains were obtained on complete media with spectinomycin (35 μg/ml) at frequencies of 10^{-7} to 10^{-8} per recipient. By Southern blot analyses, the transposons appeared to be randomly dispersed in both genomes. Strain 3048 causes disease on citrus and common bean. Auxotrophic mutants of strain 3048 were recovered at a frequency of 2.6%. Nonauxotrophic mutations in strain 3048 affecting pathogenicity on both hosts ($Path^-$) or only one host (host species specific, Hss^-) were recovered at frequencies of ca. 0.36% and 0.33%, respectively. X.c. pv. translucens strain 216.2 causes disease on barley, oats, rye, triticale, and wheat. Pathogenicity tests of 216.2 insertional derivatives are in progress.

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CLONING AND PRELIMINARY CHARACTERIZATION OF AN *HRP* GENE CLUSTER FROM *ERWINIA AMYLOVORA*. R. J. Laby; C. H. Zumoff; B. J. Sneath; D. W. Bauer; and S. V. Beer. Department of Plant Pathology, Cornell University, Ithaca, NY 14853

Several transposon-induced mutants of *E. amylovora*, deficient in pathogenicity to pear and unable to elicit the hypersensitive response (HR) in tobacco (Hrp-) could not be complemented with previously identified cosmids and plasmids. To complement these mutants, a cosmid library of wild-type *E. amylovora* DNA was screened in two ways. After conjugating the library into an Hrp^- mutant, cosmid pCPP430 was identified by restoration of pathogenicity on immature pear fruit. Cosmids pCPP440 and pCPP450 were identified by hybridization with subclones of the previously identified plasmids. These two cosmids complement for pathogenicity all but three Hrp^- mutants, while pCPP430 complements all (20) Hrp^- mutants. Each of the three cosmids enables *E. coli* to elicit the HR and has ca. 40 kb of DNA in common. Mutagenesis with *Tn5-uidA* is underway to further elucidate the organization and regulation of the transcriptional units.

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THE CLONING OF AN AVIRULENCE GENE IN XANTHOMONAS CAMPESTRIS pv. VESICATORIA THAT DETERMINES HYPERSENSITIVITY IN TOMATO. B. I. Canteros, G. V. Minsavage, and R. E. Stall. Plant Pathology Department, University of Florida, Gainesville, FL 32611.

Several strains of Xanthomonas campestris pv. vesicatoria (Xcv) from pepper failed to cause disease in leaves of tomato cultivar Bonny Best when inoculated along with abrasion by carborundum. A DNA library of one of the strains was constructed in pLAFR-3. One fragment in the library encoded for a hypersensitive reaction in tomato after strains that were compatible with tomato were complimented with the fragment. Mutagenesis by Tn-5 insertion into a 1.7 kbp subclone abolished its function. The cloned gene ($avrBs_P$) was located by Southern hybridization analysis on a plasmid of about 40 kbp that is present in many strains of Xcv. The $avrBs_P$-encoding fragment hybridized to DNA of several Xcv strains that were avirulent to tomato but not to DNA of several pathogenic strains. The $avrBs_P$ gene was linked to another avirulence gene ($avrBs_3$) on the plasmid in most strains.

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MOLECULAR CLONING OF AN EXO-PECTATE LYASE GENE FROM ERWINIA CHRYSANTHEMI AND CHARACTERIZATION OF THE GENE PRODUCT. Alan D. Brooks[1], Alan Collmer[2], and Steven Hutcheson[1]. [1]Botany Dept., University of Maryland, College Park, MD 20742 and [2]Plant Pathology Dept., Cornell University, Geneva, NY 14456.

Erwinia chrysanthemi EC16 mutant UM1005 produces no extracellular endo-pectate lyase (PL) because of deletions in the known *pel* genes, but the mutant still macerates plant tissues. In an attempt to identify the remaining macerating factor(s), a gene library of UM1005 was constructed in E. coli and screened for pectolytic activity. A structural gene for an exo-pectate Lyase (exo-PL) was identified in this library (pPNL5). Exo-PL was purified to apparent homogeneity from E. coli DH5α pPNL5 and found to have an apparent molecular weight of 76,000 daltons and an isoelectric point of 8.6. Purified exo-PL had optimal activity between pH 7.5 - 8.0, and could utilize pectate, citrus pectin, and highly methyl-esterified Link pectin as substrates. A PL^- exo-PL^- mutant of EC16 was constructed that retained pathogenicity on chrysanthemum equivalent to that of UM1005.

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MANGANESE DETECTION BY ELECTRON SPIN RESONANCE DURING WOOD DECAY BY BROWN- AND WHITE-ROT FUNGI. B. L. ILLMAN. U.S.D.A., Forest Service, Forest Products Laboratory, One Gifford Pinchot Drive, Madison, Wisconsin 53705-2398 USA.

Changes in manganese were detected in fungal degraded wood by electron spin resonance spectroscopy (ESR). Samples of wood were colonized by one of two white-rot fungi, Coriolus versicolor or Phanerochaete chrysosporium, or one of two brown-rot fungi, Gloeophyllum trabeum or Postia placenta. ESR spectra for the paramagnetic divalent manganous ion, Mn(II), were collected at weekly intervals for six weeks from wood samples of cottonwood (Populus deltoides), Douglas-fir (Pseudotsuga menziesii), sweetgum (Liquidambar styraciflua) and redwood (Sequoia sempervirens), with and without the presence of the fungi. Changes in the degree of the Mn(II) ESR spectra of each wood species were dependent upon the presence and the species of the decay fungus. In general, the magnitude of the change in the manganese signal correlated with susceptibility of wood species to decay by the fungi.

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INHIBITORY EFFECT OF TRICYCLAZOLE AND OTHER MELANIN INHIBITORS ON GREEN PIGMENTATION OF PENICILLIUM SPP. AND ASPERGILLUS SPP. Wheeler, M. H. and M. A. Klich, USDA, ARS, Cotton Pathology Research Unit, Rt. 5, Box 805, College Station, TX 77840 and USDA, ARS, SRRC, P. O. Box 19687, New Orleans, LA 70179.

Five compounds that inhibit melanin synthesis in a number of brown to black imperfect and ascomycetous fungi were used to treat fungi that produce green conidial pigments. Tricyclazole, chlobenthiazone, pyroquilon, fthalide and 2,3,4,5,6-pentachlorobenzyl alcohol, added to malt extract agar at 8 or 30 µg/ml, prevented normal green pigmentation in nine Penicillium spp. and in three of six Aspergillus spp. The compounds did not prevent green pigmentation in Gliocladium virens or Trichoderma harzianum. Those fungi whose pigment was affected by the five inhibitors were lighter green or brown in color. Chlobenthiazone at 30 µg/ml was the only treatment that strongly inhibited fungal growth and sporulation. These results suggest that many Penicillium spp. and Aspergillus spp. may contain reductase enzymes similar to those in the melanin pathway of many brown to black fungi.

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COMPARISONS OF DEVELOPMENT AND MORPHOLOGY BETWEEN SCLEROTIA AND BASIDIAL CYMES IN *RHIZOCTONIA SOLANI* KUHN ANASTOMOSIS GROUP ONE. X.B. Yang, C.S. Kousik, G.T. Berggren, and J.P. Snow. Dept. of Plant Path. & and Crop Physi. La. Ag. Expt. Sta., LSU Ag. Center, Baton Rouge, LA 70803.

Initiation and growth of sclerotia and basidial cymes in *Rhizoctonia solani* Kuhn AG1 were studied using light and scanning electron microscopy. The AG-1-IB and AG-1-IC intraspecific groups produced both microsclerotia and the basidial stage on water agar. Growth of sasakii-type sclerotia of AG-1-IA intraspecific group is a result of interweaving of mycelium and is referred to as 'loose type'. Microsclerotia of intraspecific groups IB and IC are initiated from a lateral point, develop by lateral branching, and are referred to as lateral types. The initiation and development of basidial cymes and microsclerotia is morphologically similar. Lateral growth of microsclerotia may be related to sexual reproduction of the fungus (Willetts 1972, Bio. Rev. 47:515-536).

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Comparison of pepper isolates of Phytophthora capsici from New Mexico to other solanaceous and non-solanaceous isolates. J. Y. Uchida and M. Aragaki. Department of Plant Pathology, University of Hawaii, Honolulu.

Thirty isolates of Phytophthora capsici obtained from pepper in the Rio Grande Valley near Las Cruces, New Mexico where the type culture originated in 1922, were compared to isolates from tomato and pepper from Hawaii and Mexico. Fifteen other isolates obtained in Hawaii from other hosts were also studied. Phytophthora capsici cultures from pepper and tomato from New Mexico and elsewhere: (1) did not produce chlamydospores; (2) varied in the production of sporangial fans; (3) were A1, A2, or homothallic; (4) produced sporangia variable in L:D ratios with long, variable pedicels; and (5) virulent to pepper. Phytophthora capsici cultures from other hosts formed a distinct group which: (1) produced chlamydospores; (2) frequently failed to form gametangia; (3) promptly produced sporangial fans; (4) produced narrow sporangia with long pedicels; and (5) were avirulent to pepper.

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MITOCHONDRIAL AND PLASMID DNAS IN *TRICHODERMA*. Robert J. Meyer. SBML, USDA-ARS, Beltsville, MD 20705.

The *Trichoderma* species aggregates have been difficult to subdivide into biological species by morphological characters. The nucleic acids of these species are being examined for new characters that could help define the biological species. Analysis of mitochondrial DNA (mtDNA) preparations revealed two possible characters: highly varied mtDNA restriction patterns and mitochondrial plasmids. Despite the variability in the mtDNA restriction patterns, it was possible to subdivide the strains into groups. Two of these groups correlated with two new morphological subgroups of the *Trichoderma viride* species aggregate. The plasmid DNAs were unique to each strain and the patterns generated did not correspond to the groups based on mtDNA restriction patterns. This result suggested that the mtDNAs and plasmid DNAs evolved independently. Nuclear ribosomal DNA restriction maps are being examined to see if they give a corroborative or dissimilar grouping of these strains.

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INFLUENCE OF FERTILIZATION AND PLANT AGE ON DETECTION OF ACREMONIUM COENOPHIALUM. B. L. Randall-Schadel, K. D. Gwinn, A. M. Gavin, R. A. Shelby, and L. W. Dalrymple. NC Dept. of Agric., P.O. Box 27647, Raleigh, NC 27611, Univ. of TN, Knoxville, TN 37901, Auburn Univ., Auburn, AL 26849.

Samples from 4 tall fescue seed lots were evaluated in a regional referee for viability of A. coenophialum by 7 laboratories. Results had an unacceptable level of variation. Since fertilization and the age of the plant varied among laboratories, this study was designed to examine the effects of these variables on endophyte detection. Treatments were 3 levels of fertilization, 3 ages of plants and 3 seed lots. The experiment was performed twice. Each treatment was evaluated at two locations using either ELISA or microscopic assay. ELISA and microscopic assay were highly correlated ($r = .93131$, $P = .0001$). Within individual seedlots, no variation in endophyte infection was found among nitrogen levels or plant ages. Endophyte infection levels of seed lots were statistically different ($P = .0001$).

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DEVELOPMENT OF AN ANTISERUM TO DETECT GREENING DISEASE OF CITRUS. R-J Chippindall and V H Whitlock, Department of Microbiology, University of the Witwatersrand, P O Wits, 2050, South Africa.

Antisera raised against laboratory cultures of a bacterium initially thought to be the causative agent of citrus greening disease were found to be unreliable in distinguishing between healthy and greening-infected material in various assays. In order to avoid using the cultured organism as immunogen, a polyclonal antiserum was raised against a partially-purified extract of the greening organism from citrus. Extracts were prepared by homogenization and enzymatic digestion of plant material followed by differential and gradient centrifugation. After exhaustive cross-adsorption against healthy plant material, the antiserum was able to detect greening using the ELISA and immunoblot assays. Preparation of samples for blotting by alkali leaching of bacteria from infected tissue was found to be particularly effective. This antiserum is currently being used to identify greening-infected material using the immunoblotting procedure.

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A SEMI-SELECTIVE MEDIUM FOR PHOMA MACDONALDII (PMSA). P. A. Donald and J. R. Venette. Department of Plant Pathology, North Dakota State University, Fargo, ND 58103.

A technique for detecting Phoma macdonaldii in its ecosystem has been lacking. P. macdonaldii conidia and ascospores are formed on sunflower stalk debris. P. macdonaldii is difficult to recover from debris because it competes poorly with other organisms in culture. Soil extract medium selective for P. betae was modified to support growth of P. macdonaldii and reduce growth of contaminants. To test the medium, infected sunflower stalk debris was finely ground and the powder was mixed 1:1 to 1:10 (stalk volume:nonsterile field soil volume). Portions of the mixtures were plated on PMSA. P. macdonaldii was recovered from all ground stalk assays and from all mixtures through 1:4. The pathogen was recovered from 80% of the assays with stalk:soil ratios of 1:8 and 1:10. P. macdonaldii was not recovered from field soil alone. When conidia from culture were plated on PMSA, at least $1X10^3$ conidia/ml were needed for growth to be observed.

618

DIAGNOSTIC MEDIA FOR THE DETECTION OF FUNGI (BOTRYTIS CINEREA) RESISTANT TO VINCLOZOLIN AND BENOMYL. T.R. Bardinelli, E.J. Butterfield, and T.L. Jones. BASF Corporation, Agricultural Research Center, P.O. Box 13528, Research Triangle Park, NC 27709.

A diagnostic medium was developed for the detection of Botrytis cinerea strains resistant to vinclozolin and benomyl. The medium contains 0.04% (w:v) brom cresol purple, 10% 0.1N NaOH, and 2% agar. After autoclaving, filter-sterilized dextrose is added to 4% then 40 ppm vinclozolin or 10 ppm benomyl and 50 ppm streptomycin sulfate are added. Germination and growth of resistant spores causes a color change from red to yellow in 18-48 hours after inoculation. Laboratory and field tests demonstrated selectivity against fungal contaminants, making the medium useful for field monitoring of resistance. Comparisons between this method and other techniques such as

agar diffusion tests and spore germination on fungicide ammended media (PDA, MA or WA) showed excellent correlations. This medium has also been used for the detection of resistant strains of Monilinia fructicola to benomyl.

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SEROLOGICAL COMPLEXITY IN PAV-LIKE BARLEY YELLOW DWARF VIRUSES. R.E. Klein, G.N. Webby, and R.M. Lister. Dept. of Botany & Plant Path., Purdue University, West Lafayette, IN 47907

In experiments to develop panels of antibodies for detecting and discriminating isolates of barley yellow dwarf virus, leaf samples of several cereal species from various sources were screened by ELISA with polyclonal (PAbs) or monoclonal (MAbs) antibodies to a PAV-like isolate. Four MAbs were PAV-specific and three reacted with PAV as well as an MAV isolate. Most samples which reacted positively with PAV PAbs also reacted with all seven MAbs in subsequent tests. However, several samples failed to react with one or more of the PAV-specific MAbs and quantitative differences were also found among samples with MAbs that reacted with both PAV and MAV. Based on these results, PAV-like isolates could be separated into at least three groups. The results emphasize that, as with MAV (Phytopathology 78:766-770), no single MAb can reliably detect all PAV-like isolates.

620

MODELS TO ESTIMATE YIELD LOSSES IN BELL PEPPER CAUSED BY TOBACCO ETCH VIRUS EPIDEMICS. F. W. Nutter, Jr., C. W. Kuhn, and *J. N. All, Departments of Plant Pathology and *Entomology, University of Georgia, Athens, GA 30602.

Field experiments were conducted in northeast Georgia during the years 1985-1988 to quantify the effect of tobacco etch virus (TEV) epidemics on yield of Yolo Wonder B bell pepper. TEV was found to reduce both fruit number and average fruit weight/plant. Early season infection reduced yield 74% in 1986 and 73% in 1987 while late season infection reduced yield 5 and 7% in 1986 and 1987, respectively. Pepper yield increased in a logistic fashion with respect to the delay in time (days after transplanting) that individual plants exhibited TEV symptoms. Critical point (CP), multiple point (MP), and area under the TEV disease progress curve (AUDPC) models had coefficients of determination ranging from 52-85%. An additive multiple point model based on the weekly change in TEV incidence as a function of days after transplanting pepper explained more of the variation in yield (83 to 94%) than traditional CP, MP, and AUDPC models. Yearly yield losses due to TEV ranged from 15 to 50% during the 4 yr period.

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EFFECTS OF NET BLOTCH ON YIELD OF MOREX BARLEY. Y. Jin, V. D. Pederson. Department of Plant Pathology, North Dakota State University, Fargo, ND 58105.

The relationship between disease severities of barley net blotch (incited by Pyrenophora teres Drechs.) and yield of Morex barley was investigated by inoculating barley plants at different growth stages (GS) in the greenhouse. Inoculations at GS 32, GS 48 and GS 75 reduced yield 48.7, 39.4 and 13.5 percent respectively. Early inoculations significantly reduced the number of seeds per head, height of plants and dry weight of plants. A yield loss model was developed based on disease severities in the top three leaves. Collinearity among the independent variables was diagnosed using a condition number index and variance of decomposition proportion. It was removed using a ridge regression technique with a ridge trace K = 0.04 (SAS-PROC RIDGEREG). The model accounted for 92.1 percent of the total variation. A model using inoculation stages as qualitative independent variables accounted for 93.2 percent of total variation.

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EFFECTS OF VERTICILLIUM DAHLIAE AND DROUGHT STRESS ON POTATO GROWTH. R. L. Bowden, D. I. Rouse, T. T. M. Tiirinlahti, and B. D. Bowen, Dept. Plant Pathology, University of Wisconsin, Madison, WI 53706.

Effects of Verticillium wilt and drought stress on 'Russet Burbank' potato grown in Plainfield loamy sand were studied in a split-plot experiment in 1988. Main plots were overhead irrigation treatments (1 or 3 irrigations/week) and subplots were levels of V. dahliae inoculum (0, 9, or 39 propagules/gram of soil). Canopy radiation interception was reduced by drought stress between 88 and 97 days after planting (DAP) and by V. dahliae between 110 and 124 DAP. Level of V. dahliae, but not irrigation, had significant effects on incidence of V. dahliae in stem bases at 105 DAP. Main effects of irrigation and V. dahliae on tuber dry weight were significant at final harvest (145 DAP), but not at 48, 77, or 105 DAP. Yield reductions for both effects were due to lower tuber specific gravities and a smaller yield of grade A tubers. The irrigation x V. dahliae interaction was not significant.

623

SUCCESSFUL PRODUCTION OF MONOCLONAL ANTIBODIES TO THREE CARNATION VIRUSES USING AN ADMIXTURE OF ONLY PARTIALLY PURIFIED VIRUS PREPARATIONS AS IMMUNOGEN. Ramon Jordan. USDA-ARS, Florist and Nursery Crops Laboratory, Beltsville, MD 20705.

One of the stated advantages of hybridoma produced monoclonal antibodies (McAbs), when compared with conventionally produced polyclonal antibodies is the ability to produce and select target-specific McAbs even when impure antigen or antigen mixtures are used as immunogens. This 'advantage' was tested in a project designed to generate McAbs to carnation necrotic fleck (CNFV), carnation mottle (CarMV) and/or carnation latent (CarLV) viruses. An admixture of partially purified extracts from singly- and doubly-infected carnation plants was used as immunogen and individually as ELISA screening antigens (along with a similarly prepared extract from healthy plants). Extracts contained 5-20 protein bands as determined by SDS-PAGE. Forty-one antibody-secreting virus-specific (4 to CarMV, 4 to CarLV and 33 to CNFV) hybridoma clones were selected. Virus specificity was confirmed by Western-blot analysis. Selected McAbs have been used routinely in ELISA for detection of virus in sap extracts.

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WHITEFLY-MEDIATED SILVERING OF SQUASH LEAVES. N. Bharathan, W. R. Graves, K. R. Narayanan, H. H. Bryan, and R. T. McMillan, Jr. Tropical Research and Education Center, University of Florida, IFAS, 18905 SW 280 Street, Homestead, FL 33031.

A disease of unknown etiology resulting in silvering of squash leaves has become very prevalent in South Florida. Typical symptoms include vein clearing and subsequent silvering of leaves. These symptoms are observed 2-3 weeks after whitefly (Bemisia tabaci) infestation. Squash leaves were analyzed for double standard RNA (dsRNA) which are known to be foot prints of viral infection. Double stranded RNA bands of approximately 4.6 and 4.2 Kb were consistently observed in caged, whitefly-infested plants. In contrast, no dsRNA bands were observed in caged, whitefly-free plants. Further characterization of the whitefly-mediated silvering in squash leaves is in progress.

625

PARTIAL CHARACTERIZATION OF MONOCLONAL ANTIBODIES TO ZUCCHINI YELLOW MOSAIC VIRUS (ZYMV) AND WATERMELON MOSAIC VIRUS-2 (WMV-2). G. C. Wisler, C. A. Baker, D. E. Purcifull, and E. Hiebert. Department of Plant Pathology, University of Florida, Gainesville, FL 32611.

Monoclonal antibodies (MAbs) to ZYMV and WMV-2 purified virions were developed by a modified in vitro immunization procedure by fusing BALB/c spleen cells with SP2/o cells. MAbs of potential diagnostic value were evaluated by an indirect ELISA with homologous rabbit polyclonal antiserum as the trapping antibody. All virus isolates were propagated in Cucurbita pepo L. One MAb to ZYMV (MAb-Z) detected all 10 ZYMV isolates from Florida and all four from Europe. One WMV-2 MAb (MAb-W) detected all 18 WMV-2 isolates tested, including 14 from Florida, two from California, and one each from New York and New Zealand. No cross-reactions were detected between any of the ZYMV and WMV-2 isolates and their heterologous MAbs, nor were reactions obtained with leaf extracts from uninoculated plants or plants infected with papaya ringspot, squash mosaic, or cucumber mosaic viruses. Thus, MAb-Z and MAb-W appear to be useful for detection of these ZYMV and WMV-2 isolates, respectively.

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ANTIGENIC DIVERSITY OF PAPAYA RINGSPOT VIRUS (PRSV) ISOLATES DETECTED BY MONOCLONAL ANTIBODIES TO PRSV-W. C. A. Baker and D. E. Purcifull, Dept. Plant Pathology, University of Florida, Gainesville, FL 32611

Six monoclonal antibodies (MAbs) were obtained after the fusion of SP2/0 myeloma cells with spleen cells from BALB/c mice immunized with a Florida isolate of PRSV type W (PRSV-W) or its capsid protein. The MAbs were tested by indirect ELISA against 15 isolates of PRSV-W, one isolate of PRSV from papaya (PRSV-P), and the Tigre isolate (PRSV-T) from Guadeloupe. MAb-1 reacted with all 17 PRSV isolates. MAb-2 reacted with all 15 isolates of PRSV-W and with PRSV-P. MAb-3 reacted only with the 15 PRSV-W isolates. MAb-4 reacted with 13 PRSV-W isolates and with PRSV-P. MAb-5 reacted with 12 PRSV-W isolates and with PRSV-P and PRSV-T. MAb-6 reacted only with the PRSV-W isolate used for immunization. None of the MAbs reacted with the Moroccan isolate of watermelon mosaic virus. These results provide evidence for at least six different epitopes on PRSV, five of which varied among the PRSV isolates that were tested.

628

RAPID METHOD FOR MAIZE STRIPE VIRUS IDENTIFICATION. O. E. Bradfute and J. H. Tsai*, Ohio State University, OARDC, Wooster, OH 44691 and *University of Florida, IFAS, Ft. Laud. REC, Fort Lauderdale, FL 33314

Maize stripe virus (MStpV) was identified by direct observation in phase-contrast light microscopy of needle-shaped crystals in sap from symptomatic leaf areas of maize (*Zea mays* L). Crystals were similar to crystals of MStpV noncapsid protein (NCP) in morphology and in differential solubility and reacted with antiserum to NCP in immunofluorescence microscopy. Crystals could be readily found in naturally and experimentally MStpV-infected maize plants with all types of symptoms and throughout disease development, but not in maize infected by other maize viruses or mycoplasmas. This simple test should be useful in disease surveys and in monitoring breeding programs.

630

VARIATION IN TOMATO RINGSPOT VIRUS ISOLATED FROM GRAPE. D.C. Bays and S.A. Tolin, Department of Plant Pathology, Physiology and Weed Science, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061.

A comparison was made of tomato ringspot virus (TmRSV) isolated from three sources of grape, Vidal 256 in a Virginia vineyard (WR), and rootstocks SO4 and Kober 5BB in a rootstock nursery (T), all of which had been obtained from nurseries outside the state. These isolates were designated TmRSV-WR5, -TS1, and -TKB1, respectively. The isolates had similar host ranges but varied in symptom severity on cucumber. TmRSV-WR5 induced very severe mosaic and leaf curling, and often plant death, but TmRSV-TS1 and TmRSV-TKB1 were much less severe with no plant death. A single band of Mr 60,000 was obtained upon polyacrylamide gel analysis of proteins isolated from purified virus. TmRSV-WR5 was serologically similar to typical eastern North American TmRSV isolates, and reacted identically with six TmRSV antisera. However, TmRSV-TS1 and TmRSV-TKB1, formed spurs with one of the six antisera indicating serological differences.

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CHARACTERIZATION OF A GEMINIVIRUS OF PEPPER. D. C. Stenger, J. E. Duffus, USDA-ARS, Salinas, CA, 93905, and B. Villalon, Texas Agricultural Experiment Station, Weslaco, TX, 78596.

A geminivirus causing leaf curl and distortion symptoms was isolated from pepper (Capsicum annuum) grown in Texas. The Texas pepper geminivirus (TPGV) was transmitted persistently by Bemisia tabaci, and mechanically, to species of the Solanaceae. Electron microscopy of purified virions revealed typical geminate particles. Extracts from infected plants contained a supercoiled replicative form (RF) DNA species of 2.6 kilobase pairs. RF DNA was digested with EcoR I or Hind III and cloned into pUC8. Analysis of recombinant plasmids indicated that two distinct species were cloned from RF DNA. One TPGV DNA hybridized with DNA A of tomato golden mosaic virus (TGMV). No hybridization was observed between TPGV DNAs and TGMV DNA B. Both cloned TPGV DNAs were required for systemic infection of plants. TPGV is a typical whitefly transmitted, bipartite geminivirus not previously known to occur in the United States.

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SEROLOGICAL RELATIONSHIPS BETWEEN BARLEY YELLOW DWARF VIRUSES. G.N. Webby and R.M. Lister, Purdue Univ., W. Lafayette, IN 47907.

Serological differentiation indices (SDI's) for five isolates representative of a range of barley yellow dwarf virus serotypes were measured by an indirect ELISA with rabbit polyclonal antisera to each isolate and using purified viruses coated to microtiter plates in 0.1 M phosphate, pH 7.0. The isolates were: PAV (non-specifically transmitted by Sitobion avenae and Rhopalosiphum padi), MAV (specifically transmitted by S. avenae), and SGV (specifically transmitted by Schizaphis graminum), regarded as Group 1 serotypes, and RPV (specifically transmitted by R. padi) and RMV (specifically transmitted by R. maidis), regarded as Group 2 serotypes. Group 1 isolates were all closely related (SDI's 1-3), MAV being intermediate between PAV and SGV. RPV and RMV (Group 2) were distantly related to Group 1 isolates (SDI's 5-8), but only moderately related to each other (SDI's 3.5-5.5). SDI's were reduced, particularly for distantly related isolates, when antisera produced by intradermal injection were used, perhaps indicating that such antisera contained antibodies to a greater variety of epitopes.

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THE VPG OF TOBACCO ETCH VIRUS (TEV). J.F. Murphy[1], R.E. Rhoads[2], A.G. Hunt[3], and J.G. Shaw[1]. Departments of Plant Pathology[1], Biochemistry[2] and Agronomy[3], University of Kentucky Lexington, KY. 40546.

TEV RNA, purified by SDS-sucrose gradient centrifugation, was digested with RNase A and analyzed by SDS-PAGE. Silver staining revealed proteins of 49, 32 and 24 kDa. The 49 and 24 kDa proteins were detected with anti49K antibody, while the 32 kDa protein was detected with anticoat protein antibody. Further purification of the RNA removed all traces of coat protein, but not the 49 and 24 kDa proteins. Furthermore, the 49 and 24 kDa proteins did not migrate into an SDS-polyacrylamide gel when TEV RNA was not digested with RNase A. These results indicate that 1) the VPg of TEV is 24 kDa, 2) it is immunologically related to the 49K nuclear inclusion, and 3) some viral RNAs contain covalently bound 49K protein, presumably due to incomplete cleavage of the VPg.

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PROPERTIES OF THE RNA AND COAT PROTEIN OF SPRING BEAUTY LATENT VIRUS AND COMPARISON WITH THOSE OF FOUR BROMOVIRUSES. R. A. Valverde. Dept. of Plant Pathology and Crop Physiology, Louisiana Agricultural Experiment Station, Louisiana State University Agricultural Center, Baton Rouge, LA 70803.

Spring beauty latent virus (SBLV) has been proposed as a new member of the bromovirus group. Four dsRNAs (MW: 3.1, 2.6, 1.8, and 0.6 x 106), with a profile similar to but not identical with those of four bromoviruses, were obtained when plant tissue infected with SBLV was analyzed. Molecular hybridization using cDNA to SBLV RNA did not show any detectable relationship with other bromoviruses. Infectivity tests using SBLV ssRNAs indicated that it has a tripartite genome requiring RNAs 1, 2, and 3 for infectivity. Translation of RNA 4 yielded a protein (MW=23,000) which comigrated with the coat protein in SDS-polyacrylamide gels. Western blot analysis of the coat proteins of SBLV and those of four bromoviruses revealed different degrees of homology. Results presented here provide further evidence that supports placing SBLV in the bromovirus group.

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MOLECULAR CLONING OF GENOMIC RNAS OF SWEET CLOVER NECROTIC MOSAIC VIRUS (ALFALFA STRAIN). H.R. Pappu, J.A. Williams*, C. Hiruki and J.B. Bell*. Departments of Plant Science and Genetics*, University of Alberta, Edmonton, Alberta T6G 2P5, Canada.

Complementary DNA (cDNA) copies of the two genomic RNAs of sweet clover necrotic mosaic virus (alfalfa strain) were cloned into Lambda gt10. Using viral RNA-specific nucleic acid probes, the cDNA library was screened and two clones with inserts of 1.8 kb and 1.4 kb were selected that were specific to RNA-1 and RNA-2 respectively. The specificity of each clone was further confirmed by Northern analysis. Both cDNA clones were separately sub-cloned into Blue Scribe and M13 vectors. Single-stranded DNA templates prepared from recombinant M13mp18 and M13mp19 containing the 1.4 kb fragment specific to RNA-2 are being sequenced using the dideoxy chain termination method. Analysis of the sequence information and in vitro transcription studies are in progress.

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BYDV-PAV virions contain readthrough protein. [1] Waterhouse, P.M., [2]Martin, R.R. and [1]Gerlach, W.L. [1]CSIRO Div of Plant Industry, Canberra, ACT and [2]Agriculture Canada, Vancouver, B.C.

The PAV strain of barley yellow dwaft virus (BYDV-PAV) has been sequenced and the coat protein ORF shown to end with an amber termination codon (NAR 16:6097). Following the amber codon there is an ORF that could encode a protein of 50 Kd, referred to as the readthrough protein. This readthrough ORF, minus the first codon, and the coat protein ORF were each subcloned into the SmaI site of the GEX-2 expression vestor (Gene 67:31-40). Fusion proteins were purified by affinity chromatography on glutathione-agarose beads. In Westerns, BYDV-PAV antiserum prepared to whole virus reacted with the readthrough and coat protein fusion proteins. Immunoglobulins from BYDV-PAV virion antiserum that were affinity purified on readthrough fusion protein reacted with the readthrough fusion protein but not with the coat protein suggesting that the readthrough protein is present in purified virus and distinct from the coat protein.

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SEROLOGICAL GROUPING OF THE CYTOPLASMIC INCLUSIONS OF 6 STRAINS OF SUGARCANE MOSAIC VIRUS. S. G. Jensen and J. L. Staudinger, USDA and University of Nebraska, Lincoln, NE 68583.

Antisera to the 66kd cytoplasmic inclusion protein of 5 strains of sugarcane mosaic virus (SCMV) or maize dwarf mosaic virus (MDMV) were reacted in western blots against antigens of 6 virus strains. The concentration of each antigen was adjusted to a constant stain density in PAGE. The intensity of the blot reaction, which was linear with concentration, was measured using a scanning digital densitometer (Bio Image, Visage 110). These tests divided the 6 strains into 3 serological groups. Strains MDMV-A and Minn-11 formed one group, MDMV-B and I-188 the second group and KS-1 and MDMV-O the third. Inclusions within groups cross reacted strongly. Cross reactions between the first and second groups were moderate (equivalent to an antigen concentration of about 1/3). Group 3 did not cross react with the other two groups. Serogroups based on inclusion proteins agree with serogroups based on capsid proteins.

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CHARACTERISTICS OF A VIRUS ISOLATED FROM DOWNY MILDEW-INFECTED WHEAT PLANTS. R. C. Gergerich and K. S. Kim, Dept. of Plant Pathology, University of Arkansas, Fayetteville, AR 72701.

An isometric virus measuring approximately 30 nm in diameter was found in winter wheat infected with Sclerophthora macrospora (Sacc.) T. S. & N. from central Arkansas. The virus was extracted from infected wheat using 1 part tissue, 2 parts 0.05 M phosphate buffer, pH 5.5, and 2 parts chloroform followed by alternate low and high speed centrifugation. Negative staining of both leaf dips and purified preparations revealed uniform particles, and Model E analysis indicated a single 134S component. An antiserum made to purified virus reacted with sap of virus-containing downy mildew-infected wheat but not with sap from wheat infected with downy mildew alone. Reciprocal serological tests indicate that this virus is related to the type B virus of downy mildew from rice in Japan (Honkura, R., et al. 1983. Ann. Phytopath. Soc. Japan 49:653). This is the first report of this virus occurring in the U.S.A.

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Carbohydrate degrading enzymes of Enterobacter cloacae. D.P. Roberts and C.J. Sheets. USDA, ARS, Beltsville, MD 20705.

Enterobacter cloacae strain E6 is a potential biocontrol agent for Pythium ultimum. Production of carbohydrate degrading enzymes of E6 was studied to understand how E6 establishes and maintains itself in the rhizosphere. No xylanase, carboxymethylcellulase, amylase, laminarinase, chitinase, arabinogalactanase, polygalacturonase, or pectate lyase activities were detected in culture supernatants from E6 grown in basal salts + 0.1% (w/v) glycerol (BSM), BSM + 0.2% (w/v) lettuce roots, or BSM + 0.2% (w/v) of the substrate polymer of the enzyme. In contrast, a number of glycosidase activities including β-D-glucosidase, β-D-n-acetylglucosaminidase, β-D-xylosidase, α-L-arabinopyranosidase, α-D-galactosidase, β-D-galactosidase, and α-D-glucosidase were detected in extracts from E6 grown in basal salts + 0.5% glycerol. β-D-glucosidase was the major glycosidase detected in extracellular and membrane fractions and preliminary experiments suggest that these fractions contain different β-D-glucosidase enzymes.

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FIELD POPULATION DYNAMICS OF AGROBACTERIUM SPP. ASSOCIATED WITH CHERRY SEEDLINGS. V.O. Stockwell[1], L.W. Moore[1], M.D. Kawalek[1], J.E. Loper[2], [1]Department of Botany & Plant Pathology, Oregon State University, and [2]USDA-ARS, HRCL, Corvallis, OR 97331.

Agrobacterium radiobacter K84 has been employed as a biocontrol agent of crown gall disease for the past decade, yet little is known about its survival and dissemination in agricultural fields. Population dynamics of the biocontrol agent, strain K84, and the crown gall pathogen, A. tumefaciens strain B49c, were evaluated in cherry crown galls and rhizospheres. Following inoculation of cherry seedlings, the rhizosphere population size of B49c was stable over an 18 week period (10^5-10^6 cfu/g fw root), while that of K84 declined from 10^6 to 10^4 cfu/g. The population size of B49c in crown gall tissue increased from 10^5 to 10^7 cfu/g over 8 weeks, whereas that of K84 remained stable at 10^5 cfu/g. Strain K84 was not detected in soil cores taken more than 5 cm from inoculated cherry seedlings. The population size of strain K84 was stable in crown gall tissue, but declined in rhizosphere and bulk soil.

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NOVEL CHEMICAL AND BIOLOGICAL METHODS TO CONTROL POSTHARVEST BROWN ROT OF NECTARINES. J. L. Smilanick, M.-Y. Ho, D. Henson, and P. Anderson. USDA, ARS, HCRL, 2021 South Peach Avenue, Fresno, CA 93727

Nectarines were fumigated with sec-butylamine (SBA), acetaldehyde (ACH), or benzaldehyde (BZH); or treated with fungi or bacteria to reduce postharvest decay by Monilinia fructicola. All work was conducted at 20-25°C. Fumigation 1 hr with 2% ACH or 0.3% SBA reduced decay lesion area more than 90% after 3 days at 20°C. Phytotoxicity was minor and transient with SBA but moderate and persistent with ACH. BZH was phytotoxic and did not reduce decay. Aureobasidium pullulans, Exophiala mansonii, Hormonema prunorum, an unidentified pink yeast, and Pseudomonas cepacia significantly reduced decay when 10,000 or more propagules of these organisms were placed into 3x3 mm wounds at the same time as or as long as 7 hr before 100 M. fructicola spores were inoculated. Precolonization of wounds with candidate organisms improved decay suppressiveness, but decay control efficacy was variable. All but P. cepacia were isolated from the surface of nectarines.

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BIOCONTROL OF BOTRYTIS ROT OF APPLE: ELECTRON MICROSCOPY OF EFFECTIVE AND INEFFECTIVE STRAINS OF THE YEAST, DEBARYOMYCES HANSENII. M. Wisniewski, R. McLaughlin, C. W. Wilson, and E.

Chalutz. USDA-ARS, 45 Wiltshire Rd., Kearneysville, WV 25430 and Volcani Center, Bet Dagan, Israel.

A histological study was conducted using an effective (87) and an ineffective (118) strain of D. hansenii. Greater mycelial development was observed in samples treated with 118 than with 87. This was associated with more extensive wound colonization and greater production of an extracellular matrix by 87. Attachment of yeast cells on mycelia was more apparent with 87. After 48 hr, mycelia in wounds treated with 87 appeared collapsed and convoluted, while mycelia appeared normal in wounds treated with 118. By 72 hr, 118-treated wounds showed extensive tissue degradation while 87-treated wounds appeared similar to unchallenged wounds. Results indicate biocontrol by 87 may be mediated by more effective colonization, attachment and/or copious production of an extracellular matrix.

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EVALUATION OF PSEUDOMONAS FLUORESCENS FOR SUPPRESSION OF SEEDLING DISEASE IN COTTON. C. Hagedorn and N. Nelson Virginia Polytechnic Institute and State University, Blacksburg, VA 24061-0331

A strain of Pseudomonas fluorescens (EG10-53) antagonistic to Pythium ultimum and Rhizoctonia solani, the seedling pathogens of cotton (Gossypium hirsutum L) was evaluated in a peat-based carrier for disease suppression. Both disease severity (root lesions) and incidence (infected plants) declined with either higher application rates or with larger numbers of cells at any one rate. Low application rates resulted in plants that were no better than non-inoculated controls; higher rates produced plants equal or superior to fungicide treatments. Colonization studies using an antibiotic-resistant mutant and a direct-observation staining technique indicated rapid growth and colonization of EG10-53 on cotton roots. Bacterization of cotton with EG10-53 enhanced both plant stands and dry weights, and reduced disease severity on surviving plants.

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USE OF AN INSECT VECTOR TO OVERCOME THE FREE WATER REQUIREMENT OF A MYCOHERBICIDE. J. A. Liebman and R. P. Wrubel, Department of Plant Pathology, 147 Hilgard Hall, University of California, Berkeley, CA 94720.

Most plant pathogenic fungi require free water for germination and infection. This limits the effectiveness of fungi as weed biocontrol agents. In greenhouse experiments, we investigated the use of the weevil Hypurus bertrandi to vector the fungus Dichotomophthora portulacae into the leaf tissue of common purslane (Portulaca oleracea). On plants sprayed with an aqueous suspension of the fungus, 2.1 ± 1.6% (mean ± sd) of leaves developed lesions. When plants were sprayed with the fungal suspension and then placed into moist chambers, 81.2 ± 1.8% of the leaves developed disease lesions. When weevils, reared for 48 hr on purslane plants infected with D. portulacae, were placed on uninfected plants, 47.2 ± 15.4% of the leaves developed lesions. Use of the weevil vector allowed us to obtain high levels of fungal infection without having to artificially raise humidity or provide free water.

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BIOLOGICAL CONTROL OF POSTHARVEST DISEASES OF PEARS WITH PSEUDOMONAS SYRINGAE PV. LACHRYMANS. W. Janisiewicz and L. Yourman. USDA, ARS, Appalachian Fruit Research Station, Kearneysville, WV.

Pseudomonas syringae pv. lachrymans (isolate K-1) was used to control grey-mold (incited by Botrytis cinerea) on wounded pears after harvest. The fruit were dipped for 2 min in a suspension containing conidia of the pathogen (1×10^4 conidia/ml) and various concentrations of the antagonist ranging from 1.1×10^8 to 5.4×10^8 CFU/ml. Half of the treated fruit was stored at 24 C for 6 days, the other half at 2 C for 30 days, after which the diameter of rots originating from the wounds was measured. The 2.2×10^8 CFU/ml of the antagonists under both storage conditions reduced lesion size and percentage of infection. The 5.4×10^8 CFU/ml of antagonist at 24 C prevented lesion development and at 2 C permitted only sporadic lesions. Preliminary results indicate similar control of blue-mold on pears incited by Penicillium expansum.

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BIO-PRIMING SEED TREATMENT FOR BIOLOGICAL CONTROL OF PYTHIUM ULTIMUM PREEMERGENCE DAMPING-OFF IN SH2 SWEET CORN. N. W. Callan, D. E. Mathre, and J. B. Miller, Western Agricultural Research Center, Montana State University, Corvallis, MT 59828; and Dept. Plant Pathology, MSU, Bozeman, MT 59717.

Sweet corn with the sh2 gene for enhanced sugar content is highly susceptible to Pythium ultimum preemergence damping-off. Corn rhizosphere bacteria which adhered to hyphae of P. ultimum and were antagonistic to the growth of this pathogen were isolated from Bitterroot Valley soils. An isolate of Pseudomonas fluorescens, AB254, when applied to seed provided significant protection from P. ultimum damping-off in naturally-infested cold to warm soils. At least $\log_{10}$ 7 cfu per seed of AB254 was needed to achieve maximum protection. In a process we have termed "bio-priming," dry seed was coated with AB254 and then allowed to imbibe moisture under warm temperatures until a 40% moisture content was achieved. Bio-priming provided equal or better protection against P. ultimum damping-off than did metalaxyl treatment when the seeds were planted into cold soil.

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EFFECT OF Lr GENE COMBINATIONS ON RESISTANCE TO WHEAT LEAF RUST. S. German and J.A. Kolmer, University of Manitoba and Agriculture Canada, Winnipeg, MB R3C 2M9, Canada.

Wheat lines isogenic for leaf rust resistance were crossed with TcLr34 to determine if the adult resistance gene Lr34 interacts with other Lr genes to condition enhanced resistance. An average of 300 F_2 seedings from each of two families of Lr34 x seedling genes LrB, Lr2c, Lr3ka, Lr11, Lr16, Lr17, Lr18, and Lr21 were inoculated sequentially with two cultures of leaf rust avirulent and virulent to the seedling gene in each cross. F_2 progenies from Lr34 x Lr2c segregated in a 3:1 ratio for resistance: susceptibility to the culture avirulent to Lr2c; and segregated in a 9:7 ratio for resistance to the culture virulent to Lr2c. A ratio of 9:3:4 was observed when segretation to both cultures was combined. Resistance gene Lr2c apparently interacted with Lr34 to condition enhanced resistance in the seedling stage. F_2 progenies from the other crosses did not display the same level of resistance as seedlings.

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PATHOGENICITY, VIRULENCE AND CORN INBRED REACTION TO ROOT ROT CAUSED BY BIPOLARIS ZEICOLA RACE 3. P. E. Lipps and L. E. Williams, Dept. of Plant Pathology, Ohio State Univ., Wooster, OH 44691.

Isolates of B. zeicola, obtained from corn roots, crowns and stalks were identified as race 3 by inoculation onto leaves of inbreds B73, Pr, and Pr1. Regression analysis indicated a significant ($P<.001$) linear relationship between the severity of root rot and concentration of conidia in sand or soil ($\log_{10}$ conidia/g) ($R^2=.46-.77$). Differences in virulence were detected among 11 single conidial isolates and mean root rot severity ratings ranged from 0.0 to 3.4 (LSD=0.9, $P=0.05$), based on a 0-5 scale, for individual isolates in three tests. Analysis of variance indicated a significant isolate by inbred interaction for five isolates tested on 10 inbreds. Inbreds listed in increasing order of susceptibility were: W64A, B73, Mo17, Oh51A, C103, A632, H100, Oh43, Wf9, and B14.

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EFFECT OF PLANT GROWTH STAGE AND TEMPERATURE ON PATHOGENICITY OF SEPTORIA NODORUM AND S. TRITICI. S.J. Wainshilbaum and P.E. Lipps, Dept. of Plant Pathology, Ohio State Univ., Wooster, OH 44691.

Pathogenicity of S. nodorum and S. tritici was tested on two wheat cultivars (AGRA GR855 and Caldwell) at two growth stages (GS 6 and GS 10.5) and three temperatures (19, 24 and 29 C). Disease severity (% leaf area affected) was highest for S. tritici at 19 C at both growth stages and for S. nodorum at 19 and 24 C at GS 6 and 29 C at GS 10.5. For both pathogens and cultivars, disease severity was greater at GS 10.5 than at GS 6. Percentage glume area affected was 6.8 times higher for S. nodorum than for S. tritici. Both fungi produced pycnidia on glumes after incubation in humidity chambers. Results indicate that S. nodorum caused more disease on heads than S. tritici and could infect at all temperatures tested while S. tritici was more pathogenic at the lowest temperatures and on leaves.

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SURVIVAL OF SEPTORIA NODORUM ON VOLUNTEER WHEAT IN NO-TILLAGE

SOYBEANS. Barry M. Cunfer and Juju B. Manandhar, Department of Plant Pathology, Georgia Station, University of Georgia, Griffin, GA 30223.

Following harvest of 'Hunter' wheat, soybeans were planted without tillage into the standing wheat stubble. After wheat volunteers emerged, the leaf area with Septoria nodorum colonization and the disease incidence were quantified by plating all leaves from randomly selected plants on Bannon's medium. Despite dry and hot weather during early and mid-summer, S. nodorum became established immediately after volunteers emerged. The wheat seed was not infected with S. nodorum. Therefore the inoculum source was the wheat stubble. Disease incidence increased from 45 to 78% and the percentage of diseased leaf tissue increased from 5 to 12% as the summer progressed. The absolute area of wheat leaf with S. nodorum increased 6-fold between July and September as the volunteer plants grew. Volunteer wheat in no-tillage systems is a reservoir for S. nodorum throughout the summer.

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SURVIVAL OF THE RICE BLAST PATHOGEN IN THE NILE DELTA OF EGYPT. A.P.K. Reddy and O.A. Bastawsi, Rice Research and Training Center, Sakha, Kafer El-Sheikh, Egypt.

No information is available on the mode of off-season survival of Pyricularia oryzae in the Nile Delta. Seed of the 1988 crop and straw of the 1987 crop (varieties Giza 171 and Giza 172) were sampled at random in 1988. Samples were incubated on a moist blotter for 48-72 hr and observed for sporulation of the fungus. Transmission tests from seed and straw of the 1987 crop were made in a glasshouse. P. oryzae was recovered from 67 of 127 seed lots and from 14 of 24 straw piles. The degree of seed infection within a lot varied from 1-11%. Transmission tests gave 0.5-2.5% diseased seedlings. The pathogen was also transmitted to seedling from infested straw of the 1987 crop. The results indicate the fungus readily overwinters in seed and infested straw. Methods to reduce primary inoculum could be highly effective for blast management under Egyptian conditions, as rice is grown in small, noncontiguous areas in a 3-year crop rotation.

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SURVIVAL OF PUCCINIA RECONDITA AND P. GRAMINIS UREDINIOSPORES IN THE ATMOSPHERE. M. G. Eversmeyer, C. L. Kramer and L. E. Browder, USDA-ARS, Dept. of Plant Pathology and Division of Biology, Kansas State University, Manhattan, KS 66506

Urediniospores of Puccinia recondita and P. graminis were exposed to the atmosphere to determine the survival rate under various environments throughout the year. Freshly collected spores of four cultures of both P. recondita and P. graminis were placed in 10 X 10 cm nylon hangers at 3 m above the ground at Plant Pathology plots near Manhattan, Kansas in 1987-89. Spores were collected daily from the hangers and assayed for germination on water agar. Significant differences in survival, as measured by germination percentage, were found between cultures and between species. As temperature decreased from July-August highs of 30-35 C, spore survival, measured as germination greater than 5%, decreased from 25-30 days to 3-5 days when December-January temperatures were below -10 C.

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ILLUSTRATIONS OF MYCOSPHAERELLA GRAMINICOLA AND SEPTORIA TRITICI. P.R.Scott, F.R.Sanderson, and P.W.Benedikz. CAB International, Wallingford, Oxon OX10 8DE, UK.

Photographs are presented of: (1) lesions on leaves from UK wheat crops with pycnidia and conidia of Septoria tritici; (2) asci and ascospores of Mycosphaerella graminicola from weathered leaves and leaf sheaths. Both conidia and ascospores germinate on agar to produce, by a budding process, colonies consisting mainly of conidia and short hyphae. These cultured conidia are not produced in pycnidia and are shorter, broader and more irregular than pycnidial conidia from leaves. When inoculated onto wheat, they produce normal symptoms, pycnidia and conidia of S. tritici, irrespective of whether they originated from ascocarps or pycnidia, thus proving the connection between perfect and imperfect states. Ascospores are found mainly in the winter and presumably contribute to the primary inoculum for epidemics on wheat crops.

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THE USE OF REPLACEMENT SERIES TO STUDY COMPETITION BETWEEN PYRENOPHORA TRICITI-REPENTIS AND SEPTORIA NODORUM IN THE WHEAT LEAF. S. R. Adee[1], W. F. Pfender[1], and D. C. Hartnett[2], Dept. of Plant Pathology[1] and Division of Biology[2], Kansas State University, Manhattan, KS 66506.

Replacement series experiments, adapted from plant ecological studies, were used to quantify substrate partitioning and interspecific competition between Pyrenophora tritici-repentis and Septoria nodorum. For these greenhouse experiments, spore suspensions of the pathogens were sprayed either separately or in combination (at various ratios) onto the leaves of wheat plants at anthesis. Plants were placed under moist conditions to initiate infection, then returned to the greenhouse. Following ripening of the wheat seed, the senescent top three leaves were removed and placed in moist chambers; after three weeks the number of fruit bodies was counted. The results indicate that S. nodorum is more sensitive to interspecific competition than is P. tritici-repentis.

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RHIZOCTONIA ROOT ROT OF BARLEY AFFECTED BY TIMING OF GLYPHOSATE APPLICATION. R.W. Smiley, W. Uddin and K.E.L. Rhinhart, Oregon St. Univ., Columbia Basin Agr. Res. Ctr., P.O. Box 370, Pendleton, OR 97801.

Timing intervals between glyphosate application and planting of no-till spring barley were evaluated. Volunteer grasses and cereals infected by Rhizoctonia solani and Pythium spp. were present when glyphosate (1.1 kg/ha) was applied to replicated 6 x 230m plots 21, 14, 7, 3, or 0.5 day prior to seeding, and 1 day after seeding. Barley height and weight, tillering, leaf and root numbers, and root rot index were evaluated 50 days after emergence. Height and grain yield were measured at harvest. Rhizoctonia root rot increased, and plant growth decreased, as the time interval between application and seeding decreased. Yield was reduced 0, 14, 19, 22, or 24% by applications of glyphosate 21, 14, 7, 3, or 0.5 day before seeding and by 29% 1 day after seeding.

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Cylindrocladium leaf spot of Strelitzia in Hawaii. M. Aragaki, P. S. Yahata, and J. Y. Uchida. Department of Plant Pathology, University of Hawaii, Honolulu, Hawaii 96822.

In December, 1987, a severe leaf spot of potted seedlings of Strelitzia nicolai (bird-of-paradise tree) was reported in a nursery in Hilo, Hawaii. A month later, a similar disease appeared on nursery seedlings of S. reginae (bird-of-paradise). Isolations from S. nicolai yielded Cylindrocladium theae (teleomorph = Calonectria theae), whereas C. pteridis was obtained from leaf spots of S. reginae. Isolates of C. theae from S. nicolai, Metrosideros collinus, and Howea forsterana were similar in pathogenicity to seedlings of S. nicolai, but less virulent than C. pteridis. On S. reginae seedlings, C. pteridis was also more virulent than all tested isolates of C. theae. An isolate of C. pteridis obtained from leaf spots of leather-leaf fern (Rumohra adiantiformis) was pathogenic to both S. reginae and S. nicolai, and similar in virulence to C. pteridis obtained from S. reginae.

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A BRIGHT-FIELD AND SEM STUDY OF ZOYSIAGRASS AND PAEDERIA SCANDENS INFECTED WITH PUCCINIA ZOYSIAE. M. M. Kulik and P. D. Dery. USDA-ARS, BELTSVILLE, MD 20705.

Puccinia zoysiae grows intercellularly through zoysiagrass leaf mesophyll, invading parenchyma, and forming haustoria. It does not enter vascular bundles or bulliform cells; consequently, it forms longitudinal lesions, usually adaxially. Echinulate urediniospores form with paraphyses soon after infection, and are rapidly exposed. Teliospores form many weeks later, first within uredinia, then in telia appearing on both leaf surfaces. They are two-called, persistent, and pedicellate. Inconspicuous spermogonia (pycnia) develop intercellularly within leaves of Paederia scandens, the alternate host. Aecia also form intercellularly and eventually erupt through the lower leaf epidermis. Aeciospores with pulvinulate ornamentation are produced in basipetal succession.

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DRESCHLERA CLADOPHYLL BLIGHT OF CHRISTMAS CACTUS. Robert D. Raabe, Department of Plant Pathology, University of California, Berkeley, CA 94720.

A fungus on the cladophylls of Christmas cactus (*Schlumbergera truncata*) in a California nursery was determined to be *Dreschlera cactivora* (*Helminthosporium cactivorum*) based upon a description of the fungus including conidial measurements and host range. In addition to a dark, soft rot on the cladophylls, infected cladophylls frequently dropped from the plants. Inoculations of Christmas cactus plants were made using conidia produced on pea straw natural media. Inoculations were not successful on uninjured surfaces but were when tissues were injured by pinpricking. The cultivar Maria was most susceptible, cultivar Annette was less susceptible and cultivars Rita and Majestic were the least susceptible. Although Christmas cactus previously was reported as being inconsistently infected when inoculated with *D. cactivora* under experimental conditions, this is believed to be the first report of the disease occurring naturally under greenhouse conditions.

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MODELING THE GROWTH AND FUSION OF LESIONS OF XANTHOMONAS CAMPESTRIS PV. DIEFFENBACHIAE ON ANTHURIUM. J.E. Yuen, Department of Plant Pathology, 3190 Maile Way, Honolulu, HI 96822.

Modeling the increase of diseased area on individual leaves requires information about the distribution of individual lesions and their respective growth rates. For bacterial blight of anthurium (caused by *Xanthomonas campestris* pv. *dieffenbachiae*), the increase of lesion radii was found to be a linear function of time, and rates in the greenhouse were approximately 1 mm/day following artificial inoculation. This information was integrated in a computer simulation model that divides the entire leaf surface into either diseased or healthy 'leaf elements'. Simulation runs with increasing numbers of lesions, placed either randomly on the leaf surface or randomly on the leaf margin produced disease area increase curves that were initially linear over time, but then showed decreasing disease increase rates as the amount of healthy leaf area became limiting. Modifications were made to the model to enable generation of disease assessment keys.

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EFFECT OF GENOTYPE MIXTURES ON THE DEVELOPMENT OF SORGHUM LEAF BLIGHT. J. A. Sifuentes, and R. A. Frederiksen, Department of Plant Pathology and Microbiology, Texas Agricultural Experiment Station, Texas A&M University, College Station 77843.

We conducted experiments to evaluate the effect of cultivar mixtures on the development of sorghum leaf blight caused by *Exserohilum turcicum*. Three locations with different disease potential were chosen. Seed of resistant and susceptible sorghum hybrids were mixed in 1:0, 1:3, 1:1, 3:1, and 0:1 ratios. We used a randomized complete block with 8 x 8 m plots in eight replications. Maize was planted around each plot. Diseased area of the leaf was measured for the top 5-6 leaves. We evaluated resistant and susceptible plants separately. Disease severity on the resistant hybrid was not affected by the mixtures. In contrast, there were significant differences in the amount of diseased tissue on the susceptible hybrid (P=.01). An addition of 25% resistant plants to the susceptible plant population significantly reduced disease development (P=.01). Susceptible plants had a nonsignificant tendency towards higher yield in mixtures.

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LEAF RUST POPULATIONS IN THE UNITED STATES IN 1987 AND 1988. David L. Long, A. P. Roelfs, B. D. Potter and M. E. Hughes, Cereal Rust Laboratory, USDA-ARS, Department of Plant Pathology, University of Minnesota, St. Paul, MN 55108.

Surveys from 1987 and 1988 of Puccinia recondita f. sp. tritici were analyzed to determine ecological areas in which specific virulence/avirulences were common. Race occurrences are often correlated with the cultivars grown, as well as other factors. Overwintering rust populations often outcompete exogenous populations. Different overwintering areas for races TBB, MBB and MBG were indicated by comparing virulence frequencies in both years. Lr24 virulence frequency was 10% or more both years in the potential overwintering areas of the southern and central Great Plains, where acreage of cultivars with Lr24 resistance has increased. An avirulence frequency to Lr2a greater than 50% occurred in the eastern USA and central Great Plains. Overwintering cultures in the east lack Lr2a virulence. Cultivars in the southern and central Great Plains lack Lr2a, however, Lr2a virulence is common. Some northern plains cultivars possess Lr2a but virulence is low.

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USTILAGO SCITAMINEA SORUS PRODUCTION AND TELIOSPORE DISSEMINATION AND DEPOSITION IN LOUISIANA. J. W. Hoy, M. P. Grisham, and C. P. Chao, Dept. Plant Pathol. and Crop Physiol., Ag. Exp. Sta., La. State Univ. Ag. Center, Baton Rouge, LA 70803, and USDA-ARS, Sugarcane Research Unit, Houma, LA 70361.

Production of smut sori, or whips, on infected sugarcane stalks in inoculated tests began during May, increased sharply during June and July, and continued at a lower rate through October. Whip production in resistant, moderately and highly susceptible clones was compared. Smut spore counts from spore traps indicated spore concentrations increased during May and were high from June - September. Spore concentrations per cubic meter of air decreased with distance and were less than 0.5% of levels above the crop at a distance of 135 m. A consistent diurnal pattern of spore dissemination was not detected. Spore numbers deposited at the base of plants decreased sharply with distance from an inoculum source. Heavy concentrations were deposited below and directly adjacent to plants containing smut whips.

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HOST SPECIALIZATION OF PYRENOPHORA TERES ISOLATES FROM HORDEUM MURINUM SSP. LEPORINUM. M. P. Brown and R. K. Webster. Department of Plant Pathology, University of California, Davis, CA 95616.

Hordeum murinum ssp. leporinum is the only known alternative host for Pyrenophora teres naturally occurring in California. Twenty-five isolates of P. teres from this wild relative of Hordeum vulgare exhibit resistant lesion types when inoculated onto a barley differential set in the greenhouse. These isolates also differ in virulence to the wild host, cultural characteristics, and mating compatibility with isolates of P. teres from barley. No morphological distinctions between the isolates of P. teres exists. This evidence for host specialization in P. teres suggests that the role of alternative hosts in the epidemiology of barley net blotch is greatly restricted in California.

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PHYSIOLOGICAL SPECIALIZATION OF LEAF RUST ON DURUM WHEAT. J. Huerta-Espino and A. P. Roelfs, USDA-ARS, Cereal Rust Laboratory, Department of Plant Pathology, University of Minnesota, St. Paul, MN 55108.

The North American differentials were of limited value in race studies of Puccinia recondita Rob. ex Desm. f. sp. tritici collected from certain populations of Triticum turgidum L. (durum wheat). To distinguish among isolates avirulent on the Thatcher near isogenic lines, other wheats were evaluated. Selected durum wheats were evaluated using 30 single pustule isolates representing the leaf rust populations on durum wheats in Ethiopia, Burundi, Italy, Turkey, Tanzania, Bolivia and Mexico. Glossy Hugenot, DZ04-118, Maruccos 9623, Kunduru, Berkman and Local Red were susceptible to all isolates. Those with a differential response were: Mindum, Rojal de Almeria, Boohai, ELS 6404-145-2, Gaza, Haig Mouiline, Qued Zenati 2909, Peliss and Pentad. Local Red and Berkman were susceptible to the six isolates of leaf rust from bread wheat (T. aestivum L.), while others responded differentially or were resistant.

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COMPUTER-ASSISTED WHITE MOLD MANAGEMENT ON SNAPBEANS USING A PREDICTIVE MODEL. Jana Stewart and W. R. Stevenson, Dept. of Plant Pathology, University of Wisconsin-Madison, WI 53706.

Two computerized models were developed to predict white mold incidence on snapbeans based on cultural, environmental parameters and field history. A database derived from 171 fields planted to beans in Wisconsin during the period 1984-1987 was analyzed using stepwise linear regression of continuous and indicator variables. Derived models predict white mold incidence based on field disease and cropping history, irrigation frequency, row width, evapotranspiration, heat units, canopy density, rainfall/irrigation and stand density. One model, based on data collected during the 7 days prior to 10% bloom, has an R^2 value of 27.4 ($P<.0005$) while the other, based on 7 days after 10% bloom, has an R^2 value of 35.1 ($P<.0005$). The models could be used to select fields with low white mold potential and/or determine the necessity of fungicide applications during critical periods.

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APPLICATIONS OF A NEW MESOSCALE WEATHER FORECASTING TECHNIQUE TO PLANT DISEASE PREDICTION. M. H. Royer, USDA-ARS, Ft. Detrick, Bldg. 1301, Frederick, MD 21701; J. M. Russo, ZedX, Inc., P.O. Box 404, Boalsburg, PA 16827; and J. G. W. Kelley, Dept. of Entomology, Penn State Univ., Univ. Park, PA 16802

A new weather forecasting technique, Model Output Enhancement (MOE), was used to create high resolution mesoscale potato late blight disease forecasts, 24 hr in advance, for over 215,000 1 km^2 "blocks" throughout Pennsylvania. The Wallin criteria were used as a basis to approximate blight-favorable weather. The MOE used numerical output from the National Meteorological Center's Nested Grid Model (NGM), which is the United States' operational numerical synoptic weather forecast model. The NGM output was interpolated to approximately 1 km resolution, extrapolated to the surface using theoretical and observed atmospheric processes, and adjusted with digital terrain data. Planar and three-dimensional maps were created to depict likely areas where infection by the late-blight fungus may occur, 24 hr in advance.

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IMPROVED METHODS FOR STUDYING RAINDROP IMPACT AND SPLASH DISPERSAL. X. Yang, L. V. Madden, R. D. Fox*, D. L. Reichard*, and M. A. Ellis, Dept. of Plant Pathology, Ohio State Univ., and USDA/ARS*, Wooster, OH 44691.

A new drop-generating and videographic system was developed to study the processes of raindrop impact and splash dispersal. Uniform drops, 0.2-4 mm diameter, are produced with either a piezoelectric crystal or miniature metering pump. Drops can be released at heights up to 2.4 m and the angle between the impact surface and drop trajectory (vertical) can be adjusted. High-speed strobe lamps are used to backlight drops and provide very short exposure times (1 μs) for images. Eighty-eight images are captured per video field. Therefore, drop trajectory before impaction and after rebound can be captured on several successive frames. Individual images can be stored on hard or floppy disks, displayed on a high-resolution monitor, and analyzed by specialized software that can calculate size, number, position, and velocity of drops or droplets.

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SPATIAL AND TEMPORAL DISTRIBUTION OF SUDDEN DEATH SYNDROME OF SOYBEANS. J.C. Rupe, E.E. Gbur, and D.B. Marx. University of Arkansas, Fayetteville, AR 72701.

The spatial and temporal distribution of the soilborne disease, sudden death syndrome of soybean (SDS), was determined in a 22 X 122 m field for 3 years. Disease severity was assessed on a susceptible cultivar, Lee 74, planted in a uniform pattern throughout the field. Assessments were made weekly from the end of July through Sept. in 1985, 1986 and 1987, using a 0 to 5 scale with 0=no SDS and 5$\geq$ 90% of the foliage with symptoms. Disease pressure was analyzed with the geostatistical technique krieging to construct a contour map of the entire field from the SDS ratings of the Lee 74. Disease pressure was found to be spatially correlated and non-uniformly distributed across the field. These krieged values were used as a covariate in the analysis of the response of other cultivars to SDS, thus adjusting for non-uniform disease distribution.

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ROLE OF IRRIGATION, RAINFALL, AND INITIAL INOCULUM DENSITY IN THE DEVELOPMENT OF PHYTOPHTHORA ROOT AND CROWN ROT EPIDEMICS AND YIELD IN BELL PEPPER. J. B. Ristaino, Department of Plant Pathology, North Carolina State University, Raleigh, NC 27695.

Peppers were grown in two fields in which mainplots were drip irrigated 3 times/wk, weekly, or not irrigated. Subplots were either not infested or infested with graded levels of inoculum (1X, .1X, .01X) of Phytophthora capsici. When rainfall was moderate during disease development (6.5 in), disease onset occurred earlier (26 DAI) and at a faster rate in plots that were irrigated 3 times/wk than weekly or nonirrigated plots (39 DAI). At high inoculum densities (11 cfu/gm), disease reduced yield by 43, 21 and 37 percent in plots irrigated 3 times/wk, weekly, and nonirrigated plots. At the second site, where heavy rainfalls occurred (12 in), disease progressed rapidly and onset was earlier (7 DAI) than at the other site. Yields were reduced at all levels of initial inoculum and the irrigation effect was not significant. Disease incidence at harvest was negatively correlated with yield at both sites.

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NATIVE VEGETATION AS A SOURCE OF PHYTOPHTHORA SPP. IN RIVERS USED FOR IRRIGATION, S. L. von Broembsen, Dept. of Plant Pathology, Oklahoma State Univ., Stillwater, OK 74078-9947.

Rivers draining native forest and fynbos shrublands in the southern coastal regions of the Cape Province, South Africa, were sampled for Phytophthora spp. Phytophthora cryptogea, P. cinnamomi, P. citricola, and P. drechsleri were recovered from both the headwaters and lower reaches of these rivers. Zoospores were the most frequently recovered propagules and P. cryptogea was the most frequently isolated species. Levels of Phytophthora spp. in a small stream from a closed fynbos mountain catchment were monitored every other wk for two years. Low levels of propagules (0-20/L) were found in winter to mid spring (May-September), whereas 50-350 propagules/L were recovered during summer and autumn (November-April). Thus, the highest levels of Phytophthora spp. appear in headwaters of rivers during the period when these rivers are used for irrigation.

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VALIDATION OF A WEATHER-BASED FORECASTER FOR BLACK SIGATOKA OF BANANA. Wayne M. Thal and Harvey W. Spurr, Jr. USDA-ARS Crops Research Laboratory, Oxford, NC 27565 and Dept. Plant Pathology, North Carolina State University, Raleigh 27695; Hans P. Sauter and Teresa Arroyo, Banana Development Corporation of Costa Rica, San Jose.

Three weather-based forecast systems were developed to schedule fungicide applications for control of black Sigatoka on banana. These systems use hourly temperature, rainfall and relative humidity to predict infection periods. Small plot trials are being conducted at a banana plantation in Costa Rica to test the forecast systems. Fungicide applications were reduced by 2 to 3 during a 5 month period compared to a calendar spray treatment without a significant change in disease levels ($p=0.05$). One system will be selected for a large-scale test during 1989.

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DISPERSION CHARACTERISTICS OF A WHITEFLY-TRANSMITTED GEMINIVIRUS IN THE FIELD. M.R. Nelson[1] and L.J. Stowell[2], [1]Dept. of Plant Pathology, University of Arizona, Tucson, AZ, 85721, and [2]PACE Consulting, 1267 Diamond St., San Diego, CA, 92109.

Cotton leaf crumple is incited by a whitefly-transmitted geminivirus that produces very distinctive symptoms in cotton. Epidemics can be initiated easily in isolated plantings for dispersion studies. Data analyzed is from an isolated field planted in midseason to optimize an epidemic for analysis. The first 580 cotton plants in each of 10 rows were evaluated in order of position within each row. Expression of cotton leaf crumple symptoms was recorded for each plant. Statistical analysis of diseased and healthy plant distribution was carried out by breaking each row into 10 sectors of 58 plants per sector. Aggregation within each row was evaluated using variance:mean ratio, Lloyd's indices of mean crowding and mean patchiness, the k parameter of the negative binomial distribution, and ordinary runs anlysis. The variance:mean ratio averaged 4.1 with a range of 1.1 (random) to 5.1 (aggregated). Mean crowding averaged 14.7 with a range between 9.4-19.7. Mean patchiness averaged 1.3 (1.3 times more crowded than expected for a random distribution) and ranged from 1.0 (random) to 1.6 (aggregated). The k parameter averaged 3.7 and ranged from 1.6 (aggregated) to 74.9 (random). In all rows, the z score of ordinary runs analysis indicated plant-to-plant spread with a range from -5.2 to -11.6. These data indicate that cotton leaf crumple can spread from plant to plant but overall distribution in the field may range from random to aggregated.

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DELINEATION OF CLONES OF *HETEROBASIDION ANNOSUM* IN A RED PINE-WHITE PINE STAND. D.M. Rizzo and T.C. Harrington, Dept. of Botany and Plant Pathology, Univ. of New Hampshire, Durham, 03824.

Heterokaryotic isolates and single basidiospore strains of the P-type (pine-type) of *Heterobasidion annosum* were collected from a 60 X 60 m plot in a diseased *Pinus resinosa-P. strobus* stand in Durham, NH. Heterokaryons were isolated from decay in 24 trees. Isolates were paired on Hagem's medium, malt extract agar (MEA) and MEA with white pine twigs to test for vegetative compatibility. Only the twig medium was useful for differentiating clones; a sparse zone formed between heterokaryons of differing genotype. Thirteen clones were identified from 26 heterokaryotic isolates; the largest clone was approx. 10 m in diameter and colonized nine trees. Two single-basidiospore strains (representing the two mating-type alleles) were collected from each of 30 basidiocarps (from 25 trees) and paired in all combinations. Twenty-four alleles were identified, suggesting 12 clones. These clones were generally consistent with the clones identified by vegetative compatibility. Electrophoretic markers of four isozymes supported the delineation of clones based on vegetative and sexual compatibility.

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INFECTION OF LIVINGSTON PARISH, LOUISIANA, LOBLOLLY PINE PROGENY BY SPORE COMPOSITES OF CRONARTIUM QUERCUUM F. SP. FUSIFORME. C. H. Walkinshaw, and R. L. Anderson. USDA, Forest Service, Southern Forest Experiment Station, Gulfport, MS 39505, and Resistance Screening Center, Asheville, NC.

Loblolly pines (Pinus taeda L.) from Livingston Parish, LA, grow well in the deep south and have valuable resistance to fusiform rust. We have observed tests in which 85% of bulk loblolly pines are infected, but Livingston Parish progeny have only 40% infection. To evaluate the ability of field isolates to infect Livingston Parish progeny, we examined performance over many years. Over this time, using 30-gall spore composites from 20 locations form North Carolina to Texas, Livingston Parish progeny averaged 50% infection while susceptible families had 80 to 100% rust. The majority of Livingston Parish infected seedlings had galls that were thin with rough bark and resistance zones in the inner cortex. These symptoms are associated with resistance.

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OZONE CONCENTRATIONS IN REMOTE FORESTED AREAS OF NORTHCENTRAL PENNSYLVANIA. M. Simini, J. M. Skelly, and D. D. Davis, Dept. of Plant Pathology, The Pennsylvania State University, University Park, PA 16802.

The atmospheric ozone (O_3) concentration was monitored at three forested areas on the Allegheny Plateau of northcentral Pennsylvania during the 1988 growing season using a TECO Model 49 UV photometric analyzer. The test sites were located in Clear Creek State Park, Jefferson Co. (lat. 41°19'39", long. 79°02'35"), Elliott State Park, Clearfield Co. (lat. 41°07'02", long. 78°31'40") and Tiadaghton, Lycoming Co. (lat. 41°20'05", long. 77°26'57"). A composite of the number of hours that the level of O_3 exceeded 60, 80, 100, and 120 ppb for June, July, and August demonstrated the lowest O_3 concentrations were recorded at the Tiadaghton site. The NAAQS of 120 ppbh^{-1}2x was exceeded for 14, 22, and 6h at the Clear Creek, Elliott, and Tiadaghton sites, respectively. Peak 1-h O_3 at the three sites was 152, 136, and 142 ppb, respectively.

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DAMAGE TO RESIDUAL TREES FROM A MECHANIZED HARVEST DURING WINTER AND SUMMER IN A NORTHERN HARDWOOD STAND. M. T. Hennessey, W. D. Ostrofsky, and R. C. Lemin, Jr., Cooperative Forestry Research Unit, College of Forest Resources, Univ. Maine, Orono 04469.

A sugar maple-beech-yellow birch stand in northeastern Maine was mechanically thinned in 1988 using a long-reach boom feller-buncher. Damage to residual trees from thinning during January and February was compared with damage caused during July and August. A total of 667 stems were evaluated along transect lines representing each respective season. Results indicate that significantly more (P=0.09) stems were wounded during the summer (34%) than during the winter (17%). Season of harvest appears to be as critical in determining stand damage in mechanical as in conventional harvests. In addition, 1720 stems were evaluated in fixed plots established in areas harvested in summer. Data from the fixed plots were used to develop a model which relates species, crown class, diameter at breast height, and distance from skid trail, to the probability of an individual stem being injured.

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SEASONAL VARIATION IN THE DISPERSAL AND PATHOGENICITY OF SPHAEROPSIS SAPINEA IN SOUTH AFRICA. W.J. Swart and M.J. Wingfield, Departments of Plant Pathology and Microbiology, University of the Orange Free State, Bloemfontein 9300, South Africa.

Infection of Pinus spp. by Sphaeropsis sapinea through pruning or hail wounds results in cankers and blue stain of timber. Studies were undertaken to determine the time of year when S. sapinia conidia are dispersed and woody pine tissue is most susceptible to infection and colonization by this pathogen. Conidia were trapped on microscope slides coated with petroleum jelly. Trapping was conducted over a period of two years in summer, winter, and whole-year rainfall regions of South Africa. Although conidial dispersal was strongly related to the occurrence of rainfall, maximum conidial dispersal was more closely related to increased temperature than to maximum rainfall. Pinus radiata trees were artificially inoculated during autumn and spring over a period of two years. Six months after inoculations, cambial lesions were significantly longer on trees inoculated in spring than on those inoculated in autumn. Results of these studies show that season must be taken into account when pruning trees, testing pathogenicity or screening for disease resistance.

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FUNGI ASSOCIATED WITH DAMPING-OFF OF SLASH PINE SEEDLINGS. J.W. Huang and E.G. Kuhlman. Univ. of Georgia and USDA For. Serv., SEFES, Athens, GA 30602.

Thirty-five isolates from 12 taxa of fungi were tested for pathogenicity to slash pine seedlings. Isolates of Fusarium moniliforme var. moniliforme, F. oxysporum, F. fusarioides, F. solani, Alternaria alternata, Rhizoctonia solani (AG-4), Rhizoctonia-like binucleate fungi (RLBF) (CAG-3), Pythium aphanidermatum, Penicillium expansum and Cladosporium cladosporioides caused pre-emergence damping-off. Isolates of R. solani, RLBF and P. aphanidermatum also caused significant amounts of post-emergence damping-off. Three varieties of F. moniliforme caused cotyledonary infection; of these, F. m. subglutinans showed the highest virulence. F. m. moniliforme and F. m. intermedium needed higher temperatures to cause cotyledonary infection. A baiting technique using slash pine stem segments provided a rapid, sensitive and accurate means of assessing inoculum potential of populations of R. solani and RLBF in forest nursery soils.

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PHYSIOLOGICAL AND STRUCTURAL CHARACTERISTICS OF THE BROWN-ROT FUNGUS POSTIA PLACENTA. J. A. Micales, F. Green III, M. J. Larsen, and T. L. Highley. U.S. Forest Service, Forest Products Laboratory, Madison, WI, 53705.

An aberrant, monokaryotic strain of the brown-rot fungus Postia placenta, ME20, was compared with other mono- and dikaryons. Strain ME20 produced insignificant weight losses in wood and was thus used to elucidate the mechanisms of decay by brown-rot fungi. This strain was capable of producing H_2O_2, oxalic acid and the carbohydrate-degrading enzymes normally associated with decay. It failed to form ethanol-precipitable carbohydrates in liquid culture and produced an atypical hyphal sheath in wood as observed by scanning electron microscopy. This study provides additional evidence for the importance of carbohydrate metabolism and the hyphal sheath in the wood-decay process.

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ASSOCIATION OF ROOT FEEDING INSECTS WITH PROCERUM ROOT DISEASE IN CHRISTMAS TREE PLANTATIONS. R. J. Nevill and S. A. Alexander. Dept. Plant Path., Phys & Weed Sci. VPI & SU, Blacksburg

Procerum root disease (PRD), caused by Leptographium procerum, is responsible for significant economic losses in eastern white pine Christmas tree plantations. Insect split bolt traps were placed in 10 plantations, for 24 weeks starting in May 1988. Seven of the plantations had symptoms of PRD and three were asymptomatic. In asymptomatic plantations, traps were placed randomly, and in PRD plantations, traps were placed in symptomatic and asymptomatic areas. Over 200 bark beetles of various species and 300 weevils, either Hylobius pales or Pissodes nemorensis, were recovered. Bark beetles rarely carried the pathogen. Root weevils carrying L. procerum were H. pales (65%) and P. nemorensis (15%). The total number of weevils and weevils carrying L. procerum were highest in the symptomatic areas of the PRD plantations. These findings support the evidence that insect vectors are the primary means of dissemination of L. procerum in eastern white pine Christmas tree plantations.

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PATHOGENICITY AND HOST RANGE OF STRAINS OF Hypoxylon mammatum. G. Bucher and D. W. French, Department of Plant Pathology, University of Minnesota, St. Paul, MN 55108.

Hypoxylon mammatum (Wahl.) Mill. causes an economically important canker disease on trembling aspen (Populus tremuloides Michx) and also is found on other hosts as a parasite or a saprophyte. Isolates of the fungus from Salix spp. were compared with a pathogenic isolate from P. tremuloides. The isolates were grown on oat grain and used to inoculate Populus and Salix spp. One Salix isolate was found to be as pathogenic as the P. tremuloides isolate on the Populus spp. Other isolates from Salix have caused cankers on Populus spp., but these cankers were significantly smaller than the cankers caused by the P. tremuloides isolate. The P. tremuloides isolate was not able to cause cankers on the five Salix spp. tested. A saprophytic isolate from Salix was nonpathogenic on Populus spp.

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SEASONAL SPORE LIBERATION AND CANKER DEVELOPMENT BY BOTRYOSPHAERIA STEVENSII ON JUNIPERUS SPECIES. N. A. Tisserat, A. Nus, and J. C. Pair. Depts. of Plant Pathology and Horticulture, Kansas State University, Manhattan, KS 66506.

Liberation of Botryosphaeria stevensii macroconidia from cankers on Juniperus scopulorum was monitored from April through October in 1987 and 1988 with a Kramer-Collins spore trap. Peak spore release occurred during a 3-wk period between late May and early June. Few conidia were detected before or after this period. In December 1986, and May and August 1987, branches on one to three trees each of 31 cultivars, representing four Juniperus sp., were inoculated with B. stevensii. Branches inoculated in December did not develop cankers, whereas 61% and 100% of inoculations made in May and August, respectively, resulted in girdling branch cankers within 4 mo. In laboratory experiments, macroconidia germinated in 4-6 hr at 25-30 C. These results suggest that spore liberation, germination, and infection occur in late spring or summer.

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FUNGI ASSOCIATED WITH PINE SEEDLING MORTALITY ON CONVERTED AGRICULTURAL SITES. G.B. Runion, R.J. Mitchell and W.D. Kelley. School of Forestry, Auburn University, AL 36849.

To determine potential causes of pine mortality on converted agricultural sites, roots of loblolly pine seedlings removed from five such sites on three sampling dates were rinsed in 0.525 % NaOCl, sectioned, and incubated on moistened filter paper in petri dishes. Emerging fungi were established as pure cultures on potato dextrose agar before being identified. Fungi representing 25 genera were isolated from 712 seedlings examined. Fusarium spp. were isolated from 63% of the seedlings and accounted for 61% of all fungi identified. Fusarium subglutinans accounted for 31% of all fungi isolated and was isolated from 55 - 60% of seedlings from four of the test sites; it occurred on only 7% of seedlings from the other site. Macrophomina phaseolina was recovered from twice as many trees on Site 4 (42% survival) compared to the other sites (85 - 95% survival). Most of the remaining fungi were considered to be saprophytes.

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PARTIAL PURIFICATION OF A METABOLITE OF *ENDOTHIA PARASITICA* WHICH INDUCES ETHYLENE PRODUCTION IN CHESTNUT AND SCARLET OAK. F.V. Hebard and L. Shain. University of Kentucky, Lexington, KY 40546.

Filtrates from cultures of *E. parasitica* on chestnut bark broth stimulated ethylene production by bark plugs of American and Chinese chestnut and scarlet oak, in comparison to fungus-free broth. There were no differences between species in degree of stimulation. The metabolite(s) passed thru ultrafilters with 5000 MW cutoff, and was partially retained by 1000 and 500 MW filters. It was stable to lyophilization. The metabolite bound to QAE Sephadex Q-25 at pHs above 5.0, but did not bind to DEAE Sephadex A-25 between pH 3 and 9. Three peaks of activity were eluted from a QAE Sephadex column by a gradient from pH 5 to 4. The three peaks eluted at pHs 4.6, 4.4 and 4.2, indicating the presence of a carboxyl group. The metabolite did not react with ferric chloride nor absorb strongly at 280 nm, indicating that it is not a phenol. Oxalate did not stimulate ethylene production in bark plugs. This metabolite, if further purified, may be useful for eliciting blight resistance responses in Chinese chestnut. Identification may lead to further understanding of the mechanism of pathogenesis of *E. parasitica*.

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A TISSUE CULTURE SYSTEM FOR STUDYING DISEASE RESISTANCE TO PHYTOPHTHORA CINNAMOMI. J. C. Jang and F. H. Tainter, Clemson University, Clemson, SC 29634-1003.

Calli derived from embryos of Pinus taeda, P. echinata, P. taeda x P. echinata, and P. virginiana were screened for their resistance to Phytophthora cinnamomi Rands, the littleleaf disease pathogen. The optimum culture medium with 10^{-5} M, 2,4-D at 22°C gave best growth of calli and caused the least synergistic reaction between fungus and callus tissue. Three methods were used for evaluating the in vitro resistance reaction: (1) diameter growth of P. cinnamomi on the callus surface; (2) amount of intracellular hyphae and cytological change of infected callus cells; and (3) surface reaction of inoculated calli. Loblolly pine was the most resistant species and shortleaf pine the most susceptible. Resistance reactions in vivo were always correlated with a lesser growth rate of P. cinnamomi on callus, fewer intracellular hyphae, and necrosis and accumulation of phenolic compounds in callus cells.

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DEVELOPMENT OF IN VITRO AND IN VIVO SCREENING TECHNIQUES TO DETECT LITTLELEAF DISEASE RESISTANCE. J. C. Jang and F. H. Tainter, Clemson University, Clemson, SC 29634-1003.

Two-month-old sand cultured pine seedlings were inoculated with two, five, and ten zoospores/chlamydospores of Phytophthora cinnamomi Rands to determine in vivo susceptibility. Susceptibility increased with increasing dosage of both zoospores and chlamydospores for all the species tested. In order from most to least susceptible these were Pinus echinata, P. virginiana, P. taeda, and finally the P. taeda x P. echinata hybrid. Infection rates from chlamydospore inoculation were consistently higher than for zoospore inoculation in all species, indicating that the former have greater inoculum potential. In vitro inoculation was done the same as above except that the plantlets were derived from embryonic cotyledons through organogenesis in a tissue culture system. In general, the in vitro inoculation results correlated well with the in vivo results, indicating that this screening method may be suitable for incorporation into a traditional breeding program for littleleaf disease resistance.

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MLO INFECTION AND GROWTH DECLINE OF WHITE ASH IN RELATION TO STAND CHARACTERISTICS. T.C. Wigginton, P.J. Smallidge, Y. Han, J.D. Castello AND D.J. Leopold, SUNY Coll. of Env. Sci. & Forestry, Syracuse, NY 13210.

White ash (Fraxinus americana L.) trees in 50 plots in the northeastern U.S. were indexed for ash yellows MLO by DAPI to document the effects of MLO on ash growth. Two increment cores per tree were extracted from three or more white ash per plot, measured and used to calculate basal area increment. Stand composition and structure were analyzed. A recent decline occurred in basal area increment of infected trees, while the uninfected trees did not decline. MLO infection occurred in all ash age, size, and crown classes. There is no relationship between MLO infection and white ash basal area and density. Disease and broom formation are positively associated with exposed stands.

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EFFECT OF THIRAM SEED TREATMENT ON EMERGENCE OF SOUTHERN PINES. W.D. Kelley, G.B. Runion and D.H. Land. School of Forestry, Auburn University, AL 36849.

Effect of thiram (Gustafson 42S) seed treatment on seedling emergence of longleaf, loblolly, and slash pines was determined at four concentrations (0, 41.7, 83.4 and 166.8 ml/kg seed) with and without a latex sticker (6.5 ml/kg seed). For each pine species, fifty seed/treatment were placed on natural nursery soil in each of four plastic window boxes (33 x 13 x 11 cm) and covered with vermiculite. Boxes were maintained in a glasshouse and emergence counts were recorded daily for 5 wk. The test was repeated twice. Significant differences observed among runs most likely were due to temperature differences in the glasshouse. The latex sticker had virtually no effect on emergence. The highest concentration of thiram resulted in decreased emergence of longleaf and in increased emergence of loblolly, compared to other concentrations. For slash, greatest seedling emergence was recorded at the two lower rates of thiram.

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WATER LOCALIZATION PATTERNS IN LOBLOLLY PINES AS STUDIED BY MAGNETIC RESONANCE MICROSCOPY. J. S.

MacFall and G. A. Johnson, School of Forestry and Env. Stud., Dept. of Radiol., Duke Univ., Durham, NC 27710.

The technique of proton magnetic resonance microscopy was used to study water uptake patterns by roots of loblolly pine (Pinus taeda L.) transplanted into sand. Signal intensity in the image of the sand increased with water content. Water depletion zones were observed to first form around the woody taproot (within 5 hrs of transplanting), followed by zones around mycorrhizal short roots and lateral roots. These observations show the woody taproot is one of the sites of water inflow into the plant. Bands of high signal intensity could be seen within the root tissue, with distinct patterns in roots of different ages. Mycorrhizal short roots consistently appeared bright, suggesting the presence of tightly bound water. These results show the potential usefulness of this technique in the study of plant water relations.

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INTEGRATED CONTROL OF WHEAT RUSTS IN NORTHWESTERN UNITED STATES. R.F. Line, Agricultural Research Service, U.S. Department of Agriculture, Pullman, WA 99164-6430

Rusts frequently reduce annual wheat yields in the Pacific Northwest by more than 20%. Rust control guidelines for the region are based on predicting epidemics and potential losses and on utilizing resistance, fungicides, and crop management to reduce losses. Factors considered in developing the guidelines were type of rust (stripe, leaf, and/or stem rust); affect of environment and of regional and individual farm management practices on establishment, survival, and development of the rusts; prevalence and distribution of races and vulnerability (susceptibility) of cultivars to the races; kind and degree of resistance to the rusts, such as slow rusting, adult-plant, temperature sensitive, and race specific resistance; effectiveness of fungicides when applied at various rates and schedules; potential crop yield; and economic losses or benefits of using alternative control systems.

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FIELD TESTS FOR EFFICACY OF HERBICIDES TO SUPPRESS ASCOCARP PRODUCTION BY PYRENOPHORA TRITICI-REPENTIS ON WHEAT STRAW. W. F. Pfender, U. Sharma, P. Bhatt, A. Nus, and E. Adee. Dept. of Plant Pathology, Kansas State University, Manhattan, KS 66506.

Glyphosate herbicide (Roundup), previously found to suppress ascocarp production by Pyrenophora tritici-repentis in naturally-infested wheat straw under controlled conditions, was ineffective in field tests. Further experiments suggest that inadequate coverage, and loss of the herbicide from treated straw by leaching and/or microbial degradation, are responsible for the failure. A different herbicide, monocarbamide dihydrogensulfate (Enquik), suppressed ascocarp production by Pyrenophora on infested straw in laboratory and field tests. One or two applications to infested residue in the field reduced ascocarp production by 32% and 62%, respectively. Either herbicide, but particularly Enquik, could delay saprophytic activity of Pyrenophora in reduced-tillage residue, and thus permit more effective biocontrol by an added antagonist in an integrated control strategy.

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SPORE DOT ELISA FOR THE DETECTION OF VENTURIA INAEQUALIS ASCOSPORES. J. A. Bergdahl, L. P. Berkett, and A. R. Gotlieb, Department of Plant and Soil Science, University of Vermont, Burlington, VT 05405.

Effective management of Venturia inaequalis, causal agent of apple scab, is based on multiple applications of fungicide once mature ascospores are found in the orchard. Presently, there is no way to predict initial ascospore maturation in specific orchards without equipment and information not easily available to individual growers. The benefit of accurate ascospore maturity information would be to better time fungicide applications and thereby decrease applications. We have developed an enzyme-linked immunosorbent assay (ELISA) using nitrocellulose membrane strips as the solid phase to detect ascospores. This indirect ELISA modification was termed Spore Dot ELISA. The ascospores were applied to the nitrocellulose membrane in water suspension or collected directly onto the membrane from a spore tower. A dot of red-violet precipitate indicates a positive test for individual ascospores.

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EFFECTS OF FIVE GLOMUS SPP. ON TISSUE-CULTURED ASPARAGUS. C.T. Pedersen, G.R. Safir, *S. Parent and *M. Caron. Michigan State Univ., East Lansing, MI 48824 and *Premier Peat Research Center, Riviere-du-Loup, Quebec G5R 4C9, Canada.

Five species of VA mycorrhizae (VAM) were evaluated for their effects on growth of tissue-cultured asparagus. Plants were grown in a peat-based inoculum provided by Premier Peat Moss Ltd., and then transferred either to the field or to the greenhouse. Glomus clarum, G. intraradices, G. monosporum, and G. vesiculiferum increased total dry weight by approximately 200-300% in the field. G. versiforme reduced the number of buds per plant, but had no effect on plant dry weight in the field. Plant dry weight was correlated with the number of buds per crown both in the field and greenhouse, however, fungal effects on plant growth in the greenhouse were not reliable indicators of field growth. Our results demonstrate that growth of tissue-cultured asparagus can be increased or reduced by VAM depending on the fungal species used for inoculation.

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DISTRIBUTION OF VESICULAR ARBUSCULAR MYCORRHIZAL FUNGI IN SOILS OF PERNAMBUCO (BRASIL). L. C. Maia, and S. F. B. Trufem, Universidade Federal de Pernambuco, and Instituto de Botanica de Sao Paulo.

The native VAM fungi present in six soil types grown to various crops in the State of Pernambuco were surveyed. The VAM spores were separated from soil and root samples and identified. The roots were examined for hyphae, vesicles and/or arbuscules, and percentage colonization was assessed. Twenty five species of VAM belonging to the genera Acaulospora, Entrophospora, Gigaspora, Glomus, Sclerocystis and Scutellospora were found present. In general, the different genera were associated with a specific soil pH and P level. However, Acaulospora was found in soils of widely different soil pH and P levels which indicates it is more widespread in nature. Species composition, total number of spores, and root colonization varied with soil type and with the host.

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A REVISION OF THE GENUS *SCLEROCYSTIS* BERK. & BROOME. Rogerio T. Almeida and N. C. Schenck. Plant Pathology Dept., University of Florida, Gainesville, FL 32611

The genus *Sclerocystis* is considered by some authors as not taxonomically different from the genus *Glomus* Tul. & Tul. Since the establishment of *Sclerocystis* by Berkeley and Broome in 1873 as a result of their description of *S. coremioides,* 10 additional species have been described. Based on spore ontogeny and sporocarp habit, we concluded that the genus *Sclerocystis* is distinct from *Glomus* and must be maintained with one species, *S. coremioides,* with *S. coccogena* and *S. dussii* synonymous to *S. coremioides*. We consider *S. pakistanica, S. microcarpus, S. indicus* and *S. pachycaulis* synonymous with previously described *Sclerocystis* species. The species *S. rubiformis, S. sinuosa and S. clavispora* are treated as *Glomus* species. The latter two species have a unique sporocarp morphology with elongate chlamydospores arranged side by side in a single layer around a central plexus of tightly interwoven hyphae which differs from other *Glomus* species. With further study, these species may justify their placement in a separate genus.

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SCREENING OF VESICULAR-ARBUSCULAR (VA) MYCORRHIZAL FUNGI FOR TOLERANCE IN A HIGH ALUMINUM ACID SOIL. H. T. Bartolome, Plant Pathology Department, University of Florida, Gainesville, FL 32611.

Selected species of VA mycorrhizal fungi were evaluated for tolerance in an extremely acid, Al-toxic Pacolet sandy clay loam (pH 4.2, 308 ppm Al). Gigaspora and Scutellospora species had the highest spore germination and the most extensive hyphal growth. These include Gigaspora gigantea, G. margarita, Scutellospora calospora, S. heterogama, and S. pellucida. Most Acaulospora and Entrophospora species were found sensitive. Of eight isolates from these genera tested, only Acaulospora scrobiculata showed some degree of tolerance. All six isolates of Glomus mosseae and G. etunicatum failed to germinate which supports previous hypothesis that Glomus species are not adapted to acid soils. Surprisingly, however, Glomus manihotis was found to be the only predominant VA mycorrhizal fungus in this soil. This suggests that species of VA mycorrhizal fungi which are generally sensitive to extreme soil acidity and Al toxicity may have the ability to adapt to these conditions.

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EFFECT OF AN ASPARAGUS ALLELOCHEMICAL ON GLOMUS FASCICULATUM AND GROWTH OF MYCORRHIZAL ASPARAGUS SEEDLING. T.L. Wacker, G.R. Safir and C.T. Stephens, Dept. of Botany and Plant Pathology, Michigan State University, East Lansing, Mi 48824.

The effect of ferulic acid, an allelochemical produced by asparagus, on hyphal elongation and colonization of asparagus by Glomus fasciculatum was studied. Spore germination in vitro was not affected, but hyphal elongation decreased significantly with increasing ferulic acid concentration (0 to 400 µg/g). In the greenhouse, mycorrhizal colonization of roots and growth of mycorrhizal asparagus decreased significantly with increasing ferulic acid concentration, while growth of non-mycorrhizal plants was not affected by ferulic acid. Although plant tissue phosphorus levels were apparently unaffected by ferulic acid or mycorrhizal status, ferulic acid inhibition of hyphal elongation in vitro and fungal colonization in vivo suggest that ferulic acid production by asparagus can alter the symbiotic effectiveness of the fungus, and subsequently affect plant growth.

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THE EFFECT OF PERIODIC FLOODING ON INFECTION OF PEPPER BY PHYTOPHTHORA CAPSICI. J.H. Bowers and D.J. Mitchell. Dept. of Plant Pathology, Univ. of Florida, Gainesville, FL 32611.

Periodic flooding increased the mortality of pepper plants grown in soil infested with 25 oospores of Phytophthora capsici per gram of soil. Plant mortality increased as the number of 24-hr flooding periods at 10-day intervals increased. Plants grown in infested soil at a constant soil-water matric potential of -125 mbar were not infected after 37 days when plated on a selective medium. However, when infested soil at -125 mbar was periodically flooded, 20, 53, and 100% of the plants died after 1, 2, and 3 flooding periods, respectively. At -25 mbar, 0, 80, and 100% of the plants died after 1, 2, and 3 flooding periods, respectively. Only one of 15 plants was infected in soil at a constant soil-water matric potential of -25 mbar. These results reinforce field observations in which heavy rainfall with subsequent flooding of the soil was associated with increases in disease progress.

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EFFECT OF SOIL WATER MATRIC POTENTIAL ON INFECTION BY POLYMYXA BETAE AND BEET NECROTIC YELLOW VEIN VIRUS. J. S. Gerik and J. C. Hubbard. USDA-ARS, 1636 E. Alisal St., Salinas, CA 93905.

The effect of soil water matric potential on infection of sugar beet by viruliferous Polymyxa betae was studied using a loam soil from the Salinas Valley of California which was infested with the pathogens. The soil was saturated with water and the matric potential was adjusted with a soil moisture extractor to potentials of -0.1, -0.2, -0.3, -0.4, -0.6, and -1.0 bar. Sugarbeet seedlings were transplanted into saturated soil and the adjusted soils in sealed glass beakers, and incubated for 2 weeks in a growth chamber at 24 C. The plant roots were then assayed for infection by beet necrotic yellow vein virus (BNYVV) by sandwich ELISA. Plants incubated in the -0.3 bar and wetter soils were positive for BNYVV. No plants incubated in -0.4 bar or drier soil were infected with the virus. The experiment indicates that P. betae, the vector of BNYVV, is unable to infect sugarbeet roots in this soil when the matric potential is -0.4 bar or less.

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FACTORS AFFECTING PRE-EMERGENCE FLOODING DAMAGE TO SOYBEAN. R.S. Ferriss and J.M. Baker. University of Kentucky, Lexington 40546.

A procedure was developed which produces results similar to those obtained when seeds are planted in soil and then flooded, but with more efficient use of time, materials and facilities. The procedure involves placing seeds in a 0.1 % soil suspension in deionized water, incubating for 1 to 5 days, recovering the seeds, and then assessing viability by rating the seeds for germination status after 5 days incubation on cellulose germination medium. Viability was similar using autoclaved soil, baked sand, or no soil, but was reduced by use of pasteurized or untreated soils. Filtering pasteurized or untreated soil suspension through Whatman number 1 filter paper resulted in a minor increase in viability, whereas filtering through a 0.45 µm millipore filter completely eliminated the soil effect. Viability was significantly increased by seed treatment with carboxin-thiram, thiram, captan, penicillin, erythromycin, vancomycin or ampicillin, but was not affected by seed treatment with benomyl, carboxin or metalaxyl. Viability increased with decreasing soil suspension pH. Overall, these results support the involvement of soil bacteria in flooding damage.

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THE INFLUENCE OF FUSARIUM SOLANI ON POPULATIONS OF PHYTOPHTHORA CITROPHTHORA AND P. PARASITICA IN RHIZOSPHERE SOIL OF CITRUS. L.M. Dandurand and J.A. Menge, Dept. of Plant Pathology, University of California, Riverside, CA 92521.

The influence of F. solani (Fs) on populations of P. citrophthora (Pc) and P. parasitica (Pp) was studied by sequentially sampling the rhizosphere and roots of citrus which were either pre-colonized or not with Fs prior to inoculating with zoospores of Pc or Pp. Initial rhizosphere populations of Pc were significantly higher from roots pre-colonized with Fs than from non-colonized roots. Six weeks after inoculating with zoospores, Pc populations in the rhizosphere of non-colonized roots were significantly higher than from roots pre-colonized with Fs. In a second experiment, when citrus was heat-stressed, populations of Pp from roots pre-colonized with Fs were significantly lower than from non-colonized roots. Root weights and root lengths were significantly lower in plants pre-colonized with Fs than non-colonized plants.

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GROWTH OF GENETICALLY-ALTERED PSEUDOMONAS SOLANACEARUM IN RHIZOSPHERE AND NONRHIZOSPHERE SOILS. J.W. Williamson[1], P.G. Hartel[1], and M.A. Schell[2]. Depts. of Agronomy[1] and Microbiology[2], Univ. of Georgia, Athens, GA 30602

The effect of genetic alterations of the pglA gene encoding for an α-1,4-endopolygalacturonase on the growth of Pseudomonas

solanacearum in the rhizosphere and nonrhizosphere soils of tomato (Lycopersicon esculentum), common purslane (Portulaca oleracea), and pearl millet (Pennisetum glacum) was determined. Polygalacturonase production from pglA was either eliminated by marker exchange mutagenesis, or enhanced two-fold by increasing the copy number by cloning on a recombinant plasmid. Bacterial counts of all strains, including the wild types, decreased in the nonrhizosphere of all plants, and in the rhizosphere of common purslane and pearl millet. An increase in numbers was observed in the rhizosphere of tomato for all strains. Determination of the effects of the genetic alterations on generation times and on pathogenicity are currently in progress.