1991

APS

Annual

Meeting

Abstracts of Presentations

August 17-21, 1991
Adam's Mark Hotel
St. Louis, Missouri

The American Phytopathological Society

Abstracts of Presentations

The number above an abstract corresponds to its designation in the program of the 1991 APS annual meeting in St. Louis, MO, August 17-21.

1

EFFECT OF SELECTED PRE-EMERGENT HERBICIDES AND SOIL PH ON SEEDLING BLIGHT OF GRAIN SORGHUM CAUSED BY *FUSARIUM SPP*. M. A. Davis and D. J. Jardine, Dept. of Plant Pathology, Kansas State University, Manhattan 66506-5502.

Pre-emergent herbicides and soil pH were evaluated for their effect on Fusarium seedling blight development on grain sorghum. Atrazine (0.37 kg/ha), atrazine + alachlor (0.55 kg/ha), and atrazine + metolachlor (0.69 kg/ha) were applied to plots with soil adjusted to pH 4.7, 5.9, and 6.3. Stand counts were taken 2 wk after planting and root development, disease incidence and overall root health ratings was taken 2, 4, and 6 wk after planting. Seedling emergence, root development, and root health ratings were less for all herbicide treatments compared to the control (P=0.05). The atrazine + alachlor was significantly less than either of the other two herbicide treatments. Disease incidence, as measured by colonization of the roots by Fusarium spp. was significantly higher when herbicides were compared to the control. Soil pH significantly enhanced pathogen colonization and disease severity 2 wk after planting but not at 4 or 6 wk. There were no pH-herbicide interactions.

2

FIVE FUNGAL SPECIES ASSOCIATED WITH ANTHRACNOSE OF RED-PEPPER IN KOREA. K. S. Park, C. H. Kim, and E. J. Lee. Department of Plant Pathology, Agricultural Sciences Institute, Suwon 441-707, Korea.

Pathogenic fungi associated with anthracnose of red-pepper were studied with collected disease samples over Korea from 1986 to 1988. Glomerella cingulata Colletotrichum gloeosporioides, C. acutatum, C. coccodes and C. dematium were identified based on mycological characteristics. In the inoculation tests, the strongest pathogenic species on fruits was C. gloeosporioides and followed by C. acutatum. C. coccodes was pathogenic on immature green fruits, leaves and seedlings. G. cingulata was pathogenic only on mature red-fruits and C. dematium on seedlings and leaves. C. gloeosporioides was isolated most frequently. This species was found at similar frequency with C. dematium early after field-transplanting, and became predominant thereafter. C. coccodes was exclusively isolated from the diseased seedlings in seedbeds. C. acutatum and G. cingulata were occasionally found from the infected red-fruits. This result suggests that major pathogenic species responsible for recent anthracnose epidemics on red-pepper in Korea is C. gloeosporioides.

3

RELATIONSHIP BETWEEN EAR HUSK MORPHOLOGY AND FUSARIUM EAR ROT OF CORN. R.M. Davis, J.J. Farrar, T.L. Peters, F.R. Kegel, and P.A. Mauk. Depart. of Plant Pathology, Univ. of Calif., Davis 95616.

Three treatments were imposed on the ears of three corn hybrids [very susceptible, moderately susceptible, and resistant to Fusarium ear rot (F. moniliforme)] between one and two weeks after pollination: (a) husks split open to expose the developing kernels to the air; (b) parafilm wrapped around the tips of the ears in an attempt to maintain or produce tight husks; and (c) a nontreated control. At maturity yield and severity of ear rot were evaluated. The percentages of diseased kernels in the nontreated ears were 78, 49, and 6 for the susc., mod. susc., and res. hybrids, respectively. In the "split husk" treatment almost 100% of the kernels were decayed in all the hybrids. The parafilm treatment had no effect. Yields were significantly reduced in the "split husk" treatment in all hybrids compared to the nontreated control. In a plant stress experiment ear rot was reduced when ears did not fill normally, presumably because they were not subject to insect damage.

4

ALTERNATE HOSTS OF SOYBEAN SDS STRAINS OF FUSARIUM SOLANI. L. E. Gray, USDA, ARS, Department of Plant Pathology, University of Illinois, Urbana, Illinois 61801.

Fusarium solani isolates were grown on sterile oat grains at 25C for two weeks. Sterile infested oats were used to inoculate soybean, mung bean (Vigna radiata) and garden bean (Phaseolus vulgaris) in the greenhouse. Inoculum (six-infested-seeds per plant) was placed around the crown of three-week-old seedlings and covered with sterile soil. After inoculation, the plants were grown at 25C temperature in a greenhouse for five weeks and then evaluated for disease. All F. solani isolates tested caused typical SDS symptoms on soybeans. Mung beans and garden beans also were susceptible and all plants died following inoculation. The fungus was reisolated from roots and crown tissue of inoculated plants, but not from stem tissue above the soil line. These results indicate that F. solani isolates associated with soybean SDS are not host specific and readily infect other bean hosts.

5

EFFECTS OF DATE OF PLANTING ON SEVERITY OF SOYBEAN STEM CANKER. A. Y. Chambers. Department of Entomology and Plant Pathology, University of Tennessee, Jackson, TN 38301-3200.

Seven field plantings of soybeans susceptible to Diaporthe phaseolorum var. caulivora were made at 10-day intervals from early May to late June or early July during 1988-90 to evaluate the effects of planting date and associated varying environmental conditions on the severity of stem canker. In 1988, stem canker incidence and severity were low but were highest in May 2 and 12 plantings ('J77-339' line). Disease ratings in later plantings were progressively lower. Yields were highest in May plantings and steadily declined in subsequent plantings in June and early July. Stands of J77-339 were poor in 1989, and yields were not recorded. However, disease ratings were high in the first planting on May 1 and were progressively lower in plantings made during the remainder of May and in June. In 1990, disease ratings were high in the first planting on May 10 ('RA 604' cultivar) and were progressively lower in the other six plantings made during May and June. Yields were low in the May 10 planting and steadily increased through the sixth planting on June 15.

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INFLUENCE OF TOMATO SPOTTED WILT VIRUS ON YIELD OF FLORUNNER PEANUT. A. K. Culbreath, J. W. Todd and J. W. Demski, Dept.of Plant Pathology and Dept.of Entomology, University of Georgia, Coastal Plain Experiment Station, Tifton, GA 31793-0748

Florunner peanut (Arachis hypogaea) plants showing symptoms of Tomato spotted wilt virus (TSWV) were marked 5 times through the season in 1989 and 1990, beginning 6 wk after planting. At maturity, pods from individual symptomatic plants from each evaluation date, and asymptomatic control plants were harvested. Number of seed and seed yield were determined for each plant. Number of seed per plant was 21.4 and 91.9 for symptomatic and healthy plants respectively, and total seed yield was 10.7 g/plant and 45.0 g/plant for symptomatic and healthy plants respectively, in 1989. In 1990, number of seed per plant was 33.1 and 85.4 for symptomatic and healthy plants, respectively. Total yield was 11.0 g/plant for all symptomatic plants and 35.1 g/plant for control plants. In both years, number of seed per plant and yield per symptomatic plant were positively correlated with number of weeks after planting that symptoms appeared.

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SEEDLING DISEASE OF ALFALFA IN KENTUCKY CAUSED BY APHANOMYCES EUTEICHES. P. C. Vincelli, W. C. Nesmith, & J. Doney, Dept. of Plant Pathology, University of Kentucky, Lexington, 40546.

Studies were initiated to investigate the potential for seedling disease of alfalfa under controlled conditions in a soil naturally infested with Aphanomyces euteiches. Germinated seeds of 21 cultivars were planted into pots containing a Heitt silty clay loam and placed in a growth chamber at 24 C for 3 wk. Pots were irrigated either with a 5 ppm metalaxyl solution (to control Pythium and Phytophthora spp.) or a solution of 5 ppm metalaxyl + 10 ppm hymexazol (to control A. euteiches, also). In pots irrigated with metalaxyl alone, plants of cultivars susceptible to A. euteiches generally were killed or stunted greatly. When A. euteiches was controlled using hymexazol, root rot was reduced and dry weight and nodulation were increased in all cultivars. Dry weight of metalaxyl-treated seedlings increased significantly with increasing cultivar resistance to A. euteiches. This pathogen has been found in soils from 25 counties to date. Given the disease potential observed in this study, A. euteiches may be an important cause of seedling disease of alfalfa in Kentucky.

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ACCUMULATION OF AMMONIA IN COTTON INFECTED BY *VERTICILLIUM DAHLIAE*: RELATIONSHIPS TO FUNGAL VIRULENCE AND CULTIVAR RESISTANCE. A. A. Bell. USDA, ARS, Cotton Pathology Research Unit, Route 5, Box 805, College Station, TX 77845.

Ammonium ion concentrations increased in leaf and stele tissues of 12 Acala cultivars of *Gossypium hirsutum* L. in response to defoliating (V-C1) and nondefoliating (V-C2) strains of *Verticillium dahliae* Kleb. The mean ammonium ion concentrations in healthy stele and leaf of all cultivars were 1.69 and 3.79 µmoles/g dry tissue, respectively. Relative to the control, infection with defoliating and nondefoliating strains caused mean increases in ammonium ion concentrations of 226 and 75% in stele and 296 and 24% in leaves, respectively. Increases in ammonium concentrations in leaves were negatively related to cultivar resistance; a 10 fold increase occurred in susceptible cultivars infected with defoliating isolates.

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MONOCLONAL ANTIBODIES SPECIFIC FOR CONIDIA OF THE RICE BLAST FUNGUS. J. Q. Xia, F. N. Lee, L. N. Raymond, and H. A. Scott. Department of Plant Pathology and Hybridoma Laboratory, University of Arkansas, Fayetteville, AR 72701.

Four monoclonal antibodies (MAbs) were developed by fusing P3/NS1/1-Ag4-1 myeloma cells with splenocytes from mice immunized with crushed conidial suspensions of Race IB-49 of Pyricularia grisea. MAbs secreted from cell lines 4G11, 8H1 and 3E4 reacted mainly with conidial antigens, showing 4G11 bound on the surface of conidial cells whereas 8H1 and 3E4 bound on germ tubes in an immunofluorescence assay (IFA). MAb 11C6 reacted preferentially with mycelial antigen. Using ELISA MAbs 8H1 and 3E4 showed partial cross-reactions with four unrelated fungal isolates and 11C6 with three from 11 genera tested. The species-specific MAb 4G11, isotype IgG1, failed to recognize the antigens from the 11 fungal genera. Further tests against 12 isolates of P. grisea or Pyricularia spp. from rice or grasses indicated that MAb 4G11 reacted strongly with one, moderately with two, weakly with five and negatively with four of the isolates in IFA. MAb 4G11 could detect homologous conidial antigen at 15-70 ng per ml and 10-20 conidia per well by ELISA and appeared to have potential diagnostic value.

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INFLUENCE OF TEMPERATURE AND PHOTOPERIOD ON DETECTION OF *Acremonium coenophialum* IN SEEDLINGS OF TALL FESCUE. B. Randall-Schadel, K. D. Gwinn, M. J. Munster, and C. L. Campbell, NC Dept. of Agriculture, Raleigh 27611; Univ. of Tennessee, Knoxville 37901; and NC State Univ., Raleigh 27695.

Plants of tall fescue (*Festuca arundinacea*) from four seed lots (3 pasture and 1 turf-type) were grown in replicated Phytotron chambers set for spring and fall greenhouse temperatures and photoperiods. Seasonal effects on detection of *A. coenophialum* by AOSA stain and PAS-ELISA were analyzed. No significant effect of environment or plant age was found on the percent infection detected by ELISA. With the stain method, plant age and several treatment interactions (including "season" * seed lot) were significant. In part this may have been due to a reduced sensitivity of the stain method on the youngest plants. At the 6- and 8-wk harvests, stain and ELISA were in agreement on 98% of the plants, but at 4 wk (separate plants for each method), percent infection detected by the stain method was one-third less than percent infection detected by ELISA. Endophyte infection did not alter shoot length or the number of tillers per plant.

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IMMUNOCYTOLOGICAL AND CYTOCHEMICAL STUDIES OF THE INFECTION OF MAIZE ROOTS BY *FUSARIUM MONILIFORME*. L. R. Todd, Department of Plant Pathology, University of Minnesota, St. Paul.

Scanning electron microscopy and light microscopy revealed that *Fusarium moniliforme* invaded 7-day old maize (*Zea mays* L.) seedlings both through wounds and by direct penetration. When penetrating directly, hyphae adhered to the epidermis, then breached the cell wall enzymatically. The fungus rapidly invaded the cortex, first by growing between cells, then by dissolving cell walls until large areas of tissue were destroyed. *F. moniliforme* was found in the stele of roots where the endodermis was not fully formed. Direct probing of the cell wall by gold labeled endo-1,4-β-xylanase, endo-1,4-β-glucanase and 1,4-β-D-glucan cellobiohydrolase indicated a removal of both xylan and cellulose by hyphae in contact with the cell wall. Immunogold labeling of polyclonal antibodies of the same enzymes demonstrated that these enzymes are involved in wall degradation at the interface between the hyphal and plant cell walls.

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IN VITRO AND IN VIVO ACTIVITY OF TEBUCONAZOLE AGAINST MAJOR PEANUT PATHOGENS. T. B. Brenneman[1], A. P. Murphy[2], and A. S. Csinos[1], [1]Dept. of Plant Path., Coastal Plain Stn., Tifton, GA 31793, and [2]Mobay Research Farm, Tifton, GA 31794.

Tebuconazole (Teb) demonstrated excellent in vitro activity against Rhizoctonia solani AG-4 and Sclerotium rolfsii with ED_{50}'s for mycelial growth being 0.17 and 0.08 µg/ml, respectively, compared to 24.3 and 3.9 µg/ml for pentachloronitrobenzene. Teb reduced damage from Rhizoctonia limb rot and southern stem rot and increased yields approximately 50% over plots where only foliar pathogens were controlled. Teb inhibited growth and sporulation of Cercosporidium personatum in vitro at 1.0 µg/ml compared to 0.1 µg/ml for chlorothalonil. The lowest rate of Teb evaluated (188 g/ha) controlled late leafspot when applied full season (seven times) and increased yields significantly. Efficacy against C. personatum and soilborne pathogens was maintained when Teb was applied either as a block of sprays or in an alternating schedule with chlorothalonil.

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CHEMICAL CONTROL OF *SEPTORIA NODORUM* ON WHEAT IN ILLINOIS. S. Zavala-Gallardo and W. L. Pedersen. Dept. of Plant Pathology, University of Illinois, 1102 South Goodwin, Urbana 61801

Two foliar fungicides, propiconazole (Tilt, Ciba-Geigy Corp.) and flusilazole (Punch, Du Pont Agr. Chem), were used to control *Septoria nodorum* in Cardinal wheat in 1990. Field plots were 1.2 m x 3.0 m, and were arranged in a randomized complete block design with five replications. Propiconazole was applied at growth stage GS 8 (126 ml A.I./ha) and flusilazole was applied at GS 8 and/or GS 10.1 (70 or 140 ml A.I./ha). Disease severity was rated (% leaf area infected) three times, GS 8, 10.1, and 11, and control plots had 3%, 20% and 51% disease, respectively. Maximum disease control was achieved with two applications of flusilazole (140 ml A.I./ha) and yield was increased 1247 kg/ha. Propiconazole and flusilazole (one application at GS 8) reduced disease severity and increased yields comparably.

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THE EFFECT OF BENOMYL ON YIELD AND SEED QUALITY IN EARLY PLANTED SOYBEANS IN ARKANSAS. M. L. May, J. C. Rupe, and T. L. Kirkpatrick. University of Arkansas, Fayetteville, AR.

Benomyl was applied two soybean cultivars (Crawford and Williams 82) planted in April which had prompt or delayed (14 days) harvest. Williams 82 had higher yields than Crawford with both prompt and delayed harvest, but had greater yield reductions with a delay in harvest. Benomyl increased yields in Williams 82 in prompt but not delayed harvest and in Crawford in delayed but not prompt harvest in 1989. There was no effect in 1990. Benomyl reduced frogeye leaf spot severity and pod shattering. Seed germination decreased with a delay in harvest in both cultivars and seed infection by Phomopsis longicolla increased. Seed germination was 9% and 0% in 1989 and 49% and 47% in 1990 for prompt and delayed harvest, respectively.

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CONTROL OF APPLE SCAB AND POWDERY MILDEW WITH PLANT OILS. J. Northover, Agriculture Canada, Research Station, Box 6000, Vineland Station, Ontario, Canada, L0R 2E0

Plant oils high in linoleic acid (corn, grapeseed, safflower and soybean) were similar to low linoleic acid oils (canola, olive, peanut and sunflower) in providing 73-91% foliage protection of potted apple plants against Venturia inaequalis (Cooke) Wint. The oils were applied without surfactant at 1%, as homogenized emulsions in water. Oils mixed with Agral 90 (5% w/w) and sprayed as 1% emulsions, also provided 1-day prophylactic control (82%), but they were not better than Agral 90 alone (62%), and showed no 1-day therapeutic activity. In an apple orchard, 11 applications at 7-10 day intervals of soybean and canola oils emulsified with Agral 90 gave 58-70% fruit scab control; similar to Agral 90 alone, but inferior to captan. These oils were nonphytotoxic to McIntosh but caused leaf-spotting and fruit russetting of Golden Delicious. Against Podospheara leucotricha (Ell. & Ev.) Salm., oils alone as 1% emulsions in water were very effective in 1-day prophylactic and 1-day therapeutic treatments, similar to dinocap.

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CONCEPTS AND POTENTIAL PRACTICES IN FORECASTING AND CONTROLLING SHOT HOLE OF ALMOND IN CALIFORNIA. J.M. Ogawa and J.E. Adaskaveg. Dept. Plant Pathology, Univ. Calif., Davis 95616.

Cultural practices of almonds require effective control of shot hole disease caused by Wilsonomyces carpophilus. To prevent defoliation and crop losses, fungicides are applied systematically (2-4 X) during the spring. The fungus is not known to have an overwintering sexual stage and does not produce perennial infections on almond branches. Spore survival studies indicate that < 5% of the spores can survive on the surface of host tissue for 1 yr. Thus, the disease cycle is initiated each spring mostly from inoculum produced during the previous fall when no control practices are employed. These spores infest buds and twigs during tree dormancy. In the spring, primary infection is directly related to inoculum levels of the previous fall. Spores formed during the current season function as secondary inoculum and are critical for initiating disease epidemics during springs with extended rains. Therefore, control practices in four test orchards over a 3-yr period were modified to apply protective fungicides based on inoculum levels in the fall and spore production and wetness periods in the spring. Results indicate that a 50-100% reduction in the number of sprays can provide disease control equal to that of previous practices.

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GROWTH AND DEVELOPMENT OF PHYMATOTRICHUM OMNIVORUM AFFECTED BY TRIAZOLE FUNGICIDES. H. M. Escamilla and S. D. Lyda. Tecnológico de Monterrey-C. Querétaro, Departamento. de Fitotecnia, A. Postal No. 37, Querétaro, Qro.76000, México and Dept. of Plant Pathology and Microbiology, Texas A & M Univ., College Station, TX 77843, respectively.

Triazole fungicides reduced mycelial growth and formation of sclerotia of Phymtotrichum omnivorum. Mycelial growth on PDA was highly inhibited by bromoconazole, diniconazole, flusilazole and propiconazole at less than 5 µg/l. Growth was totally inhibited when the medium was ammended with 10 mg/l for all fungicides. In soil culture the rate of mycelial growth was inhibited between 40-50% by triadimenol, furconazole, triadimefon, diniconazole, penconazole and propiconazole at 10 mg ai/kg soil. Triazole fungicides significantly reduced the formation of sclerotia. Diniconazole, hexaconazole, propiconazole and bromoconazole were the most effective fungicides to reduce sclerotial biomass. Except for flusilazole, all triazole fungicides reduced the formation of sclerotia at 100 mg ai/kg soil between 95 to 100 %. Viability of sclerotia formed in soils treated with triazole fungicides was not affected significanltly.

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SCAB OF WHITE-SAPOTE CAUSED BY ELSINOE SPP. R.T. McMillan, Jr., University of Florida, IFAS, Tropical Research and Education Center, Homestead, FL 33031

Scab caused by Elsinoe spp. has been observed for many years on Casimiroa edulis Llave & Lex. in south Florida. The White-sapote flowers during the winter with fruit set in February. Elsinoe only attacks young fruit on mature trees. The lesions are few to numerous, small, pale, yellow, elevated spots. As the young fruit develop the lesions become circular to angular, up to 1 cm, grayish brown with dark irregular margins. The lesions enlarge as the fruit enlarges and the centers become covered with cracked and fissured corky tissue. Spore masses on the lesions are velvety brown during moist periods. Elsinoe has been controlled by foliar applications of benomyl, chlorothalonil and mancozeb from flowering to fruit set.

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CHEMICAL AND CULTURAL CONTROL OF DOGWOOD ANTHRACNOSE. M. Windham and A. Windham. P.O. Box 1071. Knoxville, TN 37901 and P.O. Box 110019 , Nashville, TN 37222.

A nursery field containing 20,000 dogwoods (Cornus florida) was identified where dogwood anthracnose (incidence = 93%) was uniformly distributed throughout the field. Eight fungicide treatments (ASC 66791 - 2.6 g/l, Banner 1.1 E - 0.5 ml/l, Benlate 50 DF - 1.3 g/l, Bravo 720 - 1.5 g/l, Dithane DF - 2 g/l, Lynx 2 F - 0.5 ml/l, SAN 619 - 0.03 g/l, and Systhane 2 EC - 0.5 ml/l) were evaluated for disease control using a randomized complete block design with 12 replications. In six of each treatment's reps, all diseased tissue was pruned and removed. Disease severity was rated with a canopy scale (0 = healthy to 5 = 80 - 100% of canopy diseased). Fungicidal sprays were effective in reducing symptom severity. Pruning reduced symptom severity, but was less effective than fungicides. Fungicides + pruning treatments were no better than fungicides alone.

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DEVELOPMENT OF FIRE BLIGHT EPIDEMICS AND CONTROL MEASURES IN PEAR ORCHARDS IN TURKEY. M.T. Momol[1], O. Yoğen[1], H. Basim[1], M.A. Zachowski[2], K. Rudolph[2], and L.H. Purdy[3]. [1]Akdeniz Üniversitesi, Ziraat Fakültesi, Antalya, Turkey. [2]Institut f. Pflanzenpathologie, Göttingen, Germany. [3]Plant Pathology Department, University of Florida, Gainesville, FL USA.

Severe outbreaks of fire blight caused by Erwinia amylovora have occurred on pear and quince in Turkey since 1985. Experiments were conducted on Santa Maria pears in the western Mediterranean region of Turkey to evaluate measures of chemical control and

pruning. Blighted flowers and twigs were pruned and recorded from 24 trees per treatment on each of three assessment dates. Diseased units was determined from visible symptoms. Seven sprays of a mixture of copper oxychloride and maneb applied at five-day intervals during and after blossom period gave satisfactory disease control with no phytotoxicity. Cumulative number of blighted flowers and twigs at the end of the last assessment date was 27 for copper oxychloride plus maneb, 60 for ammoniacal copper sulfate, and 154 for the unsprayed control.

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SENSITIVITY OF TWO POPULATIONS OF *VENTURIA INAEQUALIS* TO DODINE AND FLUSILAZOLE. Katherine L. Reynolds and Wolfram Köller. Dept. of Plant Pathology, Cornell University, NYSAES, Geneva, NY 14456.

The distribution of sensitivity to dodine and flusilazole was compared in two populations of *V. inaequalis*. Forty-nine monoconidial isolates were collected from an isolated orchard that has been abandoned for over 50 years, and 54 monoconidial isolates were collected from a commercial orchard in eastern NY. Fungicide sensitivity was based on mycelial growth after 4 wk on PDA containing a discriminatory concentration of each compound alone, relative to growth on unamended media. Mean relative growth of the commercial orchard isolates on 0.01 µg/ml flusilazole was significantly greater than that of the abandoned orchard isolates (39% vs. 33%, respectively). In contrast, the mean relative growth of the abandoned orchard isolates on 0.2 µg/ml dodine was significantly greater than that of the commercial orchard isolates (44% vs. 38%, respectively), suggesting possible negative cross-resistance between the two fungicides. However, analysis of the data revealed no evidence of either positive or negative cross-resistance between dodine and flusilazole.

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BIOCIDAL ACTIVITY OF SODIUM TETRATHIOCARBONATE ON SCLEROTINIA MINOR AND S. SCLEROTIORUM. M.E. Matheron and J.C. Matejka. University of Arizona, Yuma Agric. Center, Yuma, AZ 85364

Studies were initiated to examine the effect of sodium tetrathiocarbonate (STTC) on the mycelium and sclerotia of Sclerotinia minor and S. sclerotiorum. STTC at a concentration of 612 µg/ml was lethal to mycelium of both pathogens. When sclerotia were buried 2 cm below the soil surface in plastic pots and drenched with STTC at a concentration of 2,450 or 3,675 µg/ml, germination of sclerotia of S. minor was reduced 68 and 100%, respectively, compared to treatments drenched only with water. Similar concentrations of STTC had no effect on the germination of sclerotia of S. sclerotiorum. Germination of sclerotia of S. minor in soil treated with STTC at a concentration of 2,450 µg/ml was reduced 25, 36, 70, and 81% after exposure to the chemical for 8, 16, 24, or 48 hr, respectively, compared to sclerotia not exposed to STTC. The biocidal effect of STTC on sclerotia of S. minor could provide control of plant diseases caused by this pathogen.

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Use of stimulants of sclerotial germination to manage inoculum density of Sclerotium cepivorum. F. J. Crowe, J. Debons and E. Redondo. Central Oregon Agricultural Research Center, Oregon State University, P.O. Box 246, Redmond, OR 97756, and Experto Nacional de Hortalizas, Apdo. 112, Celaya, Gto. Mexico 38000.

In the absence of host crops, single applications of 75% diallyl disulfide plus related ingredients were applied to the surface of field plots naturally-infested with Sclerotium cepivorum. Applications immediately were followed by irrigation, and were timed to ensure presence of stimulant during the range of temperature needed for stimulated germination. No trial was placed in any field in which less than one year had passed since the most recent white rot incidence, to avoid constitutive dormancy of newly-formed sclerotia. All trials were randomized block experimental designs, with three to five replications, and with plot sizes $\geq$ 10 m^2. The inoculum density of sclerotia was determined monthly from each plot as per Phytopath. 70:64-69. Excluding periods too cold for sclerotial germination, germination was active in treated plots for two to three non-dormant months. The mean numbers of sclerotia remaining viable but non-germinated expressed as a percentage of the pre-treatment viable but non-germinated population were 68.5, 3.0, 1.2, and 1.2% at Walla Walla, WA; 90.4, 32.9, 5.5, and 3.1% at San Miguel de Allende, Gto. Mexico; and 92.5, 12.3, 0, and 1.6% at Nampa, ID, respectively, for rates of application of 0 (untreated control), 0.5, 5.0 and 50.0 ml/m^2. Statistical significance ($P \leq 0.05$) was found for the above data between untreated and all treated plots from all locations. These and subsequent experiments suggest that inoculum of the Allium white rot fungus may be managed using germination stimulants, if closely coordinated with the potential biological activity of the pathogen and with cropping sequence. Ongoing research emphasizes improvements in application methodology, choice of stimulants, product formulation, and regulatory concerns.

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EFFECT OF ERGOSTEROL-BIOSYNTHESIS-INHIBITING FUNGICIDES ON GROWTH AND DEVELOPMENT OF CABERNET SAUVIGNON GRAPEVINES GRAFTED ON ROOTSTOCKS 3309 AND SO-4. H. M. Escamilla and S. D. Lyda. Tecnológico de Monterrey-C. Querétaro, Departamento. de Fitotecnia, A. Postal No. 37, Querétaro, Qro.76000, México and Dept. of Plant Pathology and Microbiology, Texas A & M Univ., College Station, TX 77843, respectively.

Actively growing potted Cabernet sauvignon grapevines grafted on rootstocks 3309 or SO-4 were treated with triazole fungicides injected into the soil. The fungicides propiconazole, myclobutanil and bromoconazole exhibited growth regulation activity like that of the growth regulator paclobutrazol. The use of triazole fungicides at 0.1, 1 and 10 mg ai/kg soil did not affect the grapevine's growth components nor dry weight. Triazole fungicides at 100 mg ai/kg soil significantly reduced shoot elongation, rate of growth, number of nodes per shoot, internode length, leaf area, number of leaves, area per leaf, lateral roots and root dry weight. The effect of triazole fungicides on the rate of shoot elongation was detected one week after application. There was no differential response between fungicides and rootstocks 3309 and SO-4 .

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EFFICACY OF CERTAIN FUNGICIDES AGAINST RHIZOCTONIA SOLANI (KÜHN) AG-4 WITH CHEMICAL MANAGEMENT OF WEEDS IN COTTON FIELDS OF EGYPT. S. M. Moustafa-Mahmoud, D. R. Sumner, M. M. Ragab, and Mona M. Ragab. University of Georgia, Coastal Plain Experiment Station, Tifton, GA 31793 and Department of Plant Pathology, Cairo University, Giza, Egypt.

Field experiments, on cotton seedling disease control, showed that recommended doses of the herbicides pendimethalin, fluometuron, prometryne and oxyfluorlen affected the efficacy of the fungicides tolclofos-methyl, pencycuron and carboxin according to the percentage of plant stand 10 and 40 days after sowing. At the first location where R. solani AG-4 was isolated frequently from diseased cotton seedlings, the herbicide pendimethalin showed the greatest effect in reducing the fungicidal activity. In the second location, all herbicides (except oxyfluorlin) increased disease incidence and decreased the antifungal activity of fungicides. In In vitro experiments, herbicides reduced the mycelial growth of R. solani significantly at 50 and 100 µg/ml.

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NUCLEOLYTIC ACTIVITIY IN FLAG LEAF EXTRACTS AS INDICATOR FOR EFFECTS OF FUNGICIDES ON THE SENESCENCE OF WHEAT PLANTS
H. Scherm[1], J. Raum, and G. M. Hoffmann. Technical University of Munich, W-8050 Freising-Weihenstephan, Germany.
[1]present address: University of California, Davis 95616.

The nucleolytic activity in crude flag leaf extracts was measured to quantify the effects of systemic and protective fungicides on the senescence pattern of field grown winter wheat plants under low disease pressure. Three applications of Tebuconazole in growth stages (GS) 37, 59, and 69 caused yellowing of flag leaves, but symptom expression was cultivar-specific. Compared to the respective controls, nucleolytic activities in fungicide-treated Apollo and Monopol leaves were higher in GS 65 and lower after GS 73, indicating a short-term stress response to repeated applications of the fungicide. Anilazine applied in GS 37, 59, and 69 delayed flag leaf senescence in cv. Basalt, indicated by greatly reduced nucleolytic activities compared to untreated and Tebuconazole-treated plants. This effect may be due to the elimination of minor pathogens in the phylloplane.

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PHYSIOLOGICAL AND ULTRASTRUCTURAL EFFECTS OF FUMONISIN ON JIMSONWEED LEAVES. H.K. Abbas, R.N. Paul, C.D. Boyette; USDA-ARS-SWSL, Stoneville, MS 38776 and R.F. Vesonder; USDA-ARS, Peoria, IL 61604.

The effects of fumonisin B_1, a phytotoxin obtained from Fusarium moniliforme, were studied to determine its mechanism of action in causing physiological and ultrastructural damage. When fumonisin B_1 was applied at a rate of 3 to 1000 ppm, effects such as electrolyte leakage, reduction in chlorophyll, autolysis and photobleaching of tissues were noted in less than 12 hrs after exposure to high intensity light (480 uE m^{-2} s^{-2}, PAR) at 25° C. No damage was noted up to 72 h while incubated in darkness. However, when these dark-treated samples were placed subsequently in the light, damage occurred in 12 h. Ultrastructural damage included tonoplast disruption and progressive loss of cytoplasmic integrity. At later stages (18 h), the only remaining organelles were chloroplasts, with starch grains and intact grana stacks. These results indicate that fumonisin B_1 causes rapid, light-dependent cell membrane disruption, through an unknown mechanism.

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CHARACTERIZATION OF PERIPLASMIC CATALASE ISOZYMES FROM PSEUDOMONAS SYRINGAE PV. GLYCINEA. M.G. Klotz and S.W. Hutcheson, Univ. of Maryland, Dept. of Botany, 1210 Patterson Hall, College Park, MD 20742.

A burst of active oxygen is observed in plant tissue during the initial interaction of Pseudomonas syringae strains with susceptible and resistant hosts which may limit successful colonization. Pathogenic P. syringae strains appear to be able to enzymically modulate active oxygen levels. Since the major active oxygen species contributing to the burst is HOOH, we are studying the role of bacterial catalase in P. syringae pathogenesis. Lysates of pathogenic P. syringae strains contained at least 10-fold higher total catalase activity than lysates of saprophytic bacteria (P.fluorescens, P.putida), independent of their respective growth stages. During early log-phase of growth in KB broth 30% of total catalase activity was detected in periplasmic fluids. By using native PAGE and column chromatography, we have identified 8 apparent isozymes with catalatic activity in lysates of P.s. pv. glycinea. Four isozymes of different size, charge, and kinetic parameters ($25<K_m<130$ mM HOOH) appear to be located in the periplasm. All 4 isozymes are much smaller (<200 kDa) than catalases purified from other bacteria. The presence of catalases in the periplasm seems to be unique to fluorescent pseudomonads.

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CYTOPLASMIC TRANSFER OF MITOCHONDRIA ASSOCIATED WITH ALTERNATE OXIDASE AND HYPOVIRULENCE IN *CRYPHONECTRIA PARASITICA*. N. Mahanti[1], Dr. H. Bertrand[2], Dr. D.W. Fulbright[1]. [1]Dept. Botany and Plant Pathology, Michigan State University, East Lansing, MI 48824. [2]Dept. of Microbiology, University of Guelph, Guelph, Canada

Hypovirulent strains of Cryphonectria parasitica have been correlated with the presence of transmissible dsRNA. Transmissible hypovirulence has been found in strains that do not harbor dsRNA. Respiration assays show as much as 85% of the total O_2 consumption by dsRNA-free hypovirulent strains occur through the alternate oxidase (AO) compared to 10% and 16% in virulent and dsRNA-associated hypovirulent strains, respectively. To assess the role of mtDNA in dsRNA-free hypovirulence, we used RFLPs and chloramphenicol (cap) resistance as mitochondrial markers and pigmentation as a nuclear marker to demonstrate that mitochondria are transferred by hyphal anastomosis from a dsRNA-free hypovirulent strain to a virulent strain. The converted strain is hypovirulent, cap resistant, has RFLPs of both parents and has acquired elevated AO activity (82%). These results indicate concerted transfer of mitochondria and the dsRNA-free hypovirulent phenotype.

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PROTECTION OF COTTON LEAF PALISADE CELLS FROM LIGHT-ACTIVATED TOXICITY OF A PHYTOALEXIN BY RED EPIDERMAL CELLS. A. R. Rowlan, J. A. Hall, T. Barfield-Schneider, and M. Essenberg, Dept. of Biochemistry, Oklahoma State University, Okla. Agric. Expt. Sta., Stillwater, OK 74078-0454.

The sesquiterpenoid phytoalexin 2,7-dihydroxycadalene (DHC) of cotton leaves has been shown to have photoactivated antibacterial activity. When healthy cotton leaves were infiltrated with solutions of DHC, toxicity was manifested several days later by scattered Evans-blue-stainable palisade cells or by tissue collapse. Plants maintained in continuous darkness after infiltration exhibited less damage. In leaves of DeRidder Red, a cultivar in which some epidermal cells are red, DHC and exposure to light resulted in fewer dead palisade cells per overlying red epidermal cell than dead palisade cells per overlying colorless epidermal cell. Individual red epidermal cells exhibited UV-visible absorbance spectra, recorded by R. G. Fulcher, Univ. of Minnesota, with severalfold higher absorbance at wavelengths responsible for DHC photoactivation than colorless epidermal cells. We suggest that the red epidermal pigments which accumulate adjacent to hypersensitive lesions protect underlying cells from phytoalexin toxicity by acting as light filters.

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EFFECTS OF LIGHT ON THE INCOMPATIBLE INTERACTION BETWEEN *XANTHOMONAS ORYZAE* PV. *ORYZAE* AND RICE (*ORYZA SATIVA*). A. Guo, P.J. Reimers, and J.E. Leach. Department of Plant Pathology, Throckmorton Hall, Manhattan, KS 66506-5502.

In leaves of rice cultivars carrying gene *Xa-10* for resistance to race 2 of *Xanthomonas oryzae* pv. *oryzae*, a dark brown lesion characteristic of the hypersensitive response (HR) was observed by 24-36 h after infiltration with bacteria. An increase in the activity of a cationic peroxidase and the accumulation of lignin in the infiltration site was correlated with a decrease in bacterial multiplication in tissues undergoing the HR. When the plants were maintained in the dark after infiltration, the brown lesion did not develop, and the leaves wilted, suggesting light was required for the HR. If leaves infiltrated with bacteria were exposed to less than 16 h of light and sampled 24 h after inoculation, little cationic peroxidase activity was detected, and the HR was not observed. In leaves exposed to less than 16 h light and sampled 48 h after infiltration, the cationic peroxidase activity was much lower compared to controls, and lignin was not detected, even though the infiltration site was light brown. Thus, an interval of 16 h or more of light by 48 h after infiltration was required for the accumulation of the cationic peroxidase and the formation of lignin.

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DIFFERENTIAL ACCUMULATION OF PEROXIDASE ACTIVITY IS CORRELATED WITH RESISTANCE TO *XANTHOMONAS ORYZAE* PV. *ORYZAE* IN RICE (*ORYZA SATIVA*). P.J. Reimers, A. Guo, and J.E. Leach. Department of Plant Pathology, Throckmorton Hall, Manhattan, KS 66506-5502.

When seedling leaves of rice plants (cultivar Cas 209 carrying gene *Xa-10* for resistance to race 2 of *Xanthomonas oryzae* pv. *oryzae*) were infiltrated with bacterial cell suspensions of race 2 (incompatible), total peroxidase activity in extracts from intercellular spaces increased more than three-fold between 16 and 24 h after inoculation. The accumulation of a cationic peroxidase (pI 8.5) activity paralleled the increase in total peroxidase activity over time in the incompatible interaction, while the activity of most other peroxidase isoenzymes changed little. By 48 h after infiltration, total peroxidase and cationic peroxidase activities increased in both compatible (race 1) and control (water) treatments, but at reduced levels relative to the incompatible combination. Similar results were observed in cultivar IR-BB10, also carrying gene *Xa-10*. In cultivar IR24, the near-isogenic parent of IR-BB10 lacking gene *Xa-10*, activity of the cationic peroxidase did not accumulate until 36 to 48 h after infiltration with race 1 or race 2 bacteria. Thus, the early accumulation of the cationic peroxidase activity was correlated with the incompatible interaction between race 2 and host cultivars carrying gene *Xa-10*. Purification and further characterization of the cationic peroxidase are in progress.

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PURIFICATION AND ANTIFUNGAL ACTIVITIES OF ONE CHITINASE, TWO ß-1,3-GLUCANASES AND TWO PROTEINS ASSOCIATED WITH SYSTEMIC RESISTANCE TO BLUE MOLD OF TOBACCO INDUCED BY STEM INJECTION WITH PERONOSPORA TABACINA OR LEAF INOCULATION WITH TOBACCO MOSAIC VIRUS. S. Q. Pan, X. S. Ye and J. Kuć, Department of Plant Pathology, University of Kentucky, Lexington, Ky 40546.

Stem injection of tobacco with sporangiospores of *P. tabacina* or leaf inoculation with TMV systemically protected tobacco plants against blue mold caused by *P. tabacina*. Previous experiments indicated that one chitinase (C6), two ß-1,3-glucanases (G1 and G2) and two proteins (B1 and B2) were associated with induced resistance. G1, B1 and B2 were purified, whereas C6 and G2 were partially purified. ß-1,3-Glucanases G1 and G2 inhibited sporangiospore germination and lysed germ tubes of *P. tabacina*, suggesting that ß-1,3-glucanase participates in the multi-component resistance against blue mold. Chitinase C6 had less inhibitory activity than G1 and G2. *P. tabacina* belongs to the Oomycetes which do not contain chitin in their cell walls. B1 and B2 also had inhibited spore germination of *P. tabacina*; however, their biochemical function(s) remains to be elucidated.

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SYSTEMIC INDUCTION OF PHOSPHORYLATION OF TWO PROTEINS BY LOCALIZED INFECTION OF TOBACCO MOSAIC VIRUS IN TOBACCO. X. S. Ye, S. Avdiushko, S. Q. Pan, and J. Kuc. Department of Plant Pathology, University of Kentucky, Lexington, Ky 40546.

Inoculation of a few leaves of tobacco cultivars carrying the *N*-gene for hypersensitive reaction to TMV with TMV or stem injection with *Peronospora tabacina* induces systemic resistance and a systemic accumulation of defense-related proteins. Such systemic responses to a localized infection implicate the presence of a systemic signal which originates at the site of infection, is translocated systemically and is amplified through signal transduction. Phosphorylation of two proteins (29 and 51 kD) in uninfected leaves of plants induced with TMV were associated with induced systemic resistance. Plasma membranes were isolated from systemic leaves of control and TMV-induced plants by aqueous two phase partitioning. Incorporation of ^{32}P from ATP into membrane proteins was more than 12 times higher than into soluble proteins. The 51 kD phosphoprotein is a soluble cytoplasmic protein and the 29 kD phosphoprotein is a plasma membrane protein. The role of protein kinases and inducible phosphorylation of proteins in signal transduction in induced systemic resistance will be discussed.

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EARLY EXPRESSION OF HOST mRNA IN RESPONSE TO BARLEY POWDERY MILDEW. T.A. Clark, A.G. Smith, W.R. Bushnell, and R.J. Zeyen, Departments of Plant Pathology and Horticulture and Cereal Rust Laboratory, USDA/ARS, University of Minnesota, St. Paul, MN 55108.

Conidia of powdery mildew Erysiphe graminis f. sp. hordei produce primary germ tubes (PGTs) within 2 h, and appressorial germ tubes (AGTs) 8-10 h after inoculation. Both penetrate epicuticular surfaces shortly after formation and induce host cytoplasmic aggregate responses and papillae; but only AGTs penetrate host

cell walls, make contact with host plasma membranes and produce haustoria. We found induction of and expression of host response mRNAs paralleled PGT and AGT contact in a bimodal pattern. Northern blots of total host RNA were probed with cDNA probes made from host response mRNAs (1,2) including beta 1-3 glucanase and several with unknown functions. These mRNAs showed an initial induction at 2 h with peak expression at 6 h, and a second induction at 8-10 h with peak expression at 15 h after inoculation.

1) Davidson et al. 1987. Plant Mol. Biol. 8:77.
2) Schweizer et al. 1989. Plant Mol. Biol. 12:643.

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ALTERATION OF TOXIN REDOX STATE AS A MECHANISM OF CERCOSPORIN RESISTANCE. M. E. Daub, G. B. Leisman, and C. C. Sollod. Department of Plant Pathology, North Carolina State University, Raleigh, NC 27695-7616.

Cercospora spp. produce cercosporin (CR), a light-activated, singlet oxygen-generating toxin which is toxic to plants, mice, and many fungi, but not *Cercospora* spp. Previous work showed that CR resistance of fungi is strongly correlated with cell surface reducing power, and that reduced derivatives of CR produce less singlet oxygen and are less toxic than CR. To demonstrate that CR reduction occurs *in vivo*, resistant and sensitive fungi were incubated with CR and viewed by fluorescence microscopy. CR and reduced-CR were detected using bandpass filters which differentiated their fluorescence emission peaks (606 and 504 nm, respectively). CR in contact with hyphae of resistant fungi was in a reduced form. Reduced-CR was not detected when the fungi were killed. Sensitive fungi had limited ability to reduce CR, but were able to reduce it when incubated in the presence of reducing agents which protect against CR toxicity. These data support the hypothesis that resistant fungi protect themselves by a localized reduction of CR.

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ACTIVATION OF *ARABIDOPSIS* DEFENSE GENES WITH SPECIFIC *AVR* GENES FROM PHYTOPATHOGENIC BACTERIA. F. Shaheen, K. R. Davis. Biotechnology Center, Ohio State University, Columbus, Ohio 43210.

Infiltration of *Arabidopsis thaliana* (*A. t.*) leaves with avirulent *Pseudomonas* strains is associated with a larger, more rapid induction of phenylalanine ammonia-lyase (PAL) mRNA when compared to infiltration with virulent strains. Introduction of a putative *avr* gene from avirulent *P. syringae* pv. *tomato* strain 1065 into the virulent *P. s.* pv. *maculicola* strain 4326 causes the transconjugant to induce significantly higher levels of PAL mRNA when compared to *Psm* 4326. This suggests that PAL may be regulated by a signal pathway coupled to the recognition of *avr* gene products. We have extended these studies by examining the effects of introducing other cloned bacterial *avr* genes from *P. s.* pv. *tomato, P. s.* pv. *glycinea,* and *Xanthomonas campestris* pv. *malvacearum* into the virulent strains *Psm* 4326 and *Pst* DC3000. Our results show that some *avr* genes induce significantly higher levels of PAL and peroxidase mRNAs compared to wild type strains. Current studies are focused on determining if specific members of the PAL gene family in *A. t.* are specifically induced by bacterial *avr* genes and charcterizing the promoter elements responsible for this induction.

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SPECIFIC INDUCTION OF 5-LIPOXYGENASE ACTIVITY IN POTATO TUBER BY ARACHIDONIC ACID. R. M. Bostock[1], H. Yamamoto[2], K. E. Ricker[1], D. Choi[1], and B. L. Ward[1], [1]Department of Plant Pathology, University of California, Davis, CA, 95616; [2]Kagawa University, Miki-cho, Kagawa-Ken, Japan 761-07.

5-lipoxygenase (5-LOX) is the predominant LOX activity in potato and has been implicated in the activation of host defense responses by the fungal elicitor arachidonic acid (AA). Purified preparations of 5-LOX act on the substrate AA to produce 5-hydroperoxyeicosatetraenoic acid, the principal product, and a number of other highly reactive compounds, several of which are calcium ionophores. Understanding the role of 5-LOX in the response chain between elicitor treatment and plant responses can be approached by examining 5-LOX enzyme activity following AA treatment of potato tuber disks. Response-saturating concentrations of AA induce a rapid (within 1 hr) and transient increase in cytosolic and microsomal 5-LOX activities. Activities increase over those in untreated disks by as much as 50% within 3 hr and decline thereafter. The increase in 5-LOX enzyme activity precedes or temporally parallels increases in mRNA abundance for PAL and HMG-CoA reductase. Non-elicitor fatty acids do not induce 5-LOX, and treatments which inhibit AA-elicitor activity inhibit 5-LOX activation. Treatment of the disks with cycloheximide also abolishes the 5-LOX response. Microsomal enzyme activity is increased slightly (14%) by the presence of 1 mM Ca^{2+} in the reaction mixture.

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Superoxide dismutase activity in root-colonizing pseudomonads. R. E. Zdor, J. Katsuwon, and A. J. Anderson, Dept. of Biology, Utah State University, Logan, UT 84322-5305.

Survival of root-colonizing pseudomonads exposed to superoxide anion produced by plant roots may depend on the activity of superoxide dismutase (SOD). We found similar SOD activities in plant beneficial isolates of Pseudomonas putida from bean, P. fluorescens from wheat, and P. tolaasii from sugar beet. These species produced a SOD isozyme (possibly FeSOD based on H_2O_2 sensitivity) which migrated to the same position on non-denaturing PAGE. A second isozyme was induced by growth in the presence of manganese. A cosmid clone from a bean-colonizing P. putida genomic library endowed SOD activity to a SOD^- E. coli mutant QC774 and hybridized to the E. coli *sodB* gene. This clone encoded an activity in E. coli that migrated to the same position as the P. putida FeSOD isozyme on non-denaturing PAGE. Current work is examining this clone and its use in generating $FeSOD^-$ P. putida mutants.

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REGULATION OF CERCOSPORIN ACCUMULATION BY PLANT EXTRACTS. M. Ehrenshaft and R.G. Upchurch, ARS/USDA and Dept of Plant Pathology, NCSU, Raleigh, 27695-7616.

Cercospora spp. are significant pathogens of economically important plants. Many of these fungi produce the non-host-specific toxin, cercosporin, which is known to be a key pathogenicity determinant. While light is essential for cercosporin production, media composition also plays an important regulatory role. *C. kikuchii* strain PR, a field isolate from infected soybeans, accumulates uniformly high levels of cercosporin in two common media (PD, potato dextrose broth; and CM, salts plus yeast extract and casamino acids). In contrast, a spontaneously occurring derivative of PR, strain S2, displays strong media regulation of toxin accumulation. S2 accumulates high levels of cercosporin in PD broth, but little or no toxin in CM. Supplementation of CM with aqueous extracts from soybean, corn or tobacco leaves results in at least partial restoration of cercosporin accumulation. Autoclaving or treatment of soybean extract with proteinase K eliminates its stimulatory properties. In addition, we have identified light-enhanced cDNA clones for genes whose expression is correlated with cercosporin accumulation. The response of these genes to plant extracts is also being analyzed.

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GENETIC RELATEDNESS AMONG THREE dsRNAs ASSOCIATED WITH STRAINS OF ***CRYPHONECTRIA PARASITICA*** RECOVERED FROM THE CENTRAL APPALACHIANS S.A. Enebak[1], B.I. Hillman[2], and W.L. MacDonald[1]. [1]West Virginia University, Morgantown, WV 26506 and [2]Rutgers University, New Brunswick, NJ 08903.

Nearly 30% of the ***Cryphonectria*** (syn. ***Endothia***) ***parasitica*** strains recovered from virulent cankers in the central Appalachians contain a single segment of double-stranded (ds) RNA, approximately 12 kbp in length. Although the effects of these dsRNAs on morphology and virulence vary significantly, their sequence homology is unknown. This study examined the genetic relatedness of dsRNA from strains of ***C. parasitica*** recovered in MD, VA and WV. A cDNA library representing dsRNA from **SR-2**, (Savage River, MD) was constructed in the plasmid pUC9. Recombinant plasmids from ampicillin resistant colonies contained inserts ranging in size from 0.5 - 3.0 kbp. Five ^{32}P-labeled plasmids were used to probe dsRNA from strains **SR-2**, **HM-3** (Hankey Mt, VA), **SH-4** (Stillhouse, WV), **D^2** (Garards Fort, PA) and **Ep747** (Italian origin). In each blot, the recombinant plasmid used as a probe hybridized to is own template dsRNA and to dsRNA from **HM-3** and **SH-4**, but not to dsRNA of **D^2** and **Ep747**. This indicates that **SR-2**, **HM-3** and **SH-4**, which have different effects on the fungus, are related to one another but not closely related to **D^2** or **Ep747**. These results confirm similar studies in which **Ep747** was used as a probe.

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METAL ADSORPTION BY *ARMILLARIA* RHIZOMORPHS: A PROTECTIVE BARRIER THAT AIDS IN SURVIVAL. D.M. Rizzo, R.A. Blanchette, Dept. Plant Pathology, Univ. of Minnesota, and M.A. Palmer, USDA Forest Service, St. Paul 55108.

Armillaria rhizomorphs consist of differentiated hyphae with a melanized outer rind. Melanin is known to prevent lysis of fungal structures by hydrolytic enzymes and may protect against antagonistic microorganisms. Our studies indicate melanin also adsorbs cations from the surrounding soil environment, providing enhanced protection. Rhizomorphs of three *Armillaria* species (*A. ostoyae, A. gemina, A. calvescens*), collected in Minnesota and New Hampshire, bound exceedingly high concentrations of metal ions. Zinc, copper, lead, and aluminum were, respectively, 15-178, 4-40, 1-22 and 40-100 times more concentrated on rhizomorph surfaces than in surrounding soil. On some rhizomorphs, zinc was found in concentrations up to 1900 ppm, lead up to 670 ppm, and aluminum up to 3400 ppm. It is proposed that metal ions on the outer surfaces of rhizomorphs act to protect *Armillaria* from antagonistic fungi and serve as a survival mechanism.

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HYPOVIRULENCE AGENTS ACT SYNERGISTICALLY TO RESTRICT EXPANSION OF CHESTNUT BLIGHT CANKERS. L. Shain and J.B. Miller. Department of Plant Pathology, University of Kentucky, Lexington, KY 40546.

Cankers were initiated on American chestnut sprouts with a virulent methionine auxotrophic strain of Cryphonectria parasitica, Ep 289, in vegetative compatibility (v-c) group 71. Hypovirulent (H) strains isogenic to ATCC #38755, v-c group 40, were placed separately at the base of developing cankers 9 wk later. These H strains were Ep 713 or Ep 780 which contain French or Italian H agents, respectively, or a strain designated Ep 713-780 which contains both H agents. Movement of H agents was confirmed by an isolate exhibiting typical growth of the introduced H strain but little growth on media lacking methionine. Hypovirulence was transmitted through mycelium to encircle cankers challenged by Ep 713-780 within 3 wk. In contrast, those cankers challenged by H strains containing each H agent separately were not encircled at 9 wk. Canker expansion between 3-9 wk after challenge with Ep 713, Ep 780, and Ep 713-780 was 100%, 203%, and 24%, respectively. Selected H strains with multiple H agents, therefore, may act synergistically to enhance the biocontrol of chestnut blight.

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COMPETITIVE RELATIONSHIPS BETWEEN ARMILLARIA CALVESCENS and TRICHOLOMOPSIS PLATYPHYLLA. J.J. Worrall. SUNY College of Environmental Science and Forestry, Syracuse, NY.

Armillaria calvescens, the most common Armillaria species in New York, causes root and butt rot in northern hardwoods. Tricholomopsis platyphylla often occurs in stands with Armillaria and is ecologically similar but nonpathogenic. Although A. calvescens colonized primarily stumps and bases of dead trees, T. platyphylla also invaded fallen stems, branches and even twigs. As a result, the frequency of A. calvescens rhizomorphs was much higher in a thinned than in a neighboring unthinned plot, but that of T. platyphylla was high in both plots. The two fungi often occurred together on the same stumps in the thinned plot, but there was a highly significant inverse correlation in the percentage of stump circumference colonized by the two fungi. I hypothesize that, because T. platyphylla can use litter to maintain high rhizomorph frequency, it is able to limit invasion by A. calvescens when suitable resources become available.

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A RAPID METHOD OF DETERMINING WHETHER A CHESTNUT TREE IS SURVIVING BLIGHT DUE TO RESISTANCE OR HYPOVIRULENCE. F. V. Hebard, American Chestnut Foundation, RR 1, Box 17, Meadowview, VA 24361.

There have been no criteria for quickly assessing whether a chestnut tree is surviving blight primarily due to resistance or to hypovirulence in the blight fungus. Chestnut blight cankers can be classified as lethal or non-lethal. Lethal cankers are slightly sunken, with abundant sporulation, and reach the vascular cambium, killing encircled stems. Non-lethal cankers are characterized by swelling of host tissues under and around the cankers, little sporulation, superficial canker development, and survival of encircled stems. The boles and crowns of several types of chestnut trees were examined for the occurrence of lethal and non-lethal cankers. Blight-susceptible American chestnut trees located in MI (ten trees), CT (ten trees) and VA (two trees) that were surviving blight due to hypovirulence had non-lethal cankers on their boles, but several lethal cankers in their crowns. In contrast, ten blight-resistant Chinese chestnut trees, four of their moderately resistant hybrids with American chestnut, and four slightly resistant American chestnut trees had only non-lethal cankers in both their crowns and boles. Thus, the combination of non-lethal bole cankers with non-lethal crown cankers is a preliminary indication of survival due to resistance, whereas the combination of non-lethal bole cankers with lethal crown cankers is a preliminary indication of survival due to hypovirulence in the infecting blight fungus.

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RESISTANT RESPONSES OF LOBLOLLY PINE FAMILIES TO CRONARTIUM QUERCUUM F. SP. FUSIFORME IN THE FIELD AND GREENHOUSE. C. H. Walkinshaw. USDA, Forest Service, Southern Forest Experiment Station and Forest Pest Management, Rt. 3, Box 1249-A, Asheville, NC 28806.

Loblolly pine (Pinus taeda L.) can resist Cronartium quercuum (Berk.) Miyabe ex Shirai f. sp. fusiforme infection by preventing the formation of galls or by stopping the linear growth of galls once they form. Galls fail to form when the pathogen can not reach the host cambium or when invading hyphae cause massive necrosis in the cortex and cambium. Galls that do form, cease to elongate when linear fungal growth is inhibited by the host. This second type of resistance appears to predominate in loblolly pine families that form many branch galls. Since little is known about inhibition of gall elongation in the field, putative resistant gall reactions were examined for 8- to 13-yr-old loblolly pines. Results from the field observations are discussed in relationship to greenhouse findings on the same pine families.

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LEPTOGRAPHIUM PROCERUM INFECTION OF PINE SEEDLINGS FOLLOWING OZONE EXPOSURE. J.A. Carlson and S.A. Alexander. Department of Plant Pathology, Physiology and Weed Science, VPI&SU, Blacksburg, VA 24061-0330.

Fumigation studies were conducted on wild-type eastern white (*Pinus strobus* L.) and loblolly (*P. taeda* L.) pine seedlings to determine whether ozone exposure increased the incidence of infection by *Leptographium procerum* (Kendr.) Wingf. Roots of two-year-old seedlings were inoculated with a mixed conidial suspension of four strains of the fungus. At twenty-four hours post-inoculation and for 14 consecutive days, seedlings were fumigated in closed chambers with charcoal-filtered air or 200 ppb ozone for 5h/day, then removed to a charcoal-filtered greenhouse. Four weeks post-inoculation, roots were surface-sterilized and plated individually on selective medium to evaluate the proportion of the root system infected. The incidence of infection by *L. procerum* was not significantly different between trees receiving ozone or charcoal-filtered air.

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SEASONAL FLUCTUATION IN ANTHRACNOSE INFECTION OF CORNUS FLORIDA SEEDLINGS EXPOSED TO NATURAL INOCULATION. Kerry O. Britton, U.S.D.A. Forest Service, SEFES, Green St., Athens, GA 30602

Dogwood seedlings were exposed under infected trees in two sites at Coweeta Hydrologic Laboratory in SW NC. Every 2 wk from Apr to Oct of 1989 and 1990, 25 healthy seedlings in pots were exposed at each site. Following exposure, seedlings were removed to an incubation room, and two wk later, the percent leaf area infected (LAI) and twig dieback were estimated. In 1989, no significant infection occurred on seedlings exposed prior to 6 June. Seedlings exposed between 6 Jun and 9 Oct averaged >10% LAI in 1989, possibly enhanced by consistent summer rains. In 1990, the first infections occurred a little earlier, 15-29 May. Drought occurred from 11 Jun - 7 Jul and infection was near zero. After rain resumed in mid-July, LAI averaged 20%. Heavy infections appeared to follow leaf wetness periods (WP) of > 48 hr in 1990, except in Sep, when no previous WP > 48 had occurred in 7 wk and WPs of 41 and 93 hr resulted in only 5.5% LAI. In both years, very little infection occurred until 1 month after bloom. Disease severity was dependent on consistent rainfall.

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EFFECTS OF ACID RAIN TREATMENT ON THE FLOWERING DOGWOOD (*CORNUS FLORIDA* L.) LEAF SURFACE: AN SEM STUDY. D.A. Brown, M.T. Windham, E.T. Graham, R.N. Trigiano, R.L. Anderson, and P. Berrang, The University of Tennessee, P.O. Box 1071, Knoxville, TN 37901-1071 and US Forest Service, Asheville, NC 28802 and Dry Branch, GA 31020.

Acidic rainfall has a potential for influencing anthracnose severity in flowering dogwoods (*Cornus florida* L.) of the southeastern USA. One-year-old, nursery-grown flowering dogwood seedlings were treated with 1 cm simulated acid rain weekly for a ten-week period in 1990. The simulated rain was composed of a mixture of salts considered typical of rain in the southeastern USA. Simulated rain pH was adjusted to 5.5, 4.5, 3.5, and 2.5 with sulfuric and nitric acids. Leaf disk samples were cut from the margin, tip, and mid-vein of acid rain-treated trees and prepared for scanning electron microscopy. Damage observed in response to acid rain treatment included cuticular cracking, desiccation, and the erosion of trichome surfaces. Increased degradation of dogwood trichomes was observed with decreasing pH for all samples. However, the degree to which leaf mid-vein samples were damaged by acid rain treatment did not appear as great as samples from the leaf margin and tip. Cuticular erosion due to acid rain has the potential to predispose dogwoods in the southeastern USA to anthracnose caused by *Discula* sp.

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FACTORS AND SPORE EXUDATES EFFECTING GERMINATION TYPE OF FUSIFORM RUST BASIDIOSPORES. P.C. Spaine and S. Kaneko. USDA, Forest Service, SEFES, Athens, GA 30602 and Forestry and Forest Products Research Institute, Kukizaki, Inashiki-gun, Ibaraki-305, JAPAN.

Germination type of basidiospores of Cronartium quercuum f. sp. fusiforme were examined under various washing intervals and

agar, pH, and spore exudate concentrations. Basidiospores that were cast directly from telia predominantly germinated indirectly on agar surfaces. However, after even a few minutes washing in water, the germination type of the basidiospores was altered and the majority were found to germinate directly. Exudates from basidiospores were collected and tested on basidiospores in several bioassays. These exudates were found to increase the percent of direct germination in directly cast spores, but did not change the germination type in washed spores. The exudates inhibited germ tube length in both directly cast and washed basidiospores.

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INTERFERTILITY AMONG ISOLATES OF *ARMILLARIA TABESCENS* IN NORTH AMERICA. T.W. Darmono[1], H.H. Burdsall, Jr.[2], and T.J. Volk[2]. [1]Department of Plant Pathology, University of Wisconsin-Madison, Madison, WI 53706, and [2]Center for Forest Mycology Research, USDA-Forest Service, Forest Products Laboratory, One Gifford Pinchot Drive, Madison, WI 53705.

Monosporous haploid-haploid hypha confrontations and the Buller reaction (vegetative-monosporous confrontations) were performed to investigate the interfertility of North American isolates of *Armillaria tabescens* (Scop.:Fr.) Dennis, Orton & Hora. Isolates derived from collections from northeastern Ohio, Maryland, South Carolina, Georgia, southern Illinois, Florida, and Louisiana were all interfertile as indicated by the production of clamp connections and subsequent basidiome production in haploid-haploid confrontations and morphological changes in the mat character when using the Buller reaction. The evidence indicates that *A. tabescens* in the eastern United States is a single biological species and supports a previous conclusion that *A. tabescens* is bifactorially heterothallic.

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STEM CANKER DISEASE OF EUCALYPTUS GRANDIS IN SOUTH AFRICA CAUSED BY A SPECIES OF CONIOTHYRIUM. G.H.J. Kemp[1], M.J. Wingfield[1] and P. W. Crous.[2] [1]Department of Microbiology and Biochemistry, University of the Orange Free State, Bloemfontein and [2]Department of Plant Patholgy, University of Stellenbosch, Stellenbosch, South Africa.

A serious stem canker disease has recently been observed on stems of young, clonally propagated Eucalyptus grandis in the Natal province. This disease is characterised by small necrotic lesions on the young green bark that ultimately result in considerable stem malformation. A species of Coniothyrium is consistently found on the lesions and indications are that this fungus is presently undescribed. Inoculation onto young stems of susceptible clones resulted in symptoms typical of those observed under natural conditions. This canker disease is widespread in Natal and has resulted in the abandonment of a number of clones from the planting program. Field surveys and inoculation trials have been established to select clones with tolerance to this important disease.

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CRYPHONECTRIA CANKER OF EUCALYPTUS IN SOUTH AFRICA. M.J. Wingfield, W.J. Swart and G.H.J. Kemp. Departments of Microbiology and Plant Pathology, University of the Orange Free State, P.O. Box 339, Bloemfontein 9300 South Africa.

Cryphonectria cubensis causes a serious canker disease of Eucalyptus species in many tropical parts of the world. The pathogen has recently been found in South Africa for the first time and is associated with severe basal cankers on young trees. Its occurrence has led to concern due to the damage that it could cause to extensive plantings of clonally propagated Eucalyptus. Strategies to assess the potential threat of C. cubensis to local forestry include studies to determine the origin of the pathogen as well as the establishment of field surveys and inoculation trials. Preliminary results of these studies have shown that Cryphonectria canker is widespread in the country. Considerable variability also exists in the susceptibility of E. grandis clones and hybrids.

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MOLECULAR GENETIC RELATIONSHIPS OF *NECTRIA COCCINEA* VAR. *FAGINATA*, A PATHOGEN OF AMERICAN BEECH, TO EUROPEAN AND NORTH AMERICAN NECTRIAS. Eileen M. Mahoney, USDA Forest Service, Hamden, CT 06514, and Cornell University, Dept. Plant Pathology, Ithaca, NY 14853.

Nectria coccinea var. *faginata* (Ncf) is known as a pathogen only on American beech (*Fagus grandifolia*) infested with the scale insect, *Cryptococcus fagisuga*. Whether it was a native fungus that took advantage of the niche provided by the introduced insect, or an introduced fungus remains a question. In this study, Ncf was compared to seven other taxa of *Nectria*: *N. coccinea* (Nc), *N. galligena* (Ng), *N. cinnabarina*, *N. haematococca*, and *N. ochroleuca* from North America, plus *N. coccinea* var. *coccinea* (Ncc) and *N. viridescens* from Europe. Southern blotted DNA from genomic digests was probed with five randomly selected single-copy nuclear clones and one mtDNA clone of Ncf DNA, plus the ribosomal gene cluster of *Cochliobolus heterostrophus*. Under high-stringency conditions, the nuclear probes hybridized well with DNA from all Ncf and Ncc isolates and one Nc isolate; some restriction fragments were common between taxa. The mtDNA probe hybridized well with the Ncf, Ncc and Ng isolates; most Ncf restriction patterns were present in Ncc. The ribosomal gene cluster only detected common restriction fragments within taxa. These analyses indicate that Ncf is most closely related to Ncc and support the hypothesis that Ncf may have been introduced from Europe.

56

ROOT DISEASE-ASSOCIATED MORTALITY OF PINUS OCCIDENTALIS IN MOUNTAINOUS AREAS OF THE DOMINICAN REPUBLIC. R.S. Webb, 208 Newins-Ziegler Hall, Department of Forestry, University of Florida, Gainesville, FL 32611.

Dense stands of planted and naturally-regenerated Pinus occidentalis comprise most of the arboreal vegetative cover in mountainous watershed areas in the northern Dominican Republic. Despite governmental regulation, forest cover has decreased from approximately 22% to 12% since 1967 from illegal cutting. Additional losses have been realized recently by the increasing incidence of pine mortality centers. Individual trees die and surrounding pines exhibit crown symptoms associated with root disease. Symptomatic trees die and the centers continue to increase in spatial diameter, particularly down slope from the initial mortality locus. Preliminary sampling of roots from dead and dying pines in six mortality centers has yielded frequent cultures of Phytophthora cinnamomi and an as yet undetermined species of Verticicladiella. Pathological rotation guidelines are being formulated to minimize losses and spread.

57

ACTIVE DISCHARGE DISTANCE OF VENTURIA INAEQUALIS ASCOSPORES: RELATIONSHIP TO AIRBORNE ASCOSPORE DOSE. Donald E. Aylor, The Connecticut Agric. Exper. Sta., Box 1106, New Haven, CT 06504.

Ability to estimate the airborne dose of ascospores (AAD) of Venturia inaequalis (VI) in an apple orchard canopy from knowledge of levels of inoculum on the orchard floor is important for making decisions about timing sprays to control scab. A critical determinant of AAD is the distance that ascospores are discharged into the air. VI ascospores were discharged into still air from 0.1 to 13.2 mm. Only 1% were projected > 6.6 mm. In comparison to leaves on bare ground, apple leaves nestled within a ground cover canopy will be exposed to a much reduced wind, consisting of relatively long quiescent periods and occasional gusts. Ascospore discharge distances are short compared to the region of relatively quiescent air near the ground inside dense ground cover. Model calculations suggest that it might be possible to reduce AAD in the orchard canopy by a factor of 10 or more by allowing the ground cover to grow > 50 cm tall during the ascospore discharge season.

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DEVELOPMENT OF A RANKING SYSTEM TO ASSESS CARNATION CULTIVARS FOR RESISTANCE TO *UROMYCES DIANTHI*. M. Polek and D. M. Ferrin, Department of Plant Pathology, University of California, Riverside 92521.

Four cultivars of carnations (Improved White Sim, Yellow Candy, Nora, and Scania) were used to analyze methods of disease assessment to rank cultivars for resistance to *Uromyces dianthi*, the causal agent of rust on carnations. Plants were inoculated with a suspension of 10^5 urediospores/ml and incubated at 10, 15, or 20 C. Disease was assessed over the 2-wk period following the first emergence of pustules. Data were categorized by length of latent period, total pustules per plant, pustules per leaf, pustules per number of infected leaves, and proportion of the leaves infected. Since the cultivar rank was not consistent among individual assessment categories, the final rank was determined by summing across all categories. This method showed that Improved White Sim was most susceptible, followed by Nora, Scania, and Yellow Candy.

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UREDINIOSPORE PRODUCTION AND RELEASE IN RELATION TO RACE-NONSPECIFIC RUST RESISTANCE IN COMMON BEANS. M. T. Mmbaga and J. R. Steadman, Dept. of Plant Pathology, University of Nebraska, Lincoln, NE 68583-0722.

Six pubescent and three glabrous genotypes of Phaseolus vulgaris from diverse genetic and geographic origin were inoculated on the primary leaves with uniform amounts of urediniospores. Air turbulence was created in small chambers on each genotype and spore release was measured by the secondary infection they produced on the trifoliolate leaves and by spore counts. Sporulating capacity of the primary and trifoliolate leaves was measured on each genotype. Seven genotypes showed a moderately susceptible reaction to rust on the primary leaves and reduced uredinia size and density on the upper leaves compared to the susceptible Pinto U.I. 114. The same genotypes had low sporulating capacity and low spore release on the primary and trifoliolate leaves. Lower cellular susceptibility on the trifoliolate leaves was also indicated. Six out of the seven genotypes were pubescent. These findings have epidemiological implications that relate to race-nonspecific resistance reported for pubescent beans.

60

MODELLING INTERACTIONS BETWEEN ERYSIPHE GRAMINIS AND SEPTORIA NODORUM ON WHEAT. G. E. Weber & J. Kranz, Giessen University, Tropeninstitut, Schottstr. 2, 6300 Giessen, GERMANY.

A minimal interaction model, given logistic growth of a single pathogen, consists of modified Lotka-Volterra equations with rate, capacity and interaction parameters. An extended interaction model was created using the linked differential equations for description of a single pathogen epidemic published by JEGER (Phytopath. 72:1185-9). S. nodorum was considered as competing with E. graminis for infection sites and was therefore linked to its capacity term. By contrast E. graminis exerted a positive influence on S. nodorum which was modelled using two different approaches. It was assumed firstly that E. graminis increased S. nodorum capacity, secondly that E. graminis increased a S. nodorum rate parameter. Good model fits with nonzero interaction coefficients were obtained for greenhouse and field experiments with artificial inoculation. However further experiments are needed to determine the most appropriate interaction model

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GRAY LEAF SPOT OF CORN AS INFLUENCED BY THE AMOUNT OF SURFACE CORN RESIDUE. N. R. X. de Nazareno, P. E. Lipps and L. V. Madden, Dept. of Plant Pathology, The Ohio State University and Ohio Agricultural Res. and Dev. Center, Wooster, OH 44691.

The effect of the amount of infected corn residue on the soil surface on gray leaf spot, caused by Cercospora zeae-maydis, was studied in 1990 at Wooster, Ohio. Four levels of surface residue, simulating tillage practices (0%, 10%, 35%, and 85% coverage), were compared. Plots consisted of five rows (3.7 m long, 0.76 m apart) of the susceptible hybrid Pioneer Brand 3569 and were arranged in a randomized complete block with four replications. Treatment plots were separated by three rows of a resistant hybrid. Disease severity was estimated weekly by counting lesions on three leaves of eight tagged plants in the center row of all plots. Disease proportions were obtained by dividing the total estimated lesion area by the estimated leaf area. Analysis of variance of the areas under disease progress curves (AUDPC's) indicated a significant effect of residue level on disease severity. AUDPC values were 0.027, 0.077, 0.090, and 0.237 for the residue levels of 0%, 10%, 35%, and 85%, respectively. Disease severity for 10% and 35% residue levels did not differ significantly but were different (P=0.05) from 0% and 85% residue levels.

62 Withdrawn

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COMPUTER SIMULATION OF TROPICAL RICE-LEAF BLAST PATHOSYSTEM USING BLASTSIM.2. P.S. Teng and S.B. Calvero. International Rice Research Institute, P.O. Box, 933, Manila, Philippines.

BLASTSIM.2, a simulation model of P. grisea epidemics is written in FORTRAN for microcomputer applications. The model integrates both mathematical equations and 3-dimensional matrices to explain state and driving variables in the monocycle. Dynamic simulation is improved by coupling a dew formation model that uses as input the following -- crop, micro-, and macro-climatic factors. Model structure further differs from BLASTSIM.1 by incorporation of spore release and deposition, latency, and the receptivity factors of specific variety-blast isolate combination. Sensitivity analysis of BLASTSIM.2 was done using a 1988 lowland-irrigated experiment on blast conducted at IRRI, Los Baños. Simulated disease severity corresponded with the observed data which suggested potential use of the model for blast disease management.

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RELATIONSHIPS AMONG SPREAD, AGGREGATION, MEAN, AND LOGISTIC GROWTH OF PLANT DISEASE. X.B. Yang and D.O. TeBeest, Dept. of Plant Pathology, Univ. of Arkansas, Fayetteville, AR 72701.

From logistic model and negative binomial distribution, it was found that the reduction of disease growth rate (dx/dt) by aggregation follows $Y = 1 - (1 - x)^{1/k}$, so the reduction depends on disease mean x and the negative binomial k. Because x and k increase during the epidemic, effect of aggregation on disease growth rate depends on how quickly an aggregation distribution conveys to randomness. Data from several diseases showed that change of disease aggregation (dk/dx) during an epidemic is related to the rate of spread of a disease. For a rapidly spreading disease, high aggregation in the early season may have little effect on disease growth rate because of a small value of disease mean. Later in the season, there may still be little effect even when disease mean is high because a quick dispersion reduces the degree of aggregation (1/k).

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EFFECTS OF TEMPERATURE ON PRODUCTION OF PERITHECIA AND PYCNIDIA BY *DIAPORTHE PHASEOLORUM* VAR. *CAULIVORA*. G.B. Padgett, J.P. Snow, and G.T. Berggren. Department of Plant Pathology and Crop Physiology, Louisiana Agricultural Experiment Station, Louisiana State University Agricultural Center, Baton Rouge, LA 70803.

Experiments were conducted to evaluate the effects of temperature on the development of perithecia and pycnidia by *Diaporthe phaseolorum* (Cke. & Ell.) var. *caulivora* Athow and Caldwell (Dpc), pathogen of soybean stem canker. Soybean stems of a susceptible cultivar (Bedford) with perithecia primordia were collected during the winter from a field where stem canker epidemics had occurred during the past three years. Stems were placed in soil-filled containers (14% moisture) and subjected to one of seven temperature regimes (3, 7, 15, 20, 25, 30, and 35 C). In other experiments, autoclaved stems (Bedford) were placed in petri dishes and inoculated with Dpc before being placed in an incubator. After 41 or 54 days of incubation, perithecia and pycnidia were quantified. Pycnidia developed before perithecia and were observed only on stems incubated at 20 and 25 C. Numbers of perithecia were greatest on stems incubated at 25 C. Perithecia and pycnidia were observed on stems incubated only at 15, 20, 25, and 30 C.

66

MECHANISMS OF ALTERED BEAN RUST EPIDEMIOLOGY DUE TO INTERCROPPING WITH MAIZE. M.A. Boudreau and C.C. Mundt, Dept. of Botany and Plant Pathology, Oregon State University, Corvallis 97331.

Components of the intercropping effects of maize on rust of common bean were quantified. The effects of competition with maize and interference by maize on dispersal of rust urediniospores were evaluated in trials conducted at 3 times during both 1989 and 1990. Alteration of the infection phase of the pathogen life cycle due to intercropping and competition with maize were assessed following each experiment. Competition consistently and significantly ($P<0.10$) steepened the dispersal gradients, as described by the modified Gregory model, by 50 days after planting. Interference tended to flatten gradients in the absence of competition. Competition and interference in combination (intercrop) produced steeper gradients, with a significant ($P<0.10$) interaction between the two factors in one experiment. Competition alone had no effect on infection efficiency, though intercropping as a whole reduced rust severity by 96% ($P<0.05$) late in 1989. Microclimatic changes created by maize are likely responsible for the effect on infection efficiency, and steeper gradients may be due to increased spore escape from the plot.

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MULTIPLE DISEASES OF WHITE CLOVER: A COMPARATIVE ANALYSIS OF SPATIAL PATTERN. Scot C. Nelson and C. Lee Campbell. Department of Plant Pathology, North Carolina State University, Raleigh 27695-7616.

Incidence of bacterial (Pseudomonas andropogonis) and Cercospora (Cercospora zebrina) leaf spots and a viral disease complex were monitored for two 6-wk epidemics in 1990 on virus-susceptible (cv. Regal) and virus-resistant (Southern Regional Virus Resistant germplasm) white clover planted in eight 64-plant plots in a 10 ha clover/tall fescue pasture. Two-dimensional distance class analysis revealed clustering of infected plants for all diseases.

More Pseudomonas-infected plants were found at plot edges, in tight clusters and/or in longer runs than expected under the hypothesis of a random distribution of infected plants. Cercospora leaf spot was not found at plot edges, but infected pairs of plants occurred in close proximity. Pairs of virus-infected plants of Regal were more often proximate, at plot edges, and in longer runs than were virus-infected plants of the Southern Regional Virus Resistant germplasm.

68

THE INFLUENCE OF STRIPE RUST ON COEVOLVING WHEAT POPULATIONS. M. R. Finckh and C. C. Mundt. Dept. of Botany and Plant Pathology, Oregon State University, Corvallis, OR 97331-2902.

Twenty populations that were mixtures of four cultivars of wheat (Triticum aestivum) were grown for one to three generations at two locations. Populations were either exposed to stripe rust (Puccinia striiformis), protected from stripe rust, or exposed to alternating years of diseased and disease-free conditions. Disease severity and crop reproductive success (fitness) were determined for each genotype separately in each population. Our hypothesis was that fitness of a cultivar in a mixed population is frequency-dependent in the presence of disease because disease severity declines with decreasing cultivar frequency. Competitive interactions among the cultivars were enhanced by disease. Extinction of certain cultivars in the populations might be accelerated by stripe rust.

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EVALUATION AND VALIDATION OF THE GEORGIA LATE LEAFSPOT ADVISORY MODEL. F. W. Nutter, Jr. and A. K. Culbreath. Departments of Plant Pathology, Iowa State University and University of Georgia Tifton 31793 respectively.

Experiments were conducted at Plains and Tifton, GA, to validate and compare the Georgia Late Leafspot Advisory System with the 10-14 day calendar spray system currently recommended by the Cooperative Extension Service, and to determine the efficacy of different fungicides when applied according to the Georgia Advisory Model. Percent defoliation and the number of late leafspot lesions/leaflet were determined weekly from stems sampled from each replicate plot. Canopy temperature and hours of leaf wetness were monitored in each field using an EnviroCaster (Neogen Corp., Lansing, MI). The Georgia Advisory Model successfully predicted the initial appearance of late leafspot lesions at both locations. Although an average of 3 fewer sprays were applied using the GA model, there were no significant differences in the level of disease control, pod yield, or pod quality compared to the calendar spray schedule using chlorothalonil. The yield risk relative to mean yields (coefficient of variation) was lowest for the GA Advisory Model and highest for nonsprayed plots. All fungicides tested provided good control of late leafspot when applied according to the GA model.

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DETECTING AND MODELING PATTERNS OF LONG-TERM DYNAMICS OF PLANT PATHOSYSTEMS. X.B. Yang, Dept. of Plant Path., Univ. of Arkansas, Fayetteville; S.M. Zeng, Dept. of Plant Protection, Beijing Agri. Univ., Beijing, China.

Methods of time series were used to detect dynamic patterns in a plant pathosystem. Analysis of 41-year pandemic series of wheat stripe rust showed a possible 4-year cycle in the occurrence of pandemics in northern China pandemic system. The epidemic series of the source region was different from the series of dispersion regions. The epidemic series of four dispersal regions were identical in the cycle pattern but different in the degree of epidemics. Analysis of 63-year phenotypical diversity series of wheat stem rust showed a possible 5-year cycle in change of race composition in North America. Analysis of a 73-year yield series in a field fertilized only with farmyard manures in England showed a natural 3-year cycle in wheat yield. ARIMA models provided satisfactory long-term predictions for these systems.

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ANALYSIS OF SPATIAL PATTERNS OF DISEASE INCIDENCE IN CROPS. Gareth Hughes, School of Agriculture, University of Edinburgh, Edinburgh, EH9 3JG, Scotland, U.K.

The power-law relationship $\log(\underline{v}) = \underline{a} + \underline{b}.\log(\underline{m})$ has been used to detect aggregated spatial patterns of many organisms. However, when disease is measured as incidence (proportion of plants affected), this relationship does not always provide a good description of the relationship between sample variance ($\underline{v}$) and sample mean ($\underline{m}$). Field data show that problems arise because $\underline{v}$ may not continue to increase as $\underline{m}$ approaches a maximum, and may even decrease. In such cases, the data can better be described by power-law relationships between $\underline{v}$ and the expected binomial variance. These relationships allow a spatial interpretation similar to that of the usual form of the power-law, and have two further interesting characteristics : they suggest the beta distribution as a basis for simulating patterns of disease incidence in crops; and they can be linked to crop loss assessment models which incorporate the effect of both pattern and level of disease.

72

SIMILARITY BETWEEN COPPER RESISTANCE GENES FROM *XANTHOMONAS CAMPESTRIS* PV. *VESICATORIA* AND *PSEUDOMONAS SYRINGAE* PV. *TOMATO*. A. E. Voloudakis[1], D. A. Cooksey[1], and C. L. Bender[2], Dept. of Plant Pathology, [1]University of California, Riverside 92521, and [2]Oklahoma State University, Stillwater 74078.

Previous hybridization experiments suggested that copper resistance genes in *X. c.* pv. *vesicatoria* from Florida and Oklahoma were not related to the copper resistance operon (*cop*) from *P. s.* pv. *tomato* from California. However, we recently cloned copper resistance genes from strain 07882 of *Xcv* from California that hybridized with *cop* under conditions of lower stringency. Further hybridization and mapping studies have shown that the resistance genes from 07882 are very similar to those from the strains in Florida and Oklahoma, and that they are all related to *cop*. The similarity appears to be restricted to the first gene of the *cop* operon, *copA*. A copper-inducible protein of similar size to the *copA* product was detected by western blot analysis of proteins from Cu^r strains of *Xcv* and from a sensitive strain containing cloned resistance genes.

73

A Gene Required for Pathogenicity of *Pseudomonas syringae* pv. *syringae* on Bean Belongs to a Family of Bacterial Regulators. E. M. Hrabak & D. K. Willis, Department of Plant Pathology and USDA/ARS, University of Wisconsin, 53706.

The *lemA* gene of *P. syringae* pv. *syringae* B728a is required for disease lesion formation on bean. In addition, production of protease and the toxin, syringomycin, require a wild-type *lemA* gene. Sequence analysis of the DNA region containing the *lemA* gene revealed a large open reading frame which had similarity at both the nucleic acid and amino acid levels to a class of bacterial regulatory proteins commonly referred to as two-component regulators. Amino acid sequence motifs which are thought to be important in the biological activity of these regulators are also highly conserved in the putative *lemA* amino acid sequence.

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REGULATION OF VIRULENCE IN *PSEUDOMONAS SOLANACEARUM* BY AN ENDOGENOUS VOLATILE COMPOUND. S. J. Clough and T. P. Denny, Dept. Plant Pathology, Univ. of Georgia, Athens, GA 30602.

Similar to phenotype conversion (PC) mutants, *Pseudomonas solanacearum* mutant AW1-83 is deficient for extracellular polysaccharide (EPS) and endoglucanase (EG), two important virulence factors. When grown on split-plates, EPS production by AW1-83 was reversibly induced by a volatile compound synthesized by all 80 wild-type strains of *P. solanacearum* tested. In contrast, PC-type (*phcA*) mutants were not inducible and produced very little of the volatile inducer. AW1-83 was stimulated to produce wild-type levels of both EPS and EG when co-cultured in broth with an EG^-, EPS^-, inducer strain. Both EPS and EG production by AW1-83 were restored by a cloned 4.3-kb wild-type region that is distinct from *phcA*. Based on this and other data, we hypothesize that the endogenous volatile compound is an inducer that is necessary for PhcA to efficiently activate transcription.

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PROTEIN-DNA BINDING INTERACTIONS IN THE COPPER-INDUCIBLE EXPRESSION OF THE *COP* OPERON OF *PSEUDOMONAS SYRINGAE* PV. *TOMATO*. S. D. Mills and D. A. Cooksey, Department of Plant Pathology, University of California, Riverside, CA 92521.

The copper-inducible promoter region from the copper resistance operon (*cop*) of *Pseudomonas syringae* pv. *tomato* was used in DNA mobility-shift experiments with protein extracts from copper-resistant strains. A DNA-binding protein was identified by a protein-DNA shift, which was observed when extracts of cells grown without copper were incubated with the cloned promoter DNA. The protein appeared to be chromosomally encoded, and it bound specifically to the *cop* promoter. No shift was observed when cells were grown with copper. The copper-induced disruption of the DNA-protein complex required a trans-acting factor from the copper resistance plasmid, pPT23D. These data suggest a model for copper-inducible gene expression in which a copper-responsive factor from the plasmid causes the release of a chromosomally-encoded repressor protein.

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CHARACTERIZATION OF COPPER- AND STREPTOMYCIN-RESISTANT STRAINS OF PSEUDOMONAS SYRINGAE. G. W. Sundin and C. L. Bender, Department of Plant Pathology, Oklahoma State University, Stillwater, OK 74078-9947.

Strains of Pseudomonas syringae resistant to copper (Cu), streptomycin (Sm), or both were isolated from cottonwood, ornamental pear, and willow exhibiting tip dieback and cankers. Resistant strains contained a single plasmid which ranged in size from 60-200 kilobase pairs (kb) among strains. Plasmids encoding both Cu^r and Sm^r were transferred by conjugation from their respective donors to a sensitive strain of P. syringae at frequencies ranging from 1.4×10^{-2} to 9.3×10^{-8}. Strong homology at high stringency was demonstrated between the P. syringae plasmids and the Sm^r genes from the broad-host-range plasmid RSF1010. This homology was used to clone the P. syringae Sm^r gene(s) on a 3.8 kb PstI fragment in pBluescript II SK.

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CLONING AND CHARACTERIZATION OF CORONATINE GENES IN PLASMID p4180A FROM PSEUDOMONAS SYRINGAE pv. GLYCINEA 4180. S. A. Young, C. L. Bender, S. K. Park, Department of Plant Pathology, Oklahoma State University, Stillwater, OK 74078-9947.

We have recently demonstrated that coronatine synthesis genes reside on a 90 kb plasmid (p4180A) in Pseudomonas syringae pv. glycinea PG4180. In the present study, a cosmid library of p4180A was constructed in pLAFR3. A 54 kb cosmid clone, designated pSAY10, restored coronatine production to two coronatine-defective mutants of PG4180, indicating that pSAY10 contains some of the genes required for coronatine synthesis. pSAY10 was subjected to saturation mutagenesis with Tn5 and mutants selected were conjugated into PG4180 and recombined into p4180A by marker-exchange. Organic acids were extracted from these mutants and analyzed by reverse-phase high performance liquid chromatography to determine the effect of various Tn5 insertions on coronatine synthesis. Mutations in two regions of pSAY10 (17.9 and 2.2 kb) completely blocked the synthesis of coronafacic acid and coronatine.

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TABTOXIN BIOSYNTHESIS IS CONTROLLED BY A REGULATORY GENE THAT IS FUNCTIONALLY CONSERVED IN MANY PATHOVARS OF *PSEUDOMONAS SYRINGAE*. T. M. Barta, E. M. Hrabak, T. G. Kinscherf, and D. K. Willis. Department of Plant Pathology and USDA/ARS, University of Wisconsin, Madison, WI 53706

Pseudomonas syringae pv.*coronafaciens* was mutagenized with Tn*5* in order to isolate derivatives defective in tabtoxin production. Seven mutants were isolated that lacked tabtoxin production (Tox^-). The Tn*5* insertions in all seven strains were in *lemA*, a gene first shown to be required for the ability of *P. syringae* pv. *syringae* to form lesions on bean. All strains of *P. syringae* examined to date have DNA that hybridizes to *lemA*. Furthermore, the cloned *lemA* genes from several strains, including those that do not produce tabtoxin, restore tabtoxin production to the Tox^- mutants. According to Northern Blot analysis, *lemA* was required for the detection of a 1 kb transcript from within the tabtoxin biosynthetic gene cluster, which has been cloned as a single unit. Therefore, *lemA* appears to positively regulate the transcription of tabtoxin biosynthetic DNA.

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MOLECULAR CHARACTERISTICS OF AVIRULENCE GENE D HOMOLOGUE FROM PSEUDOMONAS SYRINGAE PV. PHASEOLICOLA. I. Yucel and N. T. Keen, Dept. of Plant Pathology, University of California, Riverside, CA 92521.

Avirulence gene D (*avr*D) from *Pseudomonas syringae* pv. *tomato* and its homologue from *P. s.* pv. *glycinea* were previously found to have 86% amino acid identity, with substitutions occurring throughout the protein. Despite the high degree of identity, the *P. s.* pv. *glycinea* homologue was unable to elicit the hypersensitive response in soybean. To elucidate regions necessary for *avr*D activity, the homologue from *P. s.* pv. *phaseolicola* was characterized and found to have the *avr*D phenotype on test cultivars of soybean. DNA sequence analysis revealed that the degree of identity between this homologue and *avr*D was similar to the comparison made above. Further sequence comparison between the homologues from *P. s.* pv. *phaseolicola* and *glycinea* disclosed a 96% amino acid identity with only five substitutions, clustered mainly at the carboxyl end of the protein. Chimeric constructs and site-directed mutagenesis were used to determine positions important in *avr*D function.

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CLONING AND CHARACTERIZATION OF *PHCA*, A REGULATORY GENE OF *PSEUDOMONAS SOLANACEARUM*. *S.M. Brumbley*, B.F. Carney and T.P. Denny, Dept. Plant Pathology, Univ. of Georgia, Athens, GA 30602

Pseudomonas solanacearum strain AW1 requires a functional copy of the regulatory gene *phcA* to maintain wild type levels of several virulence factors. Spontaneous mutation of *phcA* to a nonfunctional allele results in phenotype conversion (PC). The DNA sequence of *phcA* revealed an open reading frame of 1041 bases, which should encode a protein of about 38 kDa. The first 700 nucleotides of the gene are 53.1% homologous with *nahR*, a gene encoding a transcriptional activator in *Pseudomonas putida*. When over expressed in *Escherichia coli*, *phcA* encoded a polypeptide of approximately 38 kDa. A nonfunctional allele (phcA1) from a PC-mutant (AW1-PC) encoded a protein of approximately 35.5 kDa; truncation was due to a two base pair insertion 147 bases upstream of the carboxyl terminus of PhcA.

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A PUTATIVE DNA INVERTASE FOUND ADJACENT TO THE AVIRULENCE GENE D IN *PSEUDOMONAS SYRINGAE PATHOVAR TOMATO*. M. Stayton, T. Hanekamp, D. Kobayashi, S. Hayes, S. Tamaki, and *N. Keen, Univ. of Wyoming, Dept. of Molecular Biology, University Station P.O. Box 3944, Laramie, WY 82071, and *Univ. of California, Riverside, Dept. of Plant Pathology, Riverside, CA 92521-0122.

The bacterial plant pathogen *Pseudomonas syringae pv. tomato* elicits a hypersensitive response in all soybean (*Glycine max*) cultivars. Avirulence gene D (*avrD*) of the pathogen contributes to the induction of resistance via interaction with the soybean disease resistance gene, *Rpg4*. The *avrD* gene has been isolated and is linked to five, downstream tandem open reading frames (Kobayashi, D. et. al. (1990) *Molec. Plant-Microbe Interact.* **3**:94-102). This organization suggests that these genes may be functionally related. Here we present the DNA sequence 5.4 kb upstream of the avrD gene. Nine possible open reading frames (ORF's) have been identified. An extended region of 1.1 kb shows remarkable sequence identity with a pectate lyase-linked sequence of the soft rot bacterium *Erwinia carotovora*. Furthermore, the open reading frame (ORF 6) adjacent to the *AvrD* gene shows significant sequence identity to known DNA invertases and resolvases. Experiments confirming the functionality of the DNA invertase and its relationship to the *avrD* gene are currently in progress.

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EFFECT OF MOIST PERIOD ON RESPONSE OF WHEAT CULTIVARS TO INFECTION BY FUSARIUM GRAMINEARUM. Bai, G-H., Shaner, G., Dept. Botany and Plant Pathology, Ohm, H., Dept. of Agronomy, 1155 Lilly Hall, Purdue University, West Lafayette, IN 47907-1155

Wheat cultivars were inoculated with *Fusarium graminearum* by applying 10^4 spores to a floret of a central spikelet at early anthesis. Plants were kept moist for 16 hr/day for 0, 1, or 3 days, at ca. 24C. Symptoms developed 3 to 6 days after inoculation. Cultivars differed in percentage of scabbed spikes (incidence) and percentage of scabbed spikelets (severity). Ning 7840, Sumai 49, Sumai 3, and Fu 5115 had incidences of 70% and severities of 5% compared to 91% and 96% for Clark. Ning 8331 and Ning 8306 were moderately resistant, with an incidence of ca. 75% and a final severity less than 30%. Ning 84R10 and Morocco had a high incidence, but moderate final severity. Incidence increased and incubation period decreased as moist periods increased. Resistant and susceptible cultivars did not differ in incidence after a moist period, but did differ when there was no moist period.

Resistance to spread of the fungus within a spike, as reflected by severity, is a relatively stable characteristic of cultivars, but resistance to infection, as reflected by incidence, depends on moisture and is unstable.

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REACTION OF CORN LINES FROM UGANDA TO EXSEROHILUM TURCICUM. E. Adipala, P. E. Lipps, and L. V. Madden, Dept of Plant Pathology, Ohio State Univ., Wooster, OH 44691

Seedlings and mature plants of nine corn lines from Uganda were inoculated to evaluate their level of resistance to Exserohilum turcicum races 0, 1, 23, and 23N. Field studies were conducted with races 0 and 1 only. Resistance was consistently expressed in only one accession, cv. Babungo. Correlations between lesion type and number of lesions per plant ($r=0.73$), between lesion number and lesion length ($r=0.72$) and between lesion type and lesion length ($r=0.65$) were high and significant ($P<.001$). The correlations describing the relationship between number of conidia per lesion and lesion type ($r=0.32$) and between number of conidia per lesion and lesion length ($r=0.24$) were low and nonsignificant. Correlations among factors such as lesion type, relative increase in lesion length over time, conidial production, area under the disease progress curve and apparent infection rate were useful for evaluating resistance to E. turcicum.

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COMPARISON OF GREENHOUSE SEEDLING BLIGHT AND FIELD SCAB INDUCED BY *FUSARIUM GRAMINEARUM* IN SPRING WHEATS. R. P. Woodward and R. D. Wilcoxson, Department of Plant Pathology, University of Minnesota, St. Paul, MN 55108.

Susceptibility of ten spring wheat cultivars to *Fusarium graminearum* (*Fg*) was compared in the greenhouse and field. Cultivars were planted in autoclaved soil infested (450 CFU/g soil dw) with an *Fg* isolate from one of eight diverse geographic locations and maintained in the greenhouse for six weeks. Field scab screenings utilized repeated applications of a composite inoculum of 25 Minnesota *Fg* isolates. Disease was assessed in the greenhouse by seedling stand counts at 3 and 5 weeks after planting and by area under the disease progress curve. Significant differences ($P=0.01$) in susceptibility were noted among cultivars in both settings, yet only the two cultivars found to be most susceptible in the field reacted similarly in all greenhouse disease assessments. Greenhouse seedling blight tests may be used to eliminate highly susceptible cultivars from scab resistance breeding programs prior to field screenings.

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VARIATION OF NET BLOTCH REACTION DUE TO ISOLATES OF *PYRENOPHORA TERES* F. SP. *TERES* AND BARLEY CULTIVARS AND LINES. Tarkus Suganda and Roy D. Wilcoxson, Department of Plant Pathology, University of Minnesota, St. Paul, MN 55108.

Twenty-six single spore isolates of *P. teres* f. sp. *teres* from northern Minnesota were tested in glasshouse on 17 barleys that possessing resistance to a Minnesota isolate that was pathogenic on Larker, Morex and Robust, leading cultivars from 1962-1990. Nineteen different pathogen isolates were identified on the basis of differential reactions on barley cultivars and lines, suggesting a wide range of variation in the pathogenic potential of the pathogen. No isolate was pathogenic to all barleys tested, whereas 3 isolates were pathogenic on 5-7 of the barleys, 4 on 3-4 barleys, 7 on 2 barleys, and 5 isolates were pathogenic on only 1 barley. Cultivars and lines resistant to all isolates were Abyssinian, Algerian, Coast, Prato, ND 5883, Fr 92677, CI 5791 and CI 5822. Lines M81-111 and ND B112 were also resistant to all isolates except isolate A, and M76-160 and JR4T-2 were resistant to all isolates except isolate Z.

87

ISOLATION OF *SEPTORIA NODORUM* FROM SEED WITH A NEW SELECTIVE MEDIUM. Barry M. Cunfer and Juju B. Manandhar. Department of Plant Pathology, University of Georgia, Georgia Station, Griffin 30223.

A medium, designated SNAB (*S. nodorum* agar for barley), for selective isolation of the barley biotype of *Septoria nodorum* from barley seed contains per L of deionized water: potato dextrose agar (10 g), peptone (2 g), oxgall (1.5 g), and agar (15 g). After autoclaving, chloroneb (5 mg), $Cu(OH)_2$ (10 mg), dicloran (7.5 mg), Ciba-Geigy CGA-449 (1 mg), chloramphenicol (3.13 mg), erythromycin (3.13 mg), tetracycline HCl (12.5 mg), and neomycin SO_4 (10 mg) are added to suppress fungi and bacteria. Paraquat (67 µl) is added to reduce seed sprouting. Seeds are surface-sterilized in 0.5% NaOCl prior to plating. Recovery of *S. nodorum* averaged 23% on SNAB and 13% on oxgall agar. *S. nodorum* was easily differentiated from other fungi on SNAB and it sporulated in <10 days. *S. nodorum* did not sporulate on oxgall agar.

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EXTENSIVE OCCURRENCE OF SEEDBORNE *STAGONOSPORA NODORUM* IN NEW YORK WINTER WHEAT. D. Shah and G. C. Bergstrom, Department of Plant Pathology, Cornell University, Ithaca, NY 14853-5908.

Stagonospora nodorum (Berk.) Cast. & Germ., incitant of Septoria nodorum blotch, is the predominant foliar fungal pathogen of winter wheat in New York. Previous field surveys indicated that seedborne inoculum may be a significant factor in initiation of epidemics. In order to ascertain the extent of seedborne *S. nodorum* in New York in 1990, 50 seed lots were randomly selected from those submitted for certification (i.e., germination) testing. Each lot was screened for the percentage of seed infected by *S. nodorum* using both a wet blotter seedling symptom test and an agar fluorescence test. Two hundred random seed per lot were screened in each test. The average incidence of infected seed in the lots was ca. 23% (range 3-58%), regardless of the method used. Germination was not affected significantly by infection by *S. nodorum*. Our results suggest that most winter wheat sown in fall 1990 with seed produced in New York in summer 1990 had a potential seedborne source of inoculum of *S. nodorum*.

89

INCIDENCE OF SEEDBORNE DISEASES OF BARLEY IN EAST AZARBAIJAN, IRAN. M. Babadoost, Department of Plant Protection, College of Agriculture, University of Tabriz, Tabriz 51664, Iran.

Seedborne diseases of barley in East Azarbaijan, a northwestern province of Iran, are considered to be economically important. In a field survey in 1990, 97 winter and spring barley fields in irrigated and rain-fed areas throughout the province were visited. In this survey spot blotch, scald, barley stripe, smuts, and Fusarium diseases were found in 4, 8, 64, 68, and 100% of the fields respectively. The diseases were present in both winter and spring barley fields. Severities of spot blotch and scald were very low. Barley stripe with severity of up to 48% was estimated to cause yield losses of about 8%. Severity of smuts averaged 3.7% with the highest incidence of 12%. Fusarium spp. were found in all 30 seed samples taken from irrigated and rain-fed winter and spring barley fields; the seed infection ranging from 0.5 to 100%, with an average of 28%. Overall yield losses of barley caused by seedborne diseases in East Azarbaijan was estimated to be about 15%.

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EFFECTS OF WHEAT GENOTYPE, GROWTH STAGE , AND FOLIAR DISEASE SEVERITY ON INCIDENCE OF SEED INFECTION BY *PYRENOPHORA TRITICI-*

REPENTIS. A. M. C. Schilder and G. C. Bergstrom. Department of Plant Pathology, Cornell University, Ithaca, NY 14853-5908.

To investigate the effects of wheat genotype and growth stage on the percentage of seed infected by *Pyrenophora tritici-repentis*, the incitant of tan spot, the spikes of four cultivars differing in foliar resistance were artificially inoculated at the anthesis, milk, and dough stages in a glasshouse experiment. Inoculation at the milk stage resulted in greater seed infection than did inoculation at the other stages, and the incidence of infection was comparable (61-78%) in ND495 (susceptible), Frankenmuth (moderately susceptible), Geneva (resistant), and BR8 (resistant). The association of foliar disease development with incidence of seed infection was investigated in a small-plot field experiment using the cultivar Frankenmuth. At harvest, the percentage of seed infected was positively correlated with tan spot severity on the flag leaf at the dough stage (p=0.04). Seed infection was detected at low levels early in seed development and increased to 42 % of the seed, in some of the plots, at harvest.

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INHIBITION AND INDUCTION OF CALLOSE SYNTHESIS IN OAT PROTOPLASTS BY ANTI-VICTORIN ANTI-IDIOTYPIC ANTIBODIES. K. Akimitsu[1], L. P. Hart[1] and J. D. Walton[2]. [1]Department of Botany and Plant Pathology and [2]DOE-Plant Research Laboratory, Michigan State University, East Lansing, MI 48824.

Anti-victorin polyclonal anti-idiotypic antibodies were raised in rabbits immunized with an anti-victorin antibody-ovalbumin conjugate. Victorin-horseradish peroxidase conjugate binding in a direct ELISA was inhibited 84% by a ten-fold dilution of anti-victorin anti-idiotypic serum. Sera from non-immunized rabbits did not show significant inhibition. The anti-idiotypic serum was added with or without victorin to protoplasts of victorin susceptible and resistant cultivars of oats. The serum diluted twenty-fold induced callose synthesis only in susceptible protoplasts. When the anti-idiotypic antibodies were added simultaneously with 60 pg/ml of victorin, callose synthesis induced by victorin was inhibited 68% by the addition of the anti-idiotypic antibodies. Since anti-idiotypic antibodies are known to bind to cell surface proteins and do not enter the cell, these results suggest that a receptor of victorin exists on the surface of susceptible oat cells.

92

STRUCTURAL ANALYSIS OF TMV COAT PROTEIN AMINO ACID SUBSTITUTIONS THAT ELICIT THE N' GENE HR. J. N. Culver[1], R. Pattanayek[2], G. J. Stubbs[2], and W. O. Dawson[1], Dept. of Plant Pathology[1], University of California, Riverside, CA 92521 and Dept. of Molecular Biology[2], Vanderbilt University, Nashville, TN 37235.

Specific amino acid substitutions within the coat protein (CP) of tobacco mosaic virus (TMV) elicit the N' gene hypersensitive response (HR). To examine the role of these substitutions in HR induction, additional substitutions located throughout the TMV CP were created. Tertiary positioning of HR-eliciting amino acid substitutions were located primarily within two structural regions on opposite sides of a single CP molecule. Quarternary aggregates of CP molecules revealed the side-by-side placement of these two regions. Thus, a specific structural region of the CP may play a role in the induction of the N' gene HR. Several substitutions also affected virion stability and virus long distance movement in the plant. No correlation between these virus functions and the induction the HR was found. However, all substitutions that interfered with virion stability also inhibited virus long distance movement.

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EFFECT OF CAULIFLOWER MOSAIC VIRUS GENE VI EXPRESSION IN TRANSGENIC *NICOTIANA BIGELOVII* ON PLANT GROWTH AND HOST-VIRAL INTERACTIONS. J.E. Schoelz and W.M. Wintermantel; Department of Plant Pathology; 108 Waters Hall; University of Missouri; Columbia, MO 65211.

We have examined the effect of expression of the CaMV gene VI product in transgenic *N. bigelovii* on plant growth and on host-viral interactions. In this study, we demonstrate that the growth of transgenic *N. bigelovii* that express gene VI of CaMV strain D4 is stunted relative to nontransformed *N. bigelovii*. In one test, plants homozygous for expression of gene VI had an average weight of 0.28 g, heterozygous plants weighed an average of 7.80 g, and nontransformed controls averaged 16.06 g. We previously showed that the transgenic *N. bigelovii* that expressed gene VI of D4 allowed long-distance spread of hybrid virus H31, a CaMV virus that is limited to the inoculated leaves of nontransfomed *N. bigelovii*. Gene VI and the large intergenic region of H31 are derived from CaMV strain W260, a virus that spreads systemically in *N. bigelovii*, while genes I-V are derived from CaMV strain CM1841, a virus that does not induce any symptoms in nontransformed *N. bigelovii*. We now show that transgenic *N. bigelovii* allow CM1841 to move systemically, although the appearance of CM1841 symptoms on noninoculated leaves is delayed by approximately 13 days, relative to H31.

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IDENTIFICATION OF PATHOGENICITY DETERMINANTS OF BARLEY STRIPE MOSAIC VIRUS. M.C. Edwards, I.T.D. Petty, and A.O. Jackson. USDA-ARS Cereal Crops Research, Fargo, ND 58105-5677; Dept. of Microbiology, North Carolina State Univ., Raleigh, NC 27695; Dept. of Plant Path., Univ. of California, Berkeley, CA 94720.

Complementary DNA clones and infectious transcripts were generated from two BSMV strains which differ significantly from 2 others previously cloned and all were used to study phenotypic determinants such as pathogenicity, seed transmissibility, symptomatology, and local lesion production. Results of recombination between the ND18 and CV42 strains indicated that the determinant for pathogenicity to oats is located within a 700 base region between the Bgl II and Bst BI sites of RNA alpha. This region is also linked to the ability of CV42 RNA alpha to suppress the formation of local lesions on Chenopodium amaranticolor when coinoculated with either ND18 or CV42 RNAs beta and gamma. Symptom type and severity was associated with both RNAs alpha and gamma. RNA gamma was shown to determine seed transmissibility in barley.

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HOST RANGE AND STUNTING SEVERITY DIFFERENCES BETWEEN TWO CaMV ISOLATES ARE DETERMINED BY THREE AMINO ACID DIFFERENCES IN THE AMINO HALF OF THE P62 PROTEIN. E.J. Anderson and J.E. Schoelz; Univ. of Missouri; Dept. of Plant Pathology; 108 Waters Hall; Columbia 65211.

Wild type CaMV strain CM1841 is mild in turnips (*Brassica campestris* L. 'Just Right') and narrow in its host range while Strain W260 is a more severe, broader host range virus that is able to systemically infect *Nicotiana bigelovii*. Sequence analysis and the use of chimeric viruses generated by genomic recombination and oligonucleotide site-directed mutagenesis with these two strains allowed us to discern sequences within a 496 bp Sac I (nt 5822) to Pvu II (nt 6318) DNA segment in the 5' half of gene VI (P62) that strongly influenced both host range and disease severity. Sequence data for CM1841 and W260 revealed five nucleotide differences within 185 bp's of each other in this region of DNA that resulted in amino acid differences. If all of the amino acids in this region were derived from CM1841 in an otherwise W260 genetic background, the virus was generally unable to systemically infect *N. bigelovii* but produced extremely severe stunting and high virus concentrations in turnips. CaMV P62 may interact with both host factors and viral sequences to inversely influence host range and stunting severity.

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DEVELOPMENT OF TOBACCO MOSAIC VIRUS-BASED VECTORS FOR THE RAPID TRANSIENT EXPRESSION OF GENES IN PLANTS. Curtis A. Holt, Andrew A. Vaewhongs, Pamela K. Robeff, Leigh B. Farrell, and Roger N. Beachy. Department of Biology, Washington University, St. Louis, MO 63130

An infectious cDNA clone of TMV has been modified to permit the introduction of foreign open reading frames in place of either the viral movement protein (MP) gene or the coat protein (CP) gene. Approximately 450 nucleotides of each of these genes were deleted and replaced with unique restriction sites. Transcripts generated *in vitro* from these vectors are infectious when inoculated onto appropriate host plants: the CP-substitution vector exhibits the host range of wild-type TMV, while the MP-substitution vector will only infect transgenic host plants that express a TMV MP gene.

The *E. coli* ß-glucuronidase (GUS) gene has been inserted into each vector to monitor viral infection and determine expression levels when the gene is inserted in either position. Histochemical staining of tissues with the chromogenic GUS substrate X-gluc clearly delineated the pattern and extent of viral infection. Fluorometric assay of infected tissue using the substrate MUG provided quantitative data on levels of GUS expression.

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EPITOPE ANALYSIS OF THE CIP OF TOBACCO ETCH VIRUS. D.A. Baunoch, P. Das and V. Hari. Wayne State University Department of Biological Sciences, Detroit, Michigan 48202

Six hundred and twenty-eight overlapping six-amino acid peptides covering the entire amino acid sequence of the cylindrical inclusion protein (CIP) of TEV were synthesized by a novel procedure. These peptides were coated onto ELISA plate microtiter wells and assayed for continuous epitopes by indirect ELISA, using antibodies to SDS-PAGE purified CIP as well as antibodies to TEV-CIP inclusions. In general, the antigenic regions of the CIP corresponded with computer predictions. However, some regions were antigenic although not predicted to be so. Several epitopes specific for the inclusion body were found. Analyses were also carried out with antisera to wheat streak mosaic virus CIP (WSMV-CIP) and with Narcissus yellow stripe virus CIP (NYSV-CIP). Antigenically conserved regions were found.

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ANALYSIS OF THE ACCUMULATION OF THE HOLMES' MASKED STRAIN OF TMV IN N. TABACUM CV. XANTHI R.S. Nelson[1], G. Li[1], C.A. Holt[2], R.N. Beachy[2], [1] The Noble Foundation, P.O. Box 2180, Ardmore, OK 73402. [2] Washington University, P.O. Box 1137, St. Louis, MO 63130

We have previously determined that the Holmes' Masked (M) strain produces lesions equal in size to those produced by the U_1 strain on Xanthi NN. In addition, the accumulation of virus and movement protein in the inoculated leaves of Xanthi nn was similar to that of U_1. However, in systemically infected leaves accumulation of M strain virus and viral RNA was less than that of U_1. By immunoblot analysis we have now determined that the M strain is delayed in accumulation in petioles of inoculated leaves and accumulates only to low levels in shoot apices compared with U_1. In inoculated and systemically infected leaves the ratio of plus to minus strand genomic RNA accumulation for each strain is similar. These and other results indicate that the M strain can replicate and move cell-to-cell similarly to U_1 but cannot efficiently move either into or out of the vascular tissue.

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MUTATIONS IN THE COAT PROTEIN GENE OF BROME MOSAIC VIRUS AFFECT SYMPTOM FORMATION IN CHENOPODIUM HYBRIDUM. A. M. Dzianott, Steven Pratt and Jozef J. Bujarski, Plant Mol. Biol. Center, Northern Illinois University, DeKalb, IL 60115.

Studying the effect of the coat protein sequences of brome mosaic virus (BMV) on symptom formation we have found that single amino acid substitutions at two separate locations (64-lysine to leucine and 130-lysine to arginine) destroyed the ability of the wild-type (wt) BMV to cause a systemic mosaic on Chenopodium hybridum. In contrast, both mutations had small, if any, effect on systemic symptoms in barley plants. While the introduction of arginine at position 130 generated a stable mutant, another mutant recovered the wt phenotype after several passages through barley, either by reverting to the 64-lysine or by a second mutation of alanine to valine at position 58. Studies on the stability of this double mutant and further mutagenesis are in progress.

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IMMUNOLOGICAL STUDIES OF THE 126kd/183kd PROTEINS OF SOME STRAINS OF TMV. P. Das, D.A. Baunoch and V. Hari. Wayne State University Biological Sciences, Detroit, MI 48202

An antiserum to a 21 amino acid peptide corresponding to the N-terminal end of the 126kd/183kd protein of TMV(U1) was prepared. This serum, as well as an antiserum to a fusion protein specific for the 126kd protein of TMV(U1), was used to compare the serological reactivities of the 126kd/183kd proteins generated in plants infected by several strains of TMV (U1, U2, M GTAMV, DH, Cc). Tests were carried out by immunoblotting, peptide analysis and immunogold localization in plants. It was found that the 126kd/183kd proteins induced by some strains of TMV did not cross-react with the antibodies tested, although other strains did cross-react. Correlations were established between amino acid sequence and serological reactivities. X-bodies reacting with the antiserum to the 126kd/183kd protein were found in TMV-U1, M, and GTAMV but not in U2 and DH.

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INVESTIGATION OF RCNMV VIRAL POLYMERASE REGULATION BY RIBOSOMAL FRAMESHIFTING. K. H. Kim and S. A. Lommel. Department of Plant Pathology, NCSU, Raleigh, NC 27695.

The single-stranded RNA plant virus, red clover necrotic mosaic virus (RCNMV), regulates expression of its polymerase gene (*pol*) at the translational level by ribosomal frameshifting. Using an RCNMV infectious RNA *in vitro* transcription system, several mutants were constructed in the frameshift heptanucleotide region of the *pol* gene, and their infectivity was assayed on *Nicotiana benthamiana* plants. A mutant eliminating the upstream 27 kDa open reading frame (ORF) amber termination codon, generating an uninterrupted 88 kDa ORF, did not cause symptoms on the plants. A second mutant which expressed the 88 kDa *pol* by amber terminator readthrough was infectious. In addition, an RCNMV mutant which exchanged the frameshift element with a barley yellow dwarf virus (BYDV) frameshift heptanucleotide was also infectious. However, the progeny BYDV frameshift element containing virus reverted back to wild-type sequence. These results suggest that RCNMV *pol* expression can occur by either terminator readthrough or frameshifting. Our analysis also indicates that *pol* expression in plants must be regulated and that *pol* over expression is toxic to the host cell and/or the viral replication process.

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VERTICILLIUM DAHLIAE AND POTATO PRODUCTION FOLLOWING SOIL FUMIGATION AND ROTATION TO IMMUNE HOSTS. G. D. Easton, M. E. Nagle and M. D. Seymour. WSU-Prosser, Irrigated Agriculture Research and Extension Center, Prosser, WA 99350-9687.

Two consecutive years' cropping to the Verticillium immune hosts sudan grass, spring wheat, sweet corn, field corn, and spring wheat followed by sudan grass reduced *V. dahliae* propagules in the soil in the spring and potato stems in the fall, but did not reduce Verticillium wilt symptoms nor propagules in the soil the following spring compared to two years of potatoes. In plots previously cropped to immune hosts, the plant height and yield of potatoes were increased, but not % U.S. No. 1 tubers. The various immune crops all had about the same effect on yield. Verticillium wilt was reduced two out of three years, and yields were increased one year in soil fumigated plots. Fumigation had about the same effect on production as two consecutive years' cropping to immune hosts.

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LINEAR GROWTH ANALYSIS OF *SCLEROTINIA SCLEROTIORUM*; A METHOD FOR PREDICTING FORMULATION PARAMETERS. D. H. Long, R. V. Miller, E. J. Ford and D. C. Sands. Department of Plant Pathology, Montana State University, Bozeman, MT 59717.

Formulations incorporating various stabilizers, nutrient sources, and carriers were prepared of *S. sclerotiorum* for improving efficacy and viability of this fungus. Formulations stored at different temperatures (4C, 25C and 30C) were analyzed using linear growth rates on water agar. Shelf-lives of formulations were compared by emergence of *S. sclerotiorum* on agar medium. Emergence from a given formulation within the first 24 to 48 hours and subsequent rapid growth appeared to correlate with higher efficacies under greenhouse conditions. Effects and optimization of formulation components can be attained using this method.

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EVALUATION OF THE CAPACITY OF TALAROMYCES FLAVUS TO REDUCE BEAN ROOT ROT CAUSED BY SCLEROTIUM ROLFSII.[(1)]Lea Madi, [(2)]Talma Katan and [(1)]Y. Henis [(1)] Dept. Plant Pathol. & Microbiol., Faculty of Agric., The Hebrew University, Rehovot 76100 & [(2)]Dept. of Plant Pathology, The Volcani center, Bet Dagan 50250, Israel.

The capacity of Talaromyces flavus to reduce bean root rot caused by Sclerotium rolfsii was evaluated in greenhouse experiments. Sclerotia were either immersed in a suspension of mycelial fragments (10.6 mg/ml), conidia or ascospores (10^7/ml) of T. flavus and placed at a distance of 0.5 cm from each bean seed. Another treatment included soil amended with 0.5% wheat bran preparation of T. flavus. Pots were incubated in the greenhouse at 28 ± 2^0C. Disease incidence was recorded for 3 weeks. Best control (91% disease reduction) was obtained with either mycelium or conidia suspension, as compared to 69% using a 0.5% wheat bran preparation, and 29% with ascospores. Disease reduction by conidia of 12 T. flavus isolates was highly correlated with their mycoparasitic capacity on S. rolfsii sclerotia in petri dishes containing sandy loam soil and with their chitinase activity (r= 0.822 and 0.862, respectively). A similar correlation (r= 0.863) between mycoparasitic and biocontrol capacities was also observed in 10 progenies of a sexual cross between isolates of T. flavus (Ben^RTF-1R6 X Ben^STF-1). Lower correlation (r= 0.692) was found between antibiotic and biocontrol capacities.

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EFFECT OF THE BIOCONTROL AGENT SPORIDESMIUM SCLEROTIVORUM ON THE DISEASE PROGRESS OF SCLEROTINIA LETTUCE DROP. D. R. Fravel[1], P. B. Adams[1], and W. E. Potts[2], [1]Biocontrol of Plant Diseases Laboratory, USDA, ARS, Beltsville, MD and [2]Statistical Consulting Services, University of Maryland and USDA, Beltsville, MD 20705.

S. sclerotivorum was sprayed at 0, 0.2, 2 or 20 kg/ha onto lettuce plants infected with Sclerotinia minor. Disease incidence was monitored for the subsequent 3 fall (F) and 2 spring (S) crops without additional pathogen or antagonist applications. Logistic growth curves were fitted to the data. Within each crop, increasing rates of antagonist resulted in a positive shift of the position of the curve with respect to time. For example, in the F 1987 crop, inflection points were 40, 43, 46 and 55 days after planting for the 0, 0.2, 2 and 20 kg rates, respectively. Maximum rates (MR) of disease increase were not different among the treatments within each crop but were different between crops. MR averaged 3.4, 3.5, 2.1, 3.7 and 1.4 % / day for F 1987, S 1988, F 1988, S 1989, and F 1989, respectively. The 2 and 20 kg/ha application rates provided control in both S and F while the 0.2 kg/ha rate functioned better in the F than in the S crops.

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Geographic Distribution of Puccinia grindeliae on Gutierrezia spp. in the Southwestern U.S.A. from 1891 to 1991. J.P. McEntee and C. M. Liddell. Department of Entomology, Plant Pathology and Weed Science, New Mexico State University, Las Cruces, NM 88003.

Herbarium specimens of Gutierrezia spp., located at seven southwestern herbaria, were examined for the presence of Puccinia grindeliae. Gutierrezia spp., known as snakeweed, are noxious rangeland weeds, and the rust P. grindeliae is being studied as a potential biocontrol agent. Approximately 1000 herbarium specimens of Gutierrezia spp. collected from 1891 to 1991 throughout the western U.S.A. and Mexico were examined and about three percent were found to be infected with P. grindeliae. The earliest known collection of P. grindeliae on this host was made in 1906 in Dona Ana county, New Mexico. Field surveys in 1990 and 1991 have shown that the rust is present in a wider range of sites than represented in the herbarium collections. Surveys of locations from which rusted plants have been collected since 1891 are being conducted to determine the present status of the rust epiphytotic at these locations.

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EFFECT OF DELAYED AND INTERRUPTED DEW PERIODS ON MORTALITY OF AMARANTHUS ALBUS SEEDLINGS INOCULATED WITH APOSPHAERIA AMARANTHI.

A.S. Mintz, and G.J. Weidemann, Plant Pathology Dept., University of Arkansas, Fayetteville, 72701.

Aposphaeria amaranthi has demonstrated potential as a biocontrol for Amaranthus spp. (pigweed). Growth chamber studies were conducted to determine the effect of delayed and interrupted dew periods on mortality of Amaranthus albus (tumble pigweed). Seedlings inoculated to runoff with A. amaranthi at 3-4 x 10^6 conidia/ml were kept in a 28 C growth chamber for 4, 8, 12, 24, 48 or 72 hr and then exposed to 12 hr of dew at 28 C. None of the delayed dew periods resulted in reduced plant mortality. To determine the effect of interrupted dew periods, seedlings were given a continuous 8-hr dew period immediately after inoculation, or the period was interrupted after 4 hours by 24 hr of no dew. Mortality was reduced to 47% when the dew period was interrupted, in contrast to 100% mortality after a continuous dew period.

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EFFECTS OF CENTAUREA SPP. GROWTH HABIT ON THE POTENTIAL OF PUCCINIA JACEAE FOR BIOLOGICAL CONTROL. N. Shishkoff and W. L. Bruckart, USDA-ARS, Ft. Detrick, Bldg. 1301, Frederick, MD 21702.

Experiments were conducted in the greenhouse to determine if root biomass and rate of leaf senescence are affected only by the number of pustules produced on a plant. Isolates of P. jaceae from diffuse knapweed (DK, C. diffusa), purple starthistle (PST, C. calcitrapa), and yellow starthistle (YST, C. solstitialis) are most virulent on their original hosts, but all readily infect cornflower (C. cyanus). Five isolates of P. jaceae were compared by inoculating both the original host and cornflower. Root biomass reduction was affected most by the number of infected leaves and less by the number of pustules per leaf. The reduction in root biomass was dependent on whether plants were indeterminate in growth (like DK and PST), or whether rosettes were determinate (like YST). The YST isolate affected its host more than the other isolates. All isolates behaved similarly on cornflower.

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ASSOCIATION OF BIOTIC FACTORS WITH SOILS SUPPRESSIVE TO *RHIZOCTONIA SOLANI* IN THE TROPICS. L. Birun, A.M. Rosales and T.W. Mew. The International Rice Research Institute, P.O. Box 933, Manila, Philippines.

An initial study showed that more antagonistic bacteria were isolated from lowland paddy field with less incidence of sheath blight. Seed bacterization with bacteria from healthy and from rhizosphere soil of healthy plants provided 76% sheath blight protection, whereas little protection was given by bacteria from infected samples. Soil samples from disease-free fields appeared to be more suppressive to *R. solani*. Rhizosphere soil samples were then collected from six locations in the Philippines and were assayed for suppressiveness to *R. solani*. Forty-five percent of 104 samples were suppressive to *R. solani*. The linear mycelial growth of the pathogen was 50% less in suppressive than in conducive soils. Given the same inoculum density in pot experiments, sheath blight severity was less in suppressive than in conducive soils. The inhibitory effect was removed by sterilization but could be restored in a mixture with 20% suppressive soil.

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BIOLOGICAL CONTROL OF DAMPING-OFF OF TOMATO TRANSPLANTS USING THE NON-PLANT PATHOGEN PYTHIUM OLIGANDRUM. F.N. Martin and C. R. Semer, Plant Pathology Department, University of Florida, Gainesvile, FL 32611.

Damping-off of tomato caused by phytopathogenic Pythium spp. (primarily P. aphanidermatum and P. ultimum) was controlled by applying spore suspensions of the non-plant pathogenic species P. oligandrum to seedlings several days prior to transplanting in the field. Some isolates were as efficacious as metalaxyl, with disease levels of ca. 5% compared to 50% for untreated controls. There was a wide range of efficacy for isolates of P. oligandrum in both field and greenhouse evaluations. Isolates also were variable in cultural characteristics, such as formation of zoosporangia, spontaneous abortion of oospores, and constitutive dormancy of oospores.

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ALTERATION OF THE HOST-RANGE OF Colletotrichum malvarum. H.K. Abbas and C.D. Boyette, USDA-ARS-SWSL, Stoneville, MS 38776.

Lengthy dew periods and narrow weed control spectra limit the bioherbicidal potential of many fungi, including C. malvarum, a pathogen of prickly sida (Sida spinosa). Studies were initiated to discover methods to overcome these constraints through solid substrate formulation. The fungus was grown on autoclaved rice for 3 wks, air-dried, and milled. Crude and cell-free filtrates applied at 15g/60ml water caused infection and kill of prickly sida, spurred anoda, american and northern jointvetchs, velvetleaf, hemp sesbania, and pigweed within 24 to 48 hr, and was achieved without dew. Pathogenesis but no mortality also occurred on 17 other crop and weed species. Spores grown on PDA infected and killed prickly sida in 5-7 days with a 16 h dew but had no effect on the other plant species. These studies revealed that the host range of C. malvarum is affected by growth media and that cell-free filtrates will induce rapid disease symptoms, suggesting that a phytotoxin(s) may be responsible for the disease produced by C. malvarum.

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RESIDUAL RESISTANCE TO CROWN RUST IN OATS. J.M. Windes and W.L. Pedersen. University of Illinois, Department of Plant Pathology, Urbana, IL 61801.

Eight oat isolines, each with a single resistance gene to crown rust (Puccinia coronata f.sp. avenae or P.c.a.), were compared to the recurrent parent for residual gene resistance. Susceptible two-week old plants were inoculated with a single isolate of P.c.a. The experiment was a randomized complete block design with four replications and four samples per replication. Plants were inoculated in a settling tower for three minutes with 2.5 mg urediniospores mixed with 2.5 mg talc. The plants were placed in a mist chamber overnight and then returned to the growth chamber. Data were collected for eight consecutive days starting on the sixth day after inoculation. Inoculated leaves were photographed to determine pustule size. The experiment was repeated four times. Latent period, spore production, pustule size, and pustule number per leaf area for each isoline were compared to the recurrent parent.

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FITNESS COMPONENTS OF *PUCCINIA RECONDITA* ISOLATES ADAPTED TO SLOW-RUSTING WHEAT CULTIVARS. J.S. Lehman and G. Shaner, Dept. of Botany & Plant Pathology, Purdue University, W. Lafayette, IN 47907-1155

To study adaptation of *Puccinia recondita* to slow-rusting wheat cultivars, the fitness components latent period (LP), infection frequency (IF), uredinial area (UA), uredinial growth rate (UGR), and urediniospore production (UP) of a wild-type population (WT) and isolates (C1 and C5) derived from WT were evaluated on fast-rusting cultivar Monon and slow-rusting cultivars CI 13227, L 574, Suwon 85, and SW 72469-6. C1 and C5 were derived from WT by selection for reduced LP for 1 and 5 asexual cycles on CI 13227. C5 had a significantly shorter LP than C1 or WT, mainly due to its adaptation to CI 13227 and L 574. Initial or final UA and UGR were not statistically different for isolates. All isolates were significantly different for IF due to decreased IF of C1 on Monon, Suwon 85, and SW 72469-6. IF was negatively

correlated to LP. C5 on CI 13227 produced significantly more spores than WT or C1, but averaged across cultivars, UP differences were not significant. LP is not necessarily correlated to UA, UGR, or UP and selection for decreased LP on one cultivar might result in decreased IF on another cultivar.

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BLAST REACTION OF DURABLY RESISTANT RICE CULTIVARS IN MULTI-LOCATION TRIALS. S. W. Ahn and D. V. Seshu, International Rice Research Institute, P.O. Box 933, Manila, Philippines.

Blast reactions of 25 cultivars, 17 adapted to irrigated rice conditions and 8 to upland rice with or without durable resistance were analyzed using the results of the International Rice Blast Nursery, a miltilocation trial conducted in different countries yearly. Most of the rice cultivars identified as durably resistant to blast showed low disease severity index (DSI), almost 5.0 or less, while the DSI's of all the cultivars without durable resistance are higher than 6.0. Also the frequency of incompatible reactions of durably resistant cultivars is higher than 60% and the frequency of scores for high severity is significantly lower than that of nondurable ones. The results indicated that the combination of broadly effective qualitative resistance and a high level of partial resistance would enhance the durability of rice blast resistance.

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DEVELOPMENT OF BIPOLARIS LEAF SPOT RESISTANT BERMUDAGRASS THROUGH CELL CULTURE. P.D. Colyer[1], S.S. Croughan[2], S.S. Quisenberry[3], and M.A. Mohamed[3]. Louisiana Agricultural Experiment Station, Red River Research Station[1], Bossier City, LA 71113, Rice Research Station[2], Crowley, LA 70527, and Department of Entomology[3], Baton Rouge, LA 70803.

Tissue culture techniques were used to develop somaclonal variants of forage-type hybrid bermudagrass, Cynodon dactylon (L.) Pers. The regenerated plants were compared with the original genotypes for resistance to Bipolaris leaf spot. Screening for resistance was accomplished by floating detached leaf tissue on biologically active fractions of fungal culture filtrates for 48 hours. Filtrate used in this assay was clarified and fractionated by sequential treatment with acetone (50% v/v, 4 C), centrifugation (2000 g, 30 min), filter sterilization (0.22 microns, cellulose acetate), and batch cation exchange chromatography. Two somaclones have been identified with increased resistance to Bipolaris leaf spot and are in advance field testing for other agronomic traits.

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EVALUATION OF THE CUTICLE AS A BARRIER TO PENETRATION BY MONILINIA FRUCTICOLA IN PEACH FRUIT. J.E. Adaskaveg, A.J. Feliciano, J.M. Ogawa. Dept. of Plant Pathology, University of California, Davis 95616.

Histological studies indicated that genotypes resistant to brown rot have a thicker cuticle. Cuticle of fruit from resistant and susceptible genotypes of peach were extracted with chloroform. Average amount of extract from the resistant Bolinha genotype (0.76 mg/cm^2) was significantly more than that of the susceptible genotypes 18-8-11 (0.46), Elegant Lady (0.45), and Corona (0.44). Gas chromatography-mass spectrometry confirmed quantitative differences between these genotypes, while no qualitative differences were observed between spectra of comparable peaks for each extract. Additionally, fruit inoculations were made with or without a pretreatment of pectinase to remove pectin from the cuticle. Increased exposure to pectinase from 1 to 4 min increased susceptibility of inoculated fruit in all genotypes evaluated. In Bolinha, however, lesion size was significantly smaller than lesions formed on other peaches (Corona, 18-8-11, Elegant Lady). Regardless of genotype, small fruit were less susceptible to brown rot than large fruit; while pectinase treatments increased the susceptibility of both large and small fruit. Furthermore, increase in wetness period during inoculation increased levels of disease in all treatments.

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COMPARATIVE RESISTANCE OF SOME STRAWBERRY CULTIVARS AND SELECTIONS TO ONE ISOLATE OF COLLETOTRICHUM ACUTATUM. **C.Q. Winterbottom, W.D. Gubler and R.S. Bringhurst. Depts. of Plant Pathology and Pomology, Univ. of California, Davis, 95616.**

Colletotrichum acutatum causes a fruit rot, infects runners, and is associated with collapsing strawberry plants due to crown rot in California. Forty-nine cultivars and selections of strawberry were inoculated with a single spore isolate of C. acutatum to evaluate their relative resistance to lesion development on runners. A detached fruit assay was used in the laboratory to evaluate 26 cultivars and selections for relative severity of fruit rot. The inheritance of plant resistance also was investigated. Plants of Aido, Aliso, Capitola, Chandler, Cruz, Douglas, Fresno, Lassen were among those rated resistant. Aptos, Brighton, Donner, Fern, Hecker, Irvine, Pajaro were among those rated susceptible. Significant differences in lesion diameter 7 days after inoculation was observed among fruit of the 26 genotypes assayed, but fruit of all genotypes were susceptible. Plant resistance to C. acutatum is conferred by a major gene, with resistance dominant to susceptibility.

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SOMACLONAL VARIATION FOR INCREASING RESISTANCE IN MINT TO *VERTICILLIUM DAHLIAE*. Karen F. Toth and M. L. Lacy, Department of Botany and Plant Pathology, Michigan State University, East Lansing, Michigan 48824.

Somaclones were regenerated from callus developing on internodal stem segments of peppermint (*Mentha piperita*) cv. Murray Mitcham, moderately resistant to *Verticillium dahliae*. Somaclones were regenerated on Murashige-Skoog medium amended with 2.0 mg indole-3-butyric acid plus 1.0 mg 6-benzylamino purine (BAP) or 10.0 mg BAP plus 250 ml coconut water per liter. Somaclones were screened for resistance to *V. dahliae* in the greenhouse by placing cut ends of three to five cuttings from somaclones in conidial suspensions (10^4 spores/ml) for 15 min, then rooting in sand. Cuttings were rated for disease severity on a 1-5 scale (1 = no symptoms, 5 = severely stunted and wilted) after 4 wk. Five of 144 somaclones screened had mean disease ratings (MDR) of 1.6-2.0, compared to MDR of 3.0 for the cv. Murray Mitcham. Somaclones have also been regenerated from peppermint cv. Black Mitcham and spearmint (*M. spicata*) cv. Scotch.

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DEVELOPING STABLE BLAST RESISTANCE IN RICE AT A "HOT SPOT" SITE. F.J. Correa-Victoria and R.S. Zeigler. Centro Internacional de Agricultura Tropical, CIAT, A.A. 6713, Cali, Colombia

Since 1983 CIAT has been breeding for stable rice blast resistance at an upland "hot spot" site where blast (Pyricularia grisea) levels are consistantly high. 200 breeding lines developed at this site were evaluated as R (highly resistant with no susceptible lesion types; Standard Evaluation System ≤ 3) or PR (partially resistant with low frequency susceptible lesions; SES 4-5) and the resistance reaction followed 9 successive seasons in the field. Of the 86 R lines only 6, or 7% became susceptible (SES >5) while of the 114 PR lines 74, or 65% became susceptible after the 9 seasons. The data suggest that under severe disease pressure R lines may be more durable and offer greater protection than PR lines.

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EVIDENCE FOR TWO GENES FOR RESISTANCE TO FROGEYE LEAF SPOT IN THE SOYBEAN CULTIVAR SANTA ROSA. D. V. Phillips and H. R. Boerma, University of Georgia, Griffin, GA 30223.

The soybean cultivar Santa Rosa (SR) is highly variable and many selections from it have been used in South America, often as a source of resistance to many races of *Cercospora sojina*. Recently selections and lines derived from them have been damaged by frogeye leaf spot. Cultivar Davis, with the *Rcs3* resistance gene, continues to be resistant to all races of *C. sojina*. To determine the inheritance of resistance, SR was crossed with Blackhawk, a cultivar susceptible to all races of *C. sojina*, and with Davis. F2 progeny of the crosses were inoculated with an isolate of *C. sojina* which causes frogeye leaf spot on Blackhawk but not on SR or Davis. Progeny from the SR X Blackhawk crosses segregated in a ratio of 1 susceptible : 15 resistant, indicating two genes for resistance at independent loci. All F2 progeny from the SR X Davis crosses were resistant but too few progeny were available to determine unequivocally if either of the genes in SR was *Rcs3*. The presence of two resistance genes in Santa Rosa will influence breeding procedures.

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CHANGES IN PEROXIDASE AND CHITINASE ACTIVITIES IN CUCUMBER PLANTS IMMUNIZED AGAINST ANTHRACNOSE BY FOLIAR INOCULATIONS

WITH COLLETOTRICHUM LAGENARIUM. R. F. Dalisay and J. Kuc., Dept. of Plant Pathology, University of Kentucky, Lexington, Ky 40546-0091.

Inoculating the first true leaf of cucumber plants with C. lagenarium enhanced systemic peroxidase and chitinase activities in the upper leaves. Enzyme activities were consistently higher in induced as compared to untreated control plants. Leaves on induced plants inoculated with C. lagenarium had reduced areas of necrosis (75 - 85%) as compared to the controls. Reduction in the area of necrosis, however, did not correlate with the enhanced total activities of peroxidase and chitinase. Plants with greater total peroxidase and chitinase activities at the time of challenge inoculation and during incubation were less protected than those with lower activities of these enzymes. Polyacrylamide gel electrophoresis demonstrated that increased peroxidase activity in induced plants was associated with highly active, fast moving anodic isoforms. Detection of chitinase isoforms is in progress.

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BIOLOGICAL ACTIVITY OF A FRACTION FROM CUCUMBER WHICH INDUCES SYSTEMIC RESISTANCE IN CUCUMBER TO ANTHRACNOSE. L. FOUGHT AND J. KUC, DEPARTMENT OF PLANT PATHOLOGY, UNIVERSITY OF KENTUCKY, LEXINGTON 40546.

Cucumber leaves (WI SMR-58) were extracted with boiling 95% ethanol to obtain a water-soluble fraction. The fraction was partially purified to contain compounds with a molecular weight of ca. 200 daltons (Fraction C). Leaves 1 & 2 of cv. SMR-58 were sprayed with Fraction C (induction) and leaf 3 was subsequently inoculated with a spore suspension of Colletotrichum lagenarium (challenge). Systemic resistance to C. lagenarium was detected in leaf 3 as early as 12 hours after spraying leaves 1 & 2. The resistance persisted for 4 weeks after spraying. The activities of peroxidase, chitinase, and β-1,3-glucanase in leaf 3 of plants sprayed with Fraction C were compared to that of plants inoculated on leaves 1 & 2 with C. lagenarium. Peroxidase and chitinase, but not β-1,3-glucanase, increased in leaf 3 after both treatments prior to challenge as compared to noninduced plants. By 4 days after challenge, the activities of the three enzymes increased in leaf 3 of induced and noninduced plants. The activities were greater in the induced plants.

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IMPACT OF DROUGHT STRESS ON THE EXPRESSION OF RESISTANCE TO VERTICILLIUM ALBO-ATRUM IN ALFALFA. B.W. Pennypacker, K.T. Leath and R.R. Hill, Jr., Penn State University and USDA-ARS, U.S. Regional Pasture Research Lab, University Park, PA 16802.

The stability of V. albo-atrum (Vaa) resistance in drought-stressed alfalfa was assessed in a factorial greenhouse experiment, with clone (2 Vaa-resistant clones), pathogen (inoculated and control), and water (drought and no drought) as factors. Interactions were examined over two 6-week growth periods and the experiment was repeated. Plants were inoculated by placing either 20 µl of a spore suspension (conc. $3x10^6$) or 20 µl of sterile water on each fresh cut stub. Inoculated plants grew for 6 wk, and were harvested once prior to imposition of the water tmts. Vaa-infected plants were shorter, flowering was reduced, and the clones differed in sensitivity to Vaa. Plant height, leaf and stem dry weight, and aerial biomass were significantly reduced by drought. When plants were drought-stressed, stem dry weight was less affected by the pathogen and fewer symptoms were present. Vaa had no consistent effect on stomatal conductance but altered leaf water potential. The reduction in symptoms and decreased effect of the pathogen on stem dry weight of drought-stressed plants indicates resistance to Vaa is stable under drought stress.

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CLAVICIPITACEOUS ENDOPHYTES IN WILD HORDEUM GERM PLASM. A. D. Wilson, W. J. Kaiser, and S. L. Clement. Regional Plant Introduction Station, USDA-ARS, Pullman, Washington, 99164-6402.

Clavicipitaceous anamorphic endophytes are recognized as new sources of resistance to pests of grasses. The incidence of anamorphic endophytes in U.S. Hordeum germ plasm from the National Small Grains Collection (NSGC) in Aberdeen, Idaho, was investigated. Seeds and leaf sheaths (50-100) of several accessions of each Hordeum spp. were examined for endophytic fungi. Endophytes were found in accessions of H. bogdani, H. brevisubulatum violaceum, and H. comosum. Some endophytes were isolated and identified as Acremonium species. Accessions of H. agriocrithon, H. brachyantherum, H. bulbosum, H. californicum, H. capense, H. chilense, H. jubatum, H. lechleri, H. marinum, H. murinum, H. procerum, H. secalinum, and H. stenostachys were endophyte-free. Endophyte-infected accessions exhibited varying resistance to the Russian wheat aphid, Diuraphis noxia (Mordvilko). High aphid mortality and reduced aphid reproduction were observed on endophyte-infected plants of some accessions.

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RELATIVE PREVALENCE OF RACES OF PHYTOPHTHORA SOJAE IN OHIO DURING 1990. A. F. Schmitthenner, Dept of Plant Pathology, Ohio State Univ., Wooster, OH 44691.

Phytophthora rot of soybeans caused by Phytophthora sojae was particularly damaging during the wet season of 1990. Yield losses, based on the yield difference between near isogenic resistant cv Resnik and susceptible cv Asgrow 3127, occurred in 16 out of 40 test fields with a mean difference of 15% or 1200 kg/ha. Phytophthora was isolated from damped-off seedlings of cv Sloan, susceptible to all races, growing in soil collected from each test site. Isolates were classified to race by reaction on lines with resistant genes Rps-1, Rps-1b, Rps-1c, Rps-1k, Rps-3 and Rps-6. Out of 200 isolates classified, Race 3 was most prevalent at 25% followed by Race 7 (18%), Race 1 (15%), Race 4 (11%), Race 8 (10%), isolates capable of defeating Rps-1k (10%) and other races (21%). Races defeating Rps-1k included Race 25 and four previously undescribed race phenotypes. All races classified could be controlled by a combination of resistant genes Rps-1c and Rps-3.

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INVERT EMULSIONS ALTER HOST-SPECIFICITY OF BIOCONTROL FUNGI. C.D. Boyette, H.K. Abbas, USDA-ARS-SWSL, Stoneville, MS 38776 and R.J. Smith, Jr., USDA-ARS, Rice Res. Ext. Ctr., Stuttgart, AR 72160

The host selectivities of two fungi, Colletotrichum gloeosporioides f. sp. aeshynomene (COLLEGO^R) a commercial bioherbicide for northern jointvetch (Aeschynomene virginica), and C. truncatum NRRL #18434 (COLTRU), a candidate bioherbicide for hemp sesbania (Sesbania exaltata) were altered in greenhouse tests when spores of these fungi were applied to weeds in an emulsifiable invert formulation. COLLEGO + invert infected and killed northern jointvetch, hemp sesbania, velvetleaf (Abutilon theophrasti), and American jointvetch (Aeschynomene americana), while COLTRU + invert affected hemp sesbania and northern jointvetch only. Host-range alterations did not occur following inoculations with spore suspensions or wound-inoculations. Alterations of the host ranges also occurred in field plots. 'Tebonnet' rice was slightly affected but recovered from injury. These experiments suggest that the host-specificities of biocontrol fungi may be influenced by formulation.

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EFFECTS OF COLD TOLERANT TRICHODERMA ON PATHOGENIC FUNGI. J. H. McBeath and M. Adelman, Agriculture and Forestry Experiment Station, University of Alaska Fairbanks, Fairbanks, AK 99775.

Cold tolerant Trichoderma isolate CHS 861 and its benomyl resistant biotypes were found capable of inhibiting the growth and development of a wide range of plant pathogens. Both coiling and penetration of Trichoderma mycelia into the mycelia of Botrytis cinerea, Pythium spp., Rhizoctonia solani, Sclerotinia borealis, Typhula spp., Coprinus psychromorbidus and scleortial low temperature basidiomycete (sLTB) were observed. Mycelia "lysing" were observed on colonies of B. cinerea and sLTB when inoculated with clumps of isolate Trichoderma spores. CHS 861 and biotypes produced proteinaceous, antimycotic substances which also inhibited the growth of plant pathogenic fungi. CHS 861 can parasitize the sclerotia of S. borealis and use it as food source. Penetration of the sclerotia of S. borealis by mycelia of Trichoderma was through the nicks and cracks in the rind. Mycelial lysis was observed on the medulla cells. Sporulation of the Trichoderma has been observed both on and in the diseased sclerotia.

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TAXONOMY OF A NEW TRICHODERMA FOUND IN ALASKA. J. H. McBeath and M. Adelman, Agriculture and Forestry Experiment Station, University of Alaska Fairbanks, Fairbanks, AK 99775-0080.

A new Trichoderma sp., antagonistic to growth of a wide range of plant pathogens, was isolated from soils in Interior Alaska. Trichoderma sp. was fast growing--colonies reached 9 cm diameter in 4 days at 20°C on oatmeal agar. It displayed a marked tolerance to low temperatures. Colonies reached 9 cm in 9 days at 7°C. At 4°C, colonies grew slower, but they were well established and appeared normal. The maximum growth temperature for this fungus was 33°C. The pH for growth of the Trichoderma sp. ranged from 1.5-9.5, with an optimum of 2.5-5.5. The colonies were hyaline. Conidiophores terminated in phialides, which dispersed in num-

bers of 3 or more. Phialopsores were green in color and globose or subglobose in shape. Their size ranged from 2.4-4.1x2.4-3.5 μm. with a length:width ratio of less than 1.25. Light and scanning electron microscopy of conidia showed that the surface of the conidia was smooth. Chlamydospores were also found. Colonies of the Trichoderma sp. produced strong coconut odor on all media tested.

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ASSOCIATION OF 33.8-kDa AND 18.7-kDa POLYPEPTIDES WITH GLIOTOXIN-PRODUCING STRAINS OF THE BIOCONTROL FUNGUS GLIOCLADIUM VIRENS. C. J. Ridout and R. D. Lumsden, Biocontrol of Plant Diseases Laboratory, USDA-ARS, Beltsville, MD 20705.

Two polypeptides (33.8-kDa and 18.7-kDa) associated with gliotoxin-producing strains of Gliocladium virens were identified and purified from crude cell homogenates using two-dimensional electrophoresis. Polyclonal antisera against the purified polypeptides were prepared using a Turkey egg yolk method. The polypeptides could be detected in immuno-blots of crude cell extracts from gliotoxin-producing strains of G. virens whereas there was no reaction against cell extracts from strains that did not produce gliotoxin. This system is being used to investigate the physiology and regulation of gliotoxin production, to develop improved screening procedures for biologically active strains of G. virens, and to monitor the biocontrol activity of G. virens in soil.

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SUPPRESSION OF *RHIZOCTONIA* ROOT ROT OF FIELD PEA BY *GLIOCLADIUM VIRENS* AND ANCHOR®. S.F. HWANG and and P. CHAKRAVARTY, Plant Sciences, Alberta Environmental Centre, Bag 4000, Vegreville, AB Canada T0B 4L0

Gliocladium virens, a potential biocontrol agent, had an inhibitory effect on two isolates of *Rhizoctonia solani* (R-11 and R-16) in culture. Percent seedling survival, shoot and root dry weights, and root rot severity of field pea caused by these two isolates of *R. solani* were reduced significantly when treated with *G. virens* and/or the fungicide, Anchor®. *G. virens* alone or in combination with Anchor® increased percent seedling survival and shoot and root dry weights, and reduced root rot severity compared to Anchor® alone. The growth of R-11 and R-16 was reduced significantly by Anchor® at 50 ppm, whereas *G. virens* was not affected at concentrations up to 500 ppm. The number of colony forming units of *R. solani* was reduced significantly in the rhizosphere of field pea seedlings inoculated with *G. virens* and/or Anchor®. The concept of integrating biological and chemical treatments for controlling Rhizoctonia root rot of field pea is proposed.

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Biomass production, conidial formation and desiccation tolerance of Trichoderma harzianum in media with different water potential levels. ***X. Jin, G. E. Harman and A. G. Taylor, Cornell University, Geneva, NY 14456.***

Biomass to be used for biocontrol must (a) consist of appropriate propagules, (b) be tolerant of drying, (c) be effective in biocontrol, and (d) be produced rapidly at high levels. These criteria have been fulfilled by producing Trichoderma biomass in a modified Richard's medium (RM8) amended with osmoticant. However, the effects of medium water potential levels on biomass production of Trichoderma remain unknown. This study investigated biomass production, conidial formation and desiccation tolerance of T. harzianum in media with various water potential levels. Results indicated that addition of osmoticants to RM8 to create an osmotic potential of -2 MPa enhanced the conidial formation and CFU number after drying. Conidia so produced have excellent desiccation tolerance.

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ANTIBIOTIC PRODUCTION BY GLIOCLADIUM VIRENS AND ITS RELATION TO BIOCONTROL OF SEEDLING DISEASES. C. R. Howell and R. D. Stipanovic, USDA, ARS, Rt. 5, Box 805, College Station, TX 77845.

Strains of Gliocladium virens fall into two distinct groups. Both groups produce viridin and viridiol, and yellow pigment among the isolates is variable enough to limit its usefulness in separating them. The only consistent difference between groups is that P strains produce heptelidic acid and gliovirin, but not gliotoxin, while Q strains produce only gliotoxin. Gliotoxin is very active against Rhizoctonia solani (Rs), but only moderately active against Pythium ultimum (Pu). Viridin, viridiol, and heptelidic acid have only minor to no activity against either pathogen. Gliovirin is strongly active against Pu but has no activity against Rs. This is consistent with group biocontrol efficacy. P strains effectively suppress disease induced by Pu, while Q strains are effective against disease induced by Rs.

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CHITIN AS A FOLIAR AMENDMENT TO MODIFY MICROBIAL ECOLOGY AND CONTROL DISEASE. N. Kokalis-Burelle, P. A. Backman, R. Rodríguez-Kábana, and L. D. Ploper. Department of Plant Pathology, Auburn University, Auburn, Alabama 36849-5409.

An amorphous suspension of chitin applied to peanut foliage in the field caused highly significant shifts in populations of epiphytic microorganisms. Chitinolytic microorganisms increased from less than 1% to over 40% of the total microbial population. A chitinolytic isolate of Bacillus cereus was selected from the enriched microflora and reapplied with chitin to peanut leaves. The bacterium was sustained as actively growing vegetative cells for an extended period of time on chitin-treated leaves. A 60% reduction in early leafspot caused by Cercospora arachidicola was recorded on leaves treated with chitin and B. cereus. Scanning electron microscopy revealed colonization of the chitin particles, fungal hyphae, and spores by B. cereus. Chitin deposits may also function as a physical barrier to spore germination and germ tube penetration.

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BIOLOGICAL CONTROL OF APPLE FRUIT DISEASES BY CHAETOMIUM GLOBOSUM FORMULATIONS CONTAINING A CARBON SOURCE. R. F. Davis, P. A. Backman, R. Rodriguez-Kabana, and N. Kokalis-Burelle. Department of Plant Pathology, Alabama Agricultural Experiment Station, Auburn University, AL 36849-5409.

Field and greenhouse experiments were conducted to evaluate the biological control abilities of Chaetomium globosum (NRRL 6296) with and without an acid hydrolyzed colloidal cellulose amendment. Cellulose and a vegetable oil-based spreader-sticker (Soydex) exhibited long-term foliar tenacity. The cellulose formulation plus an oil-based sticker was applied to apple trees along with C. globosum ascospores. Light and scanning electron microscopy in conjunction with leaf-wash dilution plating revealed that C. globosum survival, growth, and disease suppression were greatly enhanced by cellulose amendments applied to apple trees. The cellulose suspension plus C. globosum ascospores reduced flyspeck (Zygophiala jamaicensis) loci 63% over the untreated control and sooty blotch (Gloeodes pomigena) as effectively as the fungicide control.

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MODIFICATION OF LEAF MICROFLORA BY FOLIAR AMENDMENTS AND EFFECTS ON DISEASES OF TOMATO, POTATO, AND APPLE. L. D. Ploper and P. A. Backman. Dept. Plant Pathology, Auburn Univ., AL 36849.

Colloidal suspensions of biopolymers were evaluated under field conditions to assess impact on populations of epiphytic microorganisms and on foliage and/or fruit diseases of tomato, potato, and apple. The insoluble polymers chitin (2 grades), chitosan, and cellulose, and the water soluble polymers carrageenan and scleroglucan were included in formulations. Evaluation of leaf and fruit samples indicated that significant changes occurred in microbial populations as a result of these amendments. Increases up to 0.86 log cfu/g tissue in total bacteria were found on amended leaves while populations of chitinolytic microorganisms increased as much as 1.4 log over untreated plants. Early blight (Alternaria solani) on tomato and potato, and flyspeck (Zygophiala jamaicensis) and sooty blotch (Gloeodes pomigena) on apple fruit were all reduced with the biocontrol formulations. Insoluble polymers with a C and N source, particularly chitin, gave the lowest disease ratings in all trials while soluble polymers were least effective.

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EFFECTS OF CHITIN AMENDMENTS AND ADDED CHITINOLYTIC MICROORGANISMS ON FOLIAR DISEASES OF TOMATO. L. D. Ploper[1], P. A. Backman[1], S. D. Cunningham[2], and M. J. Martin[2]. [1]Dept. Plant Pathology, Auburn Univ., AL, and [2]E. I. DuPont, Newark, DE.

Fifty-one chitinolytic bacterial isolates, collected from field-grown tomato plants previously sprayed with colloidal chitin, were evaluated as potential biocontrol agents against early blight of tomato, caused by Alternaria solani. Primary screening was based on chitinolytic activity, in vitro and in vivo antagonism to A. solani, and survival on nonamended tomato leaves. Nine isolates from this group were thus selected and used in tomato field trials at two Alabama locations. Tomato plots were sprayed every 7-10 days with a 0.5% colloidal chitin preparation, followed by the test isolate at 10^9 cfu/ml. Population dynamics indicated improved survival of added microorganisms by the addition of chitin (up to 0.4 log cfu/g fresh leaf tissue over nonamended plants, 15 days after application). Early blight, Septoria leaf spot and bacterial speck were controlled or suppressed with the chitin formulation; control was improved by the addition of 5 of the 9 organisms tested.

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FUSARIUM WILT OF CARNATION IN RELATION TO MICROBIAL RECOLONIZATION OF FUMIGATED SOIL. T. Isakeit, S. Tjosvold, A. R. Weinhold, J. G. Hancock, and M. N. Schroth, Department of Plant Pathology, University of California, Berkeley, CA 94720 and Cooperative Extension, University of California, Watsonville, CA 95076

The effect of amendments on recolonization of methyl bromide-fumigated soil and incidence of carnation wilt was determined. The amendments (v/v), added immediately after fumigation, were none (control), 10% compost (spent mushroom casing, two sewage sludges), or 1% wilt-suppressive soil. Plots were infested with *Fusarium oxysporum* f. sp. *dianthi* three weeks later when microbial populations had reached maximum levels. Microbial respiration rates and biomass were highest in compost treatments. At 11 months, wilt incidence was 32-58% in compost treatments, 22% in the control, and 13% in the suppressive soil treatment. Although there was more microbial biomass and activity in compost treatments, disease incidence was increased. The efficacy of the suppressive soil treatment suggests that species composition of the microflora is more important for disease suppression than total biomass or activity.

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NITROGEN SOURCE AND C:N RATIO INFLUENCES SPORULATION OF TWO POTENTIAL MYCOHERBICIDES. K.M. Howard and R.J. Bothast. USDA-ARS, NCAUR, Peoria, IL 61604.

Alternaria crassa and Alternaria cassiae are two important fungal pathogens of the noxious weeds Jimsonweed (Datura stromonium) and Sicklepod (Cassia obtusifolia), respectively. Commercial development of these fungi as mycoherbicides is inhibited by the lack of low-cost techniques for producing infective propagules. Development of a defined medium should facilitate spore production. Experiments were conducted on agar to determine effects of 12 nitrogen sources which were incorporated in a basal salts medium and formulated at carbon/nitrogen ratios of 4:1 and 10:1. Both fungi sporulated well on inorganic, simple and complex organic nitrogen sources as compared to amino acid nitrogen sources. A. cassiae produced significantly more spores per cm^2 of colony growth on several nitrogen sources formulated at a 10:1 C:N ratio whereas A. crassa produced significantly more spores on similar media formulated at a 4:1 C:N ratio. Information from this study will be used in future submerged culture studies.

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POTENTIAL OF RESIDENT EPIPHYTIC FUNGI FOR BIOLOGICAL CONTROL OF BROWN ROT BLOSSOM BLIGHT IN STONE FRUITS. H.P. Wittig, K.B. Johnson, and J.W. Pscheidt. Department of Botany and Plant Pathology, Oregon State University, Corvallis, OR 97331-2902

Antagonistic effects of Epicoccum purpurascens, Aureobasidium pullulans, Trichoderma spp., and Botrytis cinerea on establishment of Monilinia fructicola infections in cherry blossoms were assessed in field and greenhouse studies. Conidia of each fungus were applied to blossoms that were subsequently inoculated with conidia of M. fructicola. In field trials, applications of E. purpurascens, a mixture of E. purpurascens and Trichoderma spp., and A. pullulans reduced blossom blight relative to nontreated blossoms by 47%, 51% and 54% respectively, as compared to 80% and 84% reductions with the fungicides benomyl and iprodione. Quiescent M. fructicola infections in immature fruit were evaluated by dipping green cherries in the herbicide paraquat. Control of quiescent fruit infections with the mixture of E. purpurascens and Trichoderma spp. was similar to that seen on blossoms.

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INFLUENCE OF ASPERGILLUS NIGER ON AFLATOXIN PRODUCTION BY A. FLAVUS ON PEANUTS. K. L. Bowen and C. J. Mickler, Department of Plant Pathology, Auburn University, AL 36849-5409.

Aspergillus niger has been found to reduce aflatoxin production by A. flavus when grown together on corn. These two fungi commonly coexist in peanut field soils, and may be favored by similar envionmental conditions. A. niger, when inoculated prior to or at the same time as A. flavus on whole or ground raw peanuts, was found to reduce aflatoxin production by the latter fungus. Inhibition of aflatoxin production appeared to be relative to reduction of substrate pH by A. niger. At optimal temperatures (30 and 35 C), A. niger grew more rapidly (25 and 39%, respectively) than A. flavus on a 2% homogenized peanut medium. A. niger may be an ideal candidate for screening as a biocontrol agent against aflatoxin production by A. flavus.

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SOLID PHASE PURIFICATION OF DOUBLE-STRANDED RNA FROM PLANTS C. A. Powell and C. L. Elliott, AREC Ft. Pierce, FL 34954 and Diagtec, P. O. Box 992, Loxahatchee, FL 33470

A simple, rapid method for purifying dsRNA from plants without the use of liquified phenol or chloroform was developed and tested. Crude plant sap in 0.4 M Tris-acetate, 0.2% Triton-X-100 buffer, pH 8.0, containing 15% ethanol was mixed with Bio Rad N-1 cellulose. After washing with 15% ethanol-STE, the dsRNA enriched fraction was eluted with 0.4 M Tris-acetate. This fraction, which had a pale green turbid appearance, was passed through a minicolumn containing Prosorb resin to remove the remaining proteins and pigments. The dsRNA in the eluant was then bound to Nusorb (immobilized cetyltrimethylammonium bromide) to separate it from any remaining uncharged molecules. The dsRNA was then eluted with 2 ml of 2.0 M sodium acetate buffer, pH 7.0, and precipitated with ethanol. The procedure takes about 30 minutes and eliminates the need for organic solvent extraction and centrifugation. The yield and purity of dsRNAs isolated by this method equaled or exceeded that using standard phenol-chloroform solvent extraction methods.

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A VIRUS WITH PROPERTIES OF THE REOVIRIDAE FAMILY ASSOCIATED WITH HYPOVIRULENCE OF ***CRYPHONECTIA PARASITICA***. S.A. Enebak[1] B.I. Hillman[2] W.L. MacDonald[1], and A.R. Abad[3]. [1]West Virginia University, Morgantown, 26506, [2]Rutgers University, New Brunswick, NJ 08903 and [3]University of Minnesota, St. Paul 55108.

An icosahedral virus approximately 60 nm in diameter has been purified from an isolate (C-18) of ***Cryphonectria parasitica***, the chestnut blight fungus, collected from a canker in West Virginia. The virus is composed of 11 electrophorectically distinct dsRNAs, ranging in size from 1 to 5 kbp, which segregate in an all-or-none fashion in single conidial isolates. The virus alters cultural morphology, reduces virulence when compared to dsRNA-free progeny and can be transmitted into other isolates of*C. **parasitica*** via hyphal anastomosis. Using cDNA clones from a library representing the viral genome as hybridization probes, 6 of the 11 segments have been identified as being unique with respect to hybridization properties in northern blots. Spot hybridization analyses indicate that dsRNA from C-18 neither cross-hybridizes with dsRNA from 5 other ***C. parasitica*** strains, nor to the plant Reovirus wound tumor virus (WTV). Three other strains of ***C. parasitica*** have been recovered from cankers in West Virginia that contain 11 segments of dsRNA similar in banding patterns to those found in C-18. We conclude that this virus is comparable to other viruses in the family Reoviridae. This is the first report of such a virus infecting a fungus.

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PRODUCTION OF BIOLOGICALLY ACTIVE IN VITRO TRANSCRIPTS FROM cDNA CLONES OF PEANUT STUNT VIRUS (PSV) SATELLITE RNA VARIANTS. R. A. Naidu, G. B. Collins and S. A. Ghabrial. Department of Plant Pathology and Agronomy, University of Kentucky, Lexington, KY 40546.

We have recently reported the occurrence of PSV satellite RNA (satRNA) variants with symptom-modulating

properties. In order to delineate the functional domains within PSV satRNA, we have produced full length cDNA clones of structurally similar but biologically distinct satRNAs. These clones were inserted downstream from a T7 polymerase promoter in the transcription vector pGEM3Zf(+). Capped and uncapped in vitro transcripts were found to be equally infectious upon co-inoculation of tobacco or cowpea with PSV strain ER (PSV-ER). In further studies, we have determined that a western isolate of PSV (PSV-W), a satellite-free isolate which is serologically distinct from PSV-ER, supports the replication of neither the in vitro transcripts nor the naturally-occurring satRNA variants. Pseudo-recombinants involving the genomic RNAs of PSV-ER and PSV-W are being constructed in order that the satRNA replication support function may be located within the helper virus genome.

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MOLECULAR CLONING AND NUCLEOTIDE SEQUENCING OF ISOLATES OF CUCUMBER MOSAIC VIRUS FROM BANANAS AND PLANTAINS IN PUERTO RICO. **S.S. Pappu, H.R. Pappu, C.L. Niblett, and J. Bird*. Plant Pathology Dept., Univ. of Florida, Gainesville, FL 32611, *Crop Protection Dept., Univ. of Puerto Rico, Rio Piedras, PR 00928.**

Cucumber mosaic virus (CMV) infection causes serious losses in bananas and plantains in Puerto Rico and other tropical locations. CMV isolates from bananas, plantains and Commelina diffusa (a weed common under bananas and plantains) were collected in Puerto Rico and differentiated by serology, symptoms and host range. Coat protein genes of selected isolates were PCR-amplified and cloned. Analysis of the coat protein gene of isolate PR1 revealed that this isolate belongs to the CMV subgroup-I based on nucleotide and amino acid homologies. The 3' untranslated region also exhibited a high level of homology with the known CMV isolates. Sequence analysis of other isolates is in progress.

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GENETICS OF INFECTION AND SEED TRANSMISSION OF TOBACCO STREAK VIRUS (TSV) IN BEAN AND OTHER HOSTS. M. H. Walter, S. D. Wyatt, and W. J. Kaiser. Department of Plant Pathology, Washington State University, Pullman, WA 99164-6430.

Virus and host genetics of infection and seed transmission are being studied using TSV isolates Mel 40 and Mel F. Isolates Mel 40 and Mel F are seed transmitted in *Phaseolus vulgaris* cultivar Black Turtle Soup (BTS) at about 26% and 0.5% respectively. Virus RNAs 1,2,3 and 4 were eletrophoretically separated on and recovered from 1.5% "low-melt" agarose gels. Six pseudo-recombinant genomes were constructed and inoculated on *Chenopodium quinoa* and other hosts. The pseudorecombinant isolates are being characterized as to genetic stability and phenotype including seed transmission. A host inheritance study was conducted to examine TSV Mel 40 seed transmission in BTS. Inoculations with Mel 40 were made on F1 hybrid progeny from crosses of BTS and Dubelle Witte beans (Mel 40 seed transmission 0%). F2 progeny seedlings were assayed for virus infection. The lack of infection of progeny seedlings suggests that nonsusceptibility to seed transmission is dominantly inherited.

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DEVELOPMENT OF A REVERSE TRANSCRIPTION/POLYMERASE CHAIN REACTION ASSAY FOR THE DETECTION OF PLUM POX POTYVIRUS FROM MICROGRAM QUANTITIES OF TOTAL NUCLEIC ACIDS. L. Levy and A. Hadidi. USDA-ARS, NPGRL, Beltsville, MD 20705.

A Reverse Transcription/Polymerase Chain Reaction (RT/PCR) technique was developed for the detection and identification of plum pox virus (PPV) from microgram quantities of total nucleic acid extracts. Oligonucleotide primers were designed using published sequence information from the coat protein clone pPPV-NAT309. Amplified RT/PCR products from healthy and infected tissue extracts were analyzed by silver nitrate stained PAGE. The PPV origin of amplified RT/PCR products was confirmed by Southern blot hybridization with ^{32}P-labeled cRNA probe from a p4PPV309 subclone. RT/PCR products of expected size were successfully detected in PPV-infected extracts by Southern blot analysis. PPV-specific RT/PCR products were not detected in equivalently prepared healthy extracts.

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PURIFICATION AND CHARACTERIZATION OF SORGHUM STUNT MOSAIC VIRUS (SSMV). R. Creamer, Department of Plant Pathology, University of California, Riverside, CA 92521.

Sweet corn plants from Imperial County, CA showing symptoms typical of sorghum stunt mosaic were used as sources for transmission by *Graminella sonora* to Golden Bantam corn. Rhabdovirus-like particles (identified by electron microscopy) were purified using celite filtration from the symptomatic inoculated corn. Two size classes of particles were distinguished by sucrose gradient centrifugation. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) revealed identical protein profiles from both gradient fractions on a 12% gel. SSMV contained three prominent proteins with molecular weights of ca. 91, 59, and 36 kDa, and a minor protein of ca. 30 kDa. The 91-kDa protein stained for carbohydrate, suggesting that it is the G protein. After a treatment with 2% Triton X-100, 0.4 M NaCl, the 59-kDa protein (and traces of the 36 kD protein) remained insoluble indicating the presence in the viral core particle of the 59-kDa protein, and suggesting that it is the N protein. The SSMV viral RNA was estimated to be 4.2 x 10^6 migrating identically to ssRNA of beet yellows virus in a 0.8% agarose gel.

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DETECTION OF LILY SYMPTOMLESS CARLAVIRUS BY ELISA, DOT-BLOT IMMUNOASSAY AND DIRECT TISSUE BLOTTING. H. T. Hsu, J. Y. Kim, and R. H. Lawson. USDA-ARS, FNCL, Beltsville, MD 20705-2350.

Lily symptomless carlavirus (LSV) was purified from infected lilies by differential centrifugation followed by equilibrium centrifugation in cesium chloride. Virus yields averaged 8.5 mg per 100 gm tissues using an extinction coefficient of 2.5. The first bleed from rabbits after three injections of 0.25 mg each of purified LSV had ELISA endpoints of about 10^{-5}. An indirect immunological procedure was used to detect LSV antigens from direct tissue blottings on nitrocellulose membranes. A comparison of ELISA, dot-blot immunoassay and tissue blotting sensitivities showed that the dot-blot immunoassay was 16 to 32 times as sensitive as ELISA in detection of LSV. LSV was detected in bulb scales by tissue blotting in samples where sap extracts from the same scale pieces were negative in both dot-blot and ELISA.

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INHIBITION OF CAULIFLOWER MOSAIC AND TOMATO SPOTTED WILT VIRUSES BY MYCOLAMINARAN. Christina M. Heinkel, Thomas M. Zinnen, M.E.S. Hudspeth, and R. Meganathan. Plant Molecular Biology Center and Dept. of Biological Sciences, Northern Illinois University, DeKalb, IL 60115 USA.

Mycolaminaran, a B-1,3-glucan from Phytophthora megasperma, inhibits initial TMV infection in Nicotiana tabacum and Nicotiana glutinosa when mixed at 100-500 µg/ml with inoculum. TMV inoculum amended with 250 µg/ml mycolaminaran produced fewer than 10% as many lesions as unamended control inoculum when applied to Datura stramonium, another member of the Solanaceae. Mycolaminaran similarly inhibited the infection of D. stramonium by four strains of CaMV (Cabb-B, D4, CM 1841, W260). Tomato spotted wilt virus inoculum amended with 250 µg/ml mycolaminaran also produced fewer than 10% as many lesions as unamended inoculum when applied to N. glutinosa. When plants (N. tabacum 'Xanthi-nc' or N. glutinosa) were kept at 32C for 24-48 hours before inoculation, TMV inoculum amended with mycolaminaran produced 80-100% as many lesions as unamended controls. At 30C, the induced resistance was observed on developing leaves, but not on fully expanded leaves of Xanthi-nc tobacco. The resistance induced by mycolaminaran is immediate, general, temperature sensitive, and not restricted to RNA plant viruses.

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DIFFERENCES IN SUCROSE STRESS AND RESPONSES TO SALICYLIC ACID IN TOLERANT AND SUSCEPTIBLE SISTER OAT LINES INOCULATED WITH BARLEY YELLOW DWARF VIRUS STRAIN MAV-NY J. S. Lamboy[1], W. F. Lamboy[2], C. J. D'Arcy[1], and P. D. Shaw[1], Departments of [1]Plant Pathology and [2]Agronomy, University of Illinois, Urbana, IL 61801

Daily spraying with 10% sucrose increased chlorosis and stunting in both uninfected and BYDV-MAV-NY inoculated Coast Black oat plants in a preliminary fractional factorial experiment. Sap of sucrose treated plants, with or without virus, gave strongly positive results in polyclonal ELISA, although monoclonal ELISA data showed no contamination of the uninoculated plants. A pair of susceptible and tolerant sister oat lines were used in a follow-up full factorial experiment with BYDV-MAV-NY, sucrose and salicylic acid treatments. Cultivar interactions suggest that tolerance may be related to a difference both in sucrose metabolism and expression of salicylic acid induced proteins. Increased absorbance in ELISA may be due to adherency of stress related host components to the surface of immunogen virus particles.

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PEA SEEDBORNE MOSAIC VIRUS (PSbMV) DETECTION USING THE POLYMERASE CHAIN REACTION (PCR). P.D. Kohnen, W.G. Dougherty, and R.O. Hampton. Departments of Botany & Plant Pathology and Microbiology, Oregon State University, Corvallis, OR 97331-2902 U.S.A.

A sensitive and pathotype-specific PCR methodology was developed for detecting PSbMV in small (1.0 mg) tissue samples, including leaves, pollen, and seed parts. cDNA was synthesized from PSbMV RNA in crude nucleic acid preparations from pea (Pisum sativum) tissues treated with SDS and proteinase K and amplified by PCR. PSbMV RNA could be detected in tissue preparations containing 0.1 pg of added viral RNA, whereas PSbMV was not detectable in tissue extracts containing less than 50 pg of virus by optimized ELISA. In parallel assays, PSbMV was detected in infected pea embryo axes by both PCR and ELISA. However, PCR required less material for the assay.

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THE EFFECTS OF CHEMICAL AND PHYSICAL ENVIRONMENTS ON THE MORPHOLOGY OF SOIL-BORNE WHEAT MOSAIC VIRUS. R. L. GRAYSON, M. S. MAYES, AND K. T. THORHAM. VIRGINIA POLYTECHNIC INSTITUTE AND STATE UNIVERSITY, BLACKSBURG, VA.24061-0331

Futher detailed studies were undertaken to examine the effects of fixatives on viral morphology. Carbon-coated and antibody-sensitized grids (ASG) were wxposed to viral suspensions made from infected leaves of young wheat plants. Gluteraldehyde had a determental effect on the viral protein starting with an increase in the width of viral particles and continuing until the helical structure is completely lost (1-15 h). Viral particles on ASG's initially maintained their integrity for a longer period of time before dissociation of viral protein. Viral morphology in naturally infected material was lost under all physical environments tested.

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ORGANIZATION AND PARTIAL NUCLEOTIDE SEQUENCE OF THE CITRUS TRISTEZA CLOSTEROVIRUS GENOME. H.R. Pappu[1], C.L. Niblett[1], R.F. Lee[2], L.A. Calvert[3]. IFAS, University of Florida, [1]Gainesville, FL 32611, [2]Lake Alfred, FL 33850, and [3]CIAT, AA 6713, Cali, Columbia.

The citrus tristeza closterovirus (CTV) genome consists of a positive sense, single stranded RNA of about 20 kb in size. Complementary DNA copies representing 90% of the T36 isolate of CTV were cloned and their nucleotide sequence determined by the dideoxy chain termination method. Preliminary analysis of the nucleotide sequence revealed open reading frames of various lengths. Comparison with the known 50% of the nucleotide sequence of beet yellows closterovirus showed no significant homology. The possible functional roles of the CTV genes are being investigated.

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SEQUENCE DIVERSITY IN THE SURFACE-EXPOSED AMINO-TERMINAL REGIONS OF COAT PROTEINS OF SUGARCANE MOSAIC VIRUS STRAINS CORRELATES WITH NATURAL HOST SPECIFICITY. M.J. Frenkel[1], J.M. Jilka[2], C.W. Ward[1], and D.D. Shukla[1]. [1]CSIRO, Division of Biomolecular Engineering, 343 Royal Parade, Parkville, Victoria 3052, Australia; [2]University of Illinois, Urbana, IL 63017, U.S.A.

A comparison of coat protein sequences of two sugarcane mosaic virus (SCMV) strains, SCMV-SC and SCMV-MDB, showed that the two proteins are 92% identical except for the region between amino acid residues 27 and 70 of SCMV-SC. This region of SCMV-SC is smaller than the equivalent region in the SCMV-MDB (59 residues) and has only 22% identity with the SCMV-MDB sequence. To determine the distribution of this divergent region among other SCMV strains, oligonucleotide probes corresponding to portions of the divergent regions of the two strains were synthesized and used to probe SCMV strains. Our preliminary results demonstrate that the SCMV-SC and SCMV-MDB probes hybridized only with strains originating in either sugarcane (SCMV-SC, -ISIS, -Brisbane, -Bundaberg) or maize (SCMV-MDB), respectively. The probes did not hybridize with strains SCMV-BC and SCMV-Sabi originating in grasses, or with healthy plant extracts.

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DETECTION OF CITRUS VIROIDS BY ENZYMATIC cDNA AMPLIFICATION. X. Yang, A. Hadidi, and S. M. Garnsey, USDA-ARS, Beltsville, MD 20705 and USDA-ARS, Orlando, FL 32803.

Reverse transcription-polymerase chain reaction (RT-PCR) was used to detect and identify citrus exocortis viroid (CEV), citrus cachexia viroid (CCaV), also known as citrus viroid IIb, and the closely related citrus viroid IIa (CVIIa) from nucleic acid extracts of infected host. CCaV and CVIIa are members of the hop stunt viroid (HSV) group. DNA primers (19-24 nucleotides in length) specific for CEV or HSV sequence were used for cDNA synthesis and specific amplification of CEV and CCaV (or CVIIa), respectively. The size of the major product from CEV-infected tissue was the same as full length CEV (371 bp) and hybridized with SP6-generated CEV cRNA probe. The size of the major product from CCaV or CVII-infected tissue was approximately 297 bp and 303 bp, respectively, and hybridized with SP6-generated HSV cRNA probe. These products were absent from amplified extracts of uninfected tissue.

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RELATIONSHIP OF POD YIELD OF TWO PEANUT CULTIVARS TO DURATION OF HEALTHY LEAF AREA. V. M. Aquino, R. D. Berger, F. M. Shokes, and D. W. Gorbet, Plant Pathology Department, Gainesville, FL 32611, and North Florida Research and Education Center, Quincy, FL 32351 and Marianna, FL 32446.

Pod yields for Florunner and Southern Runner peanuts increased with the increase in duration of healthy leaf area (HAD). A range of HADs associated with severity of late leafspot, caused by Cercosporidium personatum, was obtained with modifications to the fungicide program. Pod yield of Florunner was slightly underestimated by a model (Phytopathology 77:393-398) developed previously from harvests of Florunner that were not always at optimal pod maturity. Southern Runner is less efficient photosynthetically than Florunner and, indeed, the pod yield of Southern Runner was overestimated by the yield model for Florunner. Better prediction of pod yield was obtained when the HAD model was modified for crop maturity at harvest of Florunner or for the photosynthetic efficiency of Southern Runner.

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CROP LOSS IN TWO SPRING OAT CULTIVARS DUE TO PUCCINIA CORONATA F.SP. AVENAE AND BYDV-PAV-IL. S. M. Bissonnette, C. J. D'Arcy, and W.L. Pedersen, Univ. of Illinois, Dept.of Plant Pathology, Champaign-Urbana 61801.

Studies on two cultivars (Ogle, Noble) were done to determine crop loss due to Puccinia coronata f.sp. avenae (P.c.a.) in the presence or absence of BYDV-PAV-IL. The experiments were repeated at two locations in 1989 and 1990. Several levels of rust severity were established in all experiments by inoculation with P.c.a. or application of Dithane M-45. For experiments including BYDV, all plots were inoculated with BYDV-PAV-IL by application of viruliferous aphids, Rhopalosiphum padi. BYDV-PAV-IL incidence was determined using ELISA. P.c.a. severity on several dates, yield, and several yield components were determined. Linear critical point regression models were developed for individual cultivars. Crop loss due to P.c.a. alone was 50 kg/ha for both Ogle and Noble. Crop loss due to P.c.a. in the presence of BYDV-PAV-IL was 20 kg/ha for both Ogle and Noble. The effect of BYDV-PAV-IL on crop loss due to P.c.a. was not additive.

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EFFECT OF IRON STATUS ON VERTICILLIUM WILT AND *IN VITRO* PRODUCTION OF SIDEROPHORES BY *VERTICILLIUM DAHLIAE*. J. Krikun, I. Barash, and A. Nachmias. Agr. Res. Org., Volcani Center, P.O. Box 6, Bet Dagan 50-250, Israel.

Several studies have indicated that *V. dahliae* produces siderophores when grown under certain conditions in liquid media. Pot and field experiments were conducted in calcareous soils of pH 8.0 - 8.5 to determine if soil-applied chelated iron or foliar sprays of microelements could alleviate Verticillium symptoms in crop species that under our conditions can suffer severe losses when colonized by the pathogen. Potato, peanut and eggplant were used. In all cases, iron applied to the soil as FeEDDHA decreased symptom development and usually increased yield. Foliar applications of microelements on potato and peanut produced similar

results. Photosynthesis was 2X higher in infected potato receiving microelements than in non-treated infected plants. Plants growing in comparable non-infested soil did not benefit from any of the treatments. Symptom suppression was not correlated with pathogen reduction in host tissues as measured by cfu in the stem.

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USE OF CATEGORIZED INFORMATION FOR CROP LOSS ANALYSIS IN TROPICAL RICE: A PERSPECTIVE. S. Savary, F. Elazegui & P.S. Teng, IRRI, P.O. Box 933, 1099, Manila, Philippines.

Analyzing crop losses in tropical rice requires that a wide range of pest constraints and production situations be considered. At IRRI, we are studying the overall multi-pest system by (a) conducting surveys in farmers' fields, and (b) establishing a data base, where the levels of pests and the production situation are experimentally manipulated. The resulting information is ordinal (qualitative) or cardinal (quantitative) by nature, and is handled using (a) categorization of cardinal variables, (b) chi-square tests, and (c) correspondence analysis. The use of categorized information, *inter alia*, is compatible with decision making in pest management. The relevance of these techniques to identify thresholds in production situations and disease interactions in rice will be discussed and compared with a more simple multi-pest system on peanut in West Africa.

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G.C. Wall and C.A. Kimmons
Agriculture Experiment Station
College of Agriculture & Life Sciences
University of Guam

B1CMV, A VIRUS DISEASE AFFECTING PRODUCTION OF YARD LONG BEANS

Yard long beans (Vigna unguiculata subsp. sesquipedalis) are an important commodity on Guam and throughout the Mariana Islands. Previous work has identified the mosaic disease so common on this crop as Black-eyed Cowpea Mosaic Virus (B1CMV). In a recent experiment, 25 healthy and diseased pairs of plants were grown in adjacent rows. The diseased plants were inoculated at the first-leaf stage. The virus disease was found to affect plant development. Not only did the inoculated plants develop typical disease symptoms like mosaic and leaf curling, but they also had a 22.3% reduction in stem diameter and a 53.7% smaller leaf canopy area initially and produced 43% fewer pod numbers with 43.9% less pod weight. Later infections showed a tendency to reduce stem diameter, leaf canopy area, pod numbers and pod weight even more.

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EFFECT OF SOIL MOISTURE ON INCIDENCE OF POTATO EARLY DYING AND TUBER YIELD. T. A. Wheeler, R. C. Rowe, and L. V. Madden. Dept. of Plant Pathology, OARDC, Ohio State University, Wooster, 44691

The influences of soil moisture on development of potato early dying is not well defined. A field microplot study was conducted to examine the effects of several irrigation regimes and density of microsclerotia of Verticillium dahliae on disease incidence (symptoms) and tuber yield. Four soil moisture treatments were maintained by trickle irrigation: high all season (HI), low all season (LO), high preflowering then switching to low postflowering (HL), and the converse (LH). Average yield for the LO water treatment was higher ($P = 0.05$) than for the HI or LH water treatments. An interaction ($P = 0.05$) was observed between microsclerotia density and water treatment with respect to onset of wilt symptoms. The incidence of disease symptoms in HI and HL was higher ($P = 0.05$) than the LO or LH water treatments. As a result of this study, work has been initiated to examine the influence of soil moisture on the infection processes.

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DUPLICATION AND CLUSTERING OF GENES AT THE PUTATIVE TOX LOCUS OF COCHLIOBOLUS CARBONUM RACE 1. D.G. Panaccione, J.S. Scott-Craig, and J.D. Walton, DOE-Plant Research Laboratory, Michigan State University, East Lansing, MI 48824-1312

The cyclic tetrapeptide HC-toxin is the primary determinant of disease in the interaction between Cochliobolus carbonum race 1 and corn. The ability to produce HC-toxin segregates as a single Mendelian trait in crosses between toxin-producing (race 1) and toxin-nonproducing (race 2) strains of the fungus. We previously identified a large contiguous region of DNA that contains the gene for HTS-1, an enzyme involved in HC-toxin biosynthesis, and that was found only in race 1 isolates of C. carbonum. New evidence demonstrates that the entire 22-kb race 1-unique region is duplicated precisely. The two copies are distinguishable only by RFLPs in the DNA that flanks them. The two copies are linked, and present in duplicate in all race 1 isolates that we have examined. Strains containing disruptions in individual copies of the HTS-1 gene have been constructed by integrative transformation with different selectable markers. Two additional genes have been located in this race 1-unique region of DNA and cDNAs corresponding to these genes have been cloned. The possible role of these two additional genes in HC-toxin biosynthesis is being investigated.

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USE OF ALLELE-SPECIFIC OLIGONUCLEOTIDE PROBES TO CHARACTERIZE RESISTANCE TO BENOMYL IN FIELD STRAINS OF VENTURIA INAEQUALIS. H. Koenraadt, A. L. Jones, and S. C. Somerville. Department of Botany and Plant Pathology and Pesticide Research Center, Michigan State University, East Lansing 48824

Sequence analysis of the beta-tubulin gene of Venturia inaequalis revealed that a different single base mutation was associated with each level of resistance in benomyl-resistant field strains. Allele-specific oligonucleotide (ASO) probes (18-mers) were developed to detect the respective point mutations. A 408-bp fragment containing the putative mutation was amplified with the polymerase chain reaction using genomic DNA as template. Amplified DNA on dot blots was hybridized sequentially with each ASO probe. With this procedure, the critical nucleotide change associated with resistance to benomyl could be detected in unknown strains within 3 days.

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A MITOCHONDRIAL rDNA REGION AS A POSSIBLE SPECIFIC PROBE FOR *VERTICILLIUM* SPECIES. Li, K.-N., German, T. L., and Rouse, D. I., Department of Plant Pathology, University of Wisconsin, 1630 Linden Dr., Madison, WI 52706.

Mitochondrial small subunit rDNA regions from species of *Verticillium, Fusarium, Aspergillus, Magnaporthe, Neurospora* and *Ustilago* were amplified by the polymerase chain reaction (PCR). PCR was consistently successful when a low-annealing temperature of 47.5°C was used with the primers. The amplified regions ranged from about 600 to 750 base pairs. RFLP analysis showed that this region differed among genera. Ongoing research is aimed at comparing the DNA sequences of the amplified region and locating a specific DNA sequence for *Verticillium* which could be used with PCR technology to detect this pathogen in plant tissues or soil.

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TRANSCRIPTION RUN-STUDIES OF mRNA TRANSCRIPTION IN VIRULENT AND HYPOVIRULENT STRAINS OF *CRYPHONECTRIA PARASITICA*. Pam Kazmierczak, Lei Zhang, Pierre Pfeiffer, and Neal K. Van Alfen. Department of Plant Pathology and Microbiology, Texas A&M University, College Station, TX 77843.

Strains of this pathogen containing dsRNA express symptoms of reduced virulence and asexual sporulation. Previous work in this laboratory has shown that the dsRNA affects accumulation of specific fungal mRNAs. Several of these mRNAs have been cloned and used to study the mechanism of reduction of these mRNAs in a dsRNA infected strain. We report here the use of transcription run-on studies using isolated nuclei from a virulent and hypovirulent strain to examine the role of transcriptional regulation in dsRNA affected hypovirulence.

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DOWN-REGULATION OF CRYPARIN mRNA IN A HYPOVIRULENT STRAIN OF *CRYPHONECTRIA PARASITICA*. Debbie K. Villalon, Daniel Rigling, Lei Zhang, and Neal K. Van Alfen. Department of Plant Pathology and Microbiology, Texas A&M University, College Station, TX 77843-2132.

An RNA virus of the ascomycetous fungus *Crypohonectria parasitica* down-regulates the expression of a number of developmentally regulated fungal genes. The phenotype of viral infection is a reduction in asexual sporulation and virulence expression. A number of genes regulated by the virus have been cloned. One of the genes encodes a cell-surface, tissue-specific lectin-like protein. This cell-surface protein has been given the trivial name cryparin. Cryparin is found on the surface of aerial hyphae and asexual fruiting bodies when growing on solid culture media. The protein is glycine rich and has an N-terminal sequence with characteristics of structural proteins (-gly-ser- repeating sequence). This protein has many similarities in physical properties to cerato-ulmin, reported to be a toxin

produced by the Dutch elm disease fungus. Using protein sequence information, the gene for cryparin was cloned after PCR amplification. The gene is expressed as mRNA beginning at 24 hr after inoculation in liquid culture and mRNA is no longer detectable after 72 hr. Viral infection significantly reduces the amount of cryparin mRNA detected.

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CLONING OF THE ß-TUBULIN GENE FROM A BENOMYL RESISTANT MUTANT OF FUSARIUM MONILIFORME AND ITS USE AS A SELECTABLE MARKER. Keying Yan and M. B. Dickman, Department of Plant Pathology, University of Nebraska, Lincoln, NE 68583-0722.

As part of our continuing studies examining the fate, stability, and movement of foreign DNA in transgenic fungi, an altered ß-tubulin gene was isolated and cloned from a UV irradiated benomyl resistant mutant of Fusarium moniliforme. To isolate the ß-tubulin gene, a plasmid library was constructed from this mutant. Using highly conserved sequences for ß-tubulin, primers were designed and PCR was used to generate a probe from total genomic DNA of the mutant. Following the screening of the library a 2.1 kb clone was selected and mapped. Constitutive expression of the gene was indicated by RNA blots and a transcript of 1.9 kb was observed. Validation of the functionality of this gene was demonstrated by transformation of wild type F. moniliforme to benomyl resistance. Studies are in progress analyzing transformed isolates with this gene with respect to fitness characters and transmission ability. Comparisons of transformants to others generated with a heterologous altered ß-tubulin gene from Neurospora crassa as well as with hygromycin resistant transformants are also being done.

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CLONING AND ANALYSIS OF ABUNDANT mRNA FROM *PUCCINIA GRAMINIS*. Z. Liu, W.R. Bushnell and L.J. Szabo, USDA/ARS, Cereal Rust Lab., Dept. of Plant Pathology, Univ. of Minnesota, St. Paul, MN 55108.

Highly expressed mRNAs were characterized from germinated uredospores of *Puccinia graminis* f. sp. *tritici*. Sixty-eight cDNA clones were isolated representing abundant mRNA species. The two most abundant homology groups, pgt11 and pgt45, accounted for 3% of the poly(A) RNA in germinated spores. Southern analysis indicated that pgt45 was a single gene and pgt11 was a four-member gene family. The pgt45 gene was expressed in all stages of the uredial cycle examined, whereas the pgt11 gene family was sporulation stage-specific. Primer extension and DNA sequencing indicated that expression of two genes of the pgt11 family accounted for 90% of its mRNA. The pgt11 gene family encodes a small polypeptide with alanine, glycine, leucine and proline representing 48% of the protein. Both pgt45 and pgt11 are potentially useful as sources of regulatory sequences for transformation vectors and the study of gene regulation.

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DIFFERENTIATION OF GROUP IV PHYTOPHTHORA SPECIES BY PCR AMPLIFICATION OF NUCLEAR RIBOSOMAL DNA INTERNAL TRANSCRIBED SPACER REGION 2. K. F. Falkenstein[1], P. W. Tooley[2], S. B. Goodwin[3], and W. E. Fry[3]. [1]Dept. of Biology, Hood College, Frederick, MD 21701, [2]USDA-ARS, Frederick, MD 21702 and [3]Dept. of Plant Pathology, Cornell University, Ithaca, NY 14853.

The polymerase chain reaction (PCR) was used to amplify nuclear ribosomal DNA ITS region 2 (between 5.8S and 28S rDNA genes) using primers ITS3 and ITS4 (White et al. 1990. PCR Protocols, Academic Press, p. 317). Digestion of the ca. 600 bp PCR product with restriction enzyme DraI produced banding patterns that differentiated P. ilicis and P. colocasiae from P. hibernalis, P. mirabilis, P. infestans, and P. phaseoli. HhaI differentiated P. hibernalis, P. colocasiae, and the other four species. MspI differentiated P. hibernalis from the other five species. Unique TaqI banding patterns were observed for P. phaseoli, P. ilicis, P. hibernalis, and P. colocasiae. P. infestans and P. mirabilis showed identical patterns with TaqI as well as the other restriction enzymes.

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DETECTION OF dsRNA IN THE DOGWOOD ANTHRACNOSE FUNGUS. S. D. McElreath and F. H. Tainter. Clemson University, Clemson, South Carolina 29634-1003.

Mycelial extracts of 12 isolates of Discula sp., the dogwood anthracnose fungus, were analyzed for the presence of double-stranded RNA (dsRNA). After phenol-chloroform extraction, samples were purified with Whatman CF-11 cellulose and analyzed by agarose gel electrophoresis. Eleven of the twelve isolates contained dsRNA. High molecular weight bands were found in four isolates. Lower molecular weight bands were present in all isolates found to contain dsRNA. The nature of the dsRNA was confirmed with nuclease treatments. One isolate did not contain any detectable dsRNA. This isolate also differed from the others in colony morphology, degree of sporulation, and sensitivity to increased temperatures.

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VARIATION IN *BEMISIA TABACI* POPULATIONS BASED ON GEOGRAPHIC ORIGIN, SILVERLEAF SYMPTOM INDUCTION, AND ESTERASE BANDING PATTERNS. J.K. Brown[1], H.S. Costa[1], and J.Bird[2]. [1]Department of Plant Pathology, University of Arizona, Tucson, AZ 85721; [2]College of Agricultural Sciences, University of Puerto Rico, Rio Piedras, PR 00928.

Populations of *Bemisia tabaci* were collected from several locations and characterized with respect to geographic origin, the ability/inability to induce silverleaf (SL) symptoms in zucchini (*Cucurbita pepo* L.), and genetic polymorphisms based upon esterase banding patterns of individual whitefly adults. *B. tabaci* collected from field-grown zucchini in Arizona (AZ), from a population reared on poinsettia (*Euphorbia pulcherrima*)(AZ), from field-grown squash (*C. maxima*) in Puerto Rico (PR), or reared on poinsettia plants (PR), were associated with SL symptom development in zucchini test plants, while *B. tabaci* collected from cotton (AZ) and *Jatropha gossypifolia* (PR) were not. Three different esterase banding patterns (A1-5, B, and C) were detected among *B. tabaci* populations examined. The ability to induce SL symptoms was consistently associated with B-type individuals, but not with A1-5 or C-types. Based on these data, our hypothesis is that either a broad-spectrum genetic change has occurred in local populations, or that a different strain of *B. tabaci* has been introduced and/or dispersed throughout the region from an unidentified source.

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ISOLATION AND VECTOR RELATIONS OF A WHITEFLY-TRANSMITTED COMPONENT OF THE SWEET POTATO VIRUS DISEASE (SPVD) COMPLEX FROM NIGERIA. R. C. Larsen, M. Laakso, and J. W. Moyer, Department of Plant Pathology, North Carolina State University, Box 7616, Raleigh, NC 27695-7616.

The host range, symptomatology, and vector relations of the whitefly-transmitted virus-like component of SPVD were studied. Symptoms in the diagnostic indicator plant *Ipomoea nil* 'Scarlet O'Hara' (L.) Roth. included chlorosis and epinasty in new leaves followed by severe stunting and dwarfing of the entire plant. Older leaves became bronze colored and brittle. The agent was graft transmissible but attempts at mechanical transmission were unsuccessful. The infectious entity was efficiently transmitted by *Bemesia tabaci* Genn. to members of the Convolvulaceae and Solanaceae with minimum acquisition and inoculation access periods of 1 hr or less. The latent period, if any, was less than 1 hr. Results of persistence studies showed that individual whiteflies remained viruliferous for at least 24 hr but less than 48 hr.

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A NEW *BEMISIA* WHITEFLY BIOTYPE IN THE DESERT SOUTHWEST AND ITS ROLE IN SYSTEMIC PHYTOTOXEMIA AND VIRUS TRANSMISSION. J. E. Duffus, S. Cohen*, and H. Y. Liu, USDA-ARS, U.S. Agricultural Research Station, Salinas, CA 93905 and *Volcani Institute of Agricultural Research, Bet-Dagan, Israel.

Bemisia whitefly transmitted virus diseases have caused staggering losses to desert southwest agriculture since 1981. In Florida, since about 1987, whitefly populations increased greatly and induced large losses to squash and tomato growers. These losses have been attributed to factors involved in whitefly feeding. Whitefly populations from the southwest desert collected in the fall of 1990 are a mixture of biotypes. The original biotype does not induce systemic phytotoxemia on squash, broccoli and other crops under natural conditions; whereas the newly found biotype does. Physiological differences in host preference, larval development and phytotoxemia clearly distinguish the biotypes. The biotypes differ significantly in their abilities to transmit viruses and this may explain epidemiological differences between *Bemisia* transmitted viruses occurring in various places in the world.

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EFFECT OF PARTIAL RESISTANCE IN OATS ON TRANSMISSION AND TITER OF FIVE BARLEY YELLOW DWARF VIRUS ISOLATES. S. M. Gray, D. M. Smith, and M. E. Sorrells, USDA/ARS and Cornell University, Ithaca, NY.

Partial resistance that acts to reduce virus titer has been shown to reduce the transmission efficiency of several stylet-borne viruses, but has not been quantified for circulative viruses. Titers of the circulative barley yellow dwarf virus (BYDV), measured by ELISA, were suppressed in a breeding line of oats (IL86-5262). Mean level of titer suppression, relative to a susceptible oat genotype ('Astro'), for 6 wks post-inoculation was 80%, 65%, 61%, 58%, and 3% for the RMV, PAV, MAV, SGV, and RPV isolates of BYDV, respectively. Transmission efficiency of PAV, MAV and SGV by single aphids was lower from the resistant tissue relative to susceptible tissue, but remained above 70%. Transmission efficiency of RMV dropped from >80% to <40%. In field trials, the incidence of RMV reached 90% in susceptible 'Astro' plots, but remained <1% in IL86-5262 plots despite heavy inoculum and vector pressure. Final disease incidence of the MAV isolate was 74% and 97% in resistant and susceptible plots, respectively. The resistance in IL86-5262 is BYDV isolate specific and emphasizes the need to use multiple isolates when screening for resistance.

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PRELIMINARY STUDIES ON THE INCIDENCE OF BEET NECROTIC YELLOW VEIN VIRUS AND BEET DISTORTION MOSAIC VIRUS IN TEXAS. G. B. Heidel, C. M. Rush, and R. E. Mock. Texas Agricultural Experiment Station, P.O. Drawer 10, Bushland, Texas 79012.

Beet necrotic yellow vein virus (BNYVV), a soil-borne virus, was found in Texas in 1985. Beet distortion mosaic virus (BDMV) was reported in 1987. Currently, no information on the incidence of either virus in Texas is available. 160 soil samples were collected from two counties. Sugar beet seed was planted in two replications of each sample. After 9-10 weeks, root tissue was serologically assayed for BNYVV. At least one replication was positive in 29% of samples tested. Both replications were positive in 13% of samples. In a second study, beets with rhizomania symptoms were collected from eight fields. Veinal yellowing and necrosis, symptoms associated with systemic BNYVV infection, were observed in leaf growth from defoliated beets. Particles similar to both BNYVV and BDMV were observed by electron microscopy in leaf dips. Leaf tissue extracts reacted positively by ELISA against BNYVV antiserum.

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PHYSICOCHEMICAL CHARACTERIZATION, IDENTIFICATION OF GENETIC RESISTANCE AND GEOGRAPHICAL DISTRIBUTION OF SOILBORNE OAT MOSAIC IN NORTH CAROLINA. L. G. Elliott, S. Leath, and S. A. Lommel Dept. Plant Pathology Box 7616 NCSU Raleigh, N.C. 27695

A soil-borne mosaic disease has been recognized on winter oats in the southeast United States for over 50 years. The etiology of the disease has not been fully determined, therefore, we have undertaken the identification and characterization of the viruses associated with typical disease symptoms. Electron microscope observations, SDS-PAGE analysis and northern and southern blot analyses indicate the presence of both oat mosaic virus (OMV) and oat golden stripe virus (OGSV) in symptomatic leaf tissue. Polyclonal antisera have been produced in rabbits to the capsid protein of both OGSV and OMV. cDNA probes have been synthesized from the RNA genome of OGSV and sequenced (similar work is presently under way with OMV). Serological and cDNA probes were used to screen wild oat (*Avena sterilis*) lines and commercial oat (*Avena sativa*) cultivars from experimental plots established in infested fields to identify sources of resistance for use in breeding programs. Tissue samples from more than 20 commercial oat plantings throughout North Carolina and Southern Georgia were also screened to determine the geographic distribution of this disease.

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SUPPRESSION OF MAIZE CHLOROTIC MOTTLE VIRUS BY SOYBEAN ROTATION IN FIRST-YEAR CORN AND FUMIGATION IN THIRD-YEAR CORN. B. Doupnik, Jr. and S. G. Jensen, University of Nebraska, Clay Center, 68933, and USDA-ARS Lincoln, 68583.

In order to determine the effect of soybean rotation and soil fumigation upon the incidence of maize chlorotic mottle virus (MCMV) in corn, replicated plots were established in a field with a long prior history of MCMV. A susceptible hybrid (Pioneer 3168) was planted in first-yr corn ground following soybeans and in fumigated (Terr-O-Gas 67; 350 lbs/A) and unfumigated third-yr corn ground. The incidence of MCMV, resulting from natural infection, was determined by ELISA from randomly selected tassel leaves (15/plot) on July 31, 1990 and yields were subsequently determined. There were highly significant differences in MCMV incidence and yield between all three treatments. The MCMV incidence in the unfumigated ground was 98.3% and the yield was 159.2 bu/A; whereas the incidence and yield from the fumigated ground and soybean rotated ground was 53.3% and 183.4 bu/A and 0.0% and 219.1 bu/A respectively. ELISA assays for other corn viruses were negative. Results show that both soybean rotation and fumigation can significantly suppress the incidence of MCMV and that infection by MCMV alone can significantly reduce yields. These data also support the hypothesis that MCMV can be soil borne and that continuous corn production greatly increases the incidence of MCMV.

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INCIDENCE AND SPREAD OF BEET YELLOWS VIRUS IN THE LOWER SACRAMENTO VALLEY OF CALIFORNIA. P. A. Mauk and D. C. Koball. University of California Cooperative Extension, 4145 Branch Center Road, Sacramento, CA 95827.

Incidence of beet yellows virus (BYV) in sugar beets was monitored in 8 fields located near Clarksburg, CA. In March of 1990, BYV was detected (using ELISA) in 0-8% of the seedlings sampled 10-14 days after seedling emergence. Detection of BYV in sugar beets requires a minimum of 10 days of incubation, thus seedlings sampled acquired the virus through cotyledons before true leaves were formed. From March through July, distribution of BYV within fields was monitored every 10-18 days by sampling plants systematically (grid design) in each field. Disease incidence in July ranged from 40-69%. Additionally, comparisons of incidence and distribution of BYV were made for fields having different planting dates. Distribution patterns of BYV were found to be both directional and random possibly indicating both short and long distance introduction by aphid vectors, respectively. Date of planting did not significantly influence incidence or pattern of distribution of BYV within fields studied.

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A CARLAVIRUS INFECTING PASSIONFRUIT IN FLORIDA. A.A. St Hill, F.W. Zettler, M.A. Petersen, and R.G. Christie, and M.S. Elliott. Plant Pathology Department, Central Expt. Sta., Centeno Arima, Trinidad & Tobago; and Departments of Plant Pathology and Agronomy, Univ. Florida, Gainesville, 32611 U.S.A.

A carlavirus similar to one described in Germany (Brandes & Wetter. 1964. Phytopathol. Z. 49:61) was detected in Passiflora incense plants in Alachua County. The virus induced local chlorotic lesions and systemic chlorosis in manually inoculated plants of Chenopodium amaranticolor and C. quinoa, and inconspicuous systemic foliar mosaic symptoms in manually inoculated plants of P. edulis, P. foetida, and P. incarnata. Cytoplasmic inclusions like those described for carlaviruses were observed in epidermal cells of infected plants stained with Azure A (Christie & Edwardson. Florida Agr. Expt. Sta. Monograph 9, 1977). Flexuous rod-shaped virus particles were seen in negatively stained leaf extracts; of 120 particles measured, 61% were between 531 and 742 nm long with a main maximum at 651 nm. Groups of rod-shaped particles, but no cylindrical inclusions, were seen in thin sections of infected tissues. In unilateral immunodiffusion the virus did not react against antisera to passionfruit crinkle, passionfruit mottle, or passionfruit woodiness potyviruses (Chang & Lin. 1989. Taiwan Plant Protection Bull. 31:409).

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REINFECTION IN THE FIELD OF INDEXED GRAPE PLANTS BY *AGROBACTERIUM TUMEFACIENS*. X-A Pu and R.N. Goodman; Dept. of Plant Pathology; 108 Waters Hall; University of Missouri; Columbia, MO 65211.

Four indexed crown-gall-free cultivars were planted in a randomly replicated block from which the cv. Chancellor had been removed during the previous winter because of severe crown gall disease. The frequency of reinfection by *Agrobacterium* was monitored in the spring and fall for three years. The indexed grapes had remained crown-gall-free after one year's growth. In samples collected in early March before root pressure forced water into the xylem vessels, only 6% of the basal stem pieces contained *Agrobacterium* and none were detected at the apices. However, in April, when root pressure propelled water upwards in the xylem, 66% of the samples contained agrobacteria. Samples taken in October and December, however, revealed less that 5% with *Agrobacterium*. The seasonal fluctuation of *Agrobacterium* detected in grapevine suggested that the major source of *Agrobacterium* inoculum reinfecting the indexed plants was from the infested soil. The symptoms of crown gall became visible two years after the indexed grapes were planted in the heavily infested soil (3,000 cells/g). Both soil treatment with the fumigant Vorlex, and an antagonistic *A. radiobacter* strain, HLB-2, reduced the reinfection as much as 66% in this field experiment.

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THE RELATIVE SENSITIVITY OF SIX MAJOR GRAPE ROOTSTOCKS TO *AGROBACTERIUM TUMEFACIENS*, BIOVARS 1 AND 3. R.N. Goodman, Department of Plant Pathology; 108 Waters Hall; University of Missouri; Columbia, MO 65211 and Eidg. Forschungsanstalt O.W.G.; Wädenswil, Switz.

The universally used rootstocks 5BB, 5C, SO4, 8B, 125AA, and 3309 were evaluated for their sensitivity to an array of *A. tumefaciens* isolates of biovar 1 and 3. The tests were conducted both *in vitro*, wherein the amount of callus produced on 1/2 B-5 medium in 16 days was measured gravimetrically, and *in planta* where the size of tumor was measured volumetrically. *In planta* inoculations were made on exposed apical and basal surfaces of grape shoot internodes in B-5 medium without hormone. Differences in apparent sensitivity to the inocula will be discussed. In addition, the inherent sensitivity of the grape species *Vitis amurensis* was also evaluated.

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BACTERIAL STREAK OF MILLET CAUSED BY XANTHOMONAS SPP. IN COLORADO. Cutis E. Swift, Carol A. Ishimaru and William M. Brown, Jr.
Department of Plant Pathology & Weed Science, Colorado State University, Fort Collins, CO 80523.

During August 1989, millet with a brown-red, water soaked, leaf streak symptom was observed in northeastern Colorado. Affected leaf tissue was dried, pulverized in phosphate buffer (ph 7.4) and streaked onto MXP agar plates. Serial dilutions of bacterial suspensions, obtained from pure cultures, were vacuum infiltrated into young (20 cm) pearl millet seedling leaves. Typical leaf streaking symptoms developed in seven days. Bacteria were reisolated onto MXP agar. Isolation and inoculation procedures were repeated with similar results. Biolog analysis confirmed the bacterium was a Xanthomonas spp. This is the first confirmed report of Xanthomonas spp. attacking millet in Colorado.

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HALO BLIGHT OF SPRING WHEAT CAUSED BY PSUEDOMONAS SYRINGAE. Vidal R. Velasco, Carol A. Ishimaru and William M. Brown, Jr. Department of Plant Pathology and Weed Science, Colorado State University, Fort Collins, CO 80523.

A severe halo blight disease was found on Yacoro Rojo spring wheat grown under sprinkler irrigation in the San Luis Valley of south central Colorado in the summers of 1989 and 1990. Bacteria were isolated from affected leaves on King's B agar, purified and multiplied on nutrient media. Yacoro Rojo seedlings (20 cm) were vacuum inoculated with a bacterial suspension obtained from pure cultures. Typical halo-like symptoms developed in 7-10 days. Bacteria were reisolated and identified as Pseudomonas syringae using the Bilog system. Symptoms were also observed on barley in the San Luis Valley. This is the first report of Psuedomonas syringae causing halo blight on wheat and/or barley.

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DIRECT DETECTION OF THE HALO BLIGHT PATHOGEN *PSEUDOMONAS SYRINGAE* PV *PHASEOLICOLA* IN BEAN SEEDS BY DNA AMPLIFICATION. D. Prossen[1], E. Hatziloukas[2,3], N. J. Panopoulos[3], and N. W. Schaad[1]. [1]Harris Moran Seed Co., San Juan Bautista, California, [2]Dept. of Plant Pathology, University of California at Berkeley, and [3]Inst. of Molec. Biol. and Biotech., FORTH, Heraklio, Crete, Greece.

Halo blight of beans caused by *P. s. phaseolicola* (PSP) is a serious seedborne disease of beans. The only practical control is to sow seed which has been assayed and found free of the pathogen. Agar plating and immunological seed assays are available, however, such methods have limited sensitivity and work poorly with lots containing *P.s. syringae* (PSS) and/or large numbers of other bacteria. Using synthetic primers to phaseolotoxin gene sequences (*tox*) we developed a DNA amplification method to specifically detect PSP. Samples of seed washings were phenol/chloroform extracted and the crude DNA and primers amplified through 30 cycles. All 13 PSP strains tested were positive. In contrast, 27 saprophytic isolates from bean seeds, 15 PSS strains, 5 other plant pathogenic pseudomonads, and single isolates of 4 other plant pathogenic bacteria were negative in these tests. Seed washings containing 1-5 cfu/ml PSP were positively identified by PCR even when other bacteria were present in large numerical excess.

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FIRST REPORT OF RHIZOMONAS SP. CAUSING CORKY ROOT OF LETTUCE IN EUROPE. Ariena H.C. van Bruggen and K.N. Jochimsen. Department of Plant Pathology, University of California, Davis, CA 95616.

Symptoms of corky root (CR) were observed on iceberg lettuce in the Netherlands and England, on prickly lettuce in Spain, and on sowthistle in Greece. Slow-growing bacteria with colony morphologies similar to strains of Rhizomonas suberifaciens or Rhizomonas sp. were isolated from lettuce seedlings inoculated with soil extracts. Strains that had DNA homology with R. suberifaciens strain CA1 or Rhizomonas sp. strain WI4 were tested for pathogenicity on 1-wk-old lettuce seedlings, cv. Salinas. Four strains from the Netherlands and one strain from Greece induced typical symptoms of CR. One strain tested positively with monoclonal antibody MAb-Rs1, specific for all strains of R. suberifaciens, in an indirect ELISA test and shared some DNA homology with CA1. The other strains were genetically related to WI4 and tested negatively with MAb-Rs1 which does not react with WI4.

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HELICONIA WILT IN HAWAII. S. Ferreira, K. Pitz, and A. Alvarez. Department of Plant Pathology, University of Hawaii, Honolulu, HI 96822, and M. Isherwood, Department of Agriculture, Honolulu, HI 96814.

Pseudomonas solanacearum was identified from severely wilted Heliconia plants from October 1989 through November, 1990 from commercial Heliconia farms on Maui, Oahu, and the island of Hawaii. A survey of 20 farms indicated that Heliconia wilt occurred on 5 farms. A total of 17 P. solanacearum isolates were obtained, 16 from H. psittacorum and 1 from H. rostrata. Pathogenicity studies conducted by injecting bacterial suspensions into rhizome of Heliconia, the pseudostem of banana, and the stem of tomato, indicate the presence of 4 pathogenic strains of P. solanacearum from Heliconia in Hawaii. Two isolates were pathogenic on Heliconia only; 10 to Heliconia and tomato; 4 to Heliconia, tomato, and banana; and 1 to Heliconia and banana out of 17 isolates tested. Although the bacterium has not been identified from banana grown in Hawaii, the pathogenic potential of 29% of the isolates to banana suggests a threat to the Hawaiian banana industry. These results also suggest that regulations governing the importation of Heliconia into the state and the intrastate movement of vegetative cuttings or rhizomes is needed.

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A NEMATICIDAL TOXIN FROM PLEUROTUS OSTREATUS NRRL 3526. O.C.H. Kwok, R. Plattner, D. Weisleder and D.T. Wicklow, National Center for Agricultural Utilization Research, USDA-ARS, 1815 N. University St., Peoria, Illinois 61604.

Pleurotus ostreatus (Jacq. ex Fr.) Kummer, a wood-rotting fungus, captures and consumes nematodes. It produces tiny droplets of toxin from minute spathulate secretory cells. Once a nematode touches the droplets, it is immobilized in as little as 30 seconds. Strain NRRL 3526 was cultured on moistened, autoclaved wheat straw for 30 days. The toxin was extracted from the culture with deionized water and purified by HPLC. The active compound, at a

concentration of 300 ppm, immobilized 95% of the test nematodes (Panagrellus redivivus) within one hour. Immobilized nematodes did not recover. The toxin has a molecular weight of 200, and was identified by NMR and mass spectrometry as trans-2-decenedioic acid.

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GENETIC CONTROL OF SUBERIN ACCUMULATION IN WOUNDED PEACH BARK. A. R. Biggs, West Virginia University, University Experiment Farm, P. O. Box 609, Kearneysville, WV 25430.

Reciprocal crosses among three peach clones were performed during spring 1985. Parental clones and seedlings from the crosses were planted at two sites in May, 1986. Wounding studies were conducted during June 1989 to quantify suberin accumulation in healthy phloem/cortex tissues adjacent to the wound site. Differences in suberin accumulation among the three parent clones were significant, whereas all environmental sources of variation and their interactions with parent clone were not significant. Suberization of V68101 was greater than NJC95 and V68051, which did not differ from each other. Suberization response in the three families involving V68101 were not different from each other, and the remaining three families also were not different from each other. The full-sib heritability of 1.05 reflects the differences among family means. The broad sense heritability of 0.42 indicates that approximately 40% of the phenotypic variability is accounted for by genotype. Genetic factors are sufficiently large to allow for selection for increased suberization response.

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EFFICACY OF NON-TARPED APPLICATIONS OF METHYL BROMIDE FOR CONTROL OF FUSARIUM WILT/ROOT-KNOT NEMATODE COMPLEX IN COTTON. J.F. Hadden. Extension Plant Pathology Department, The University of Georgia Cooperative Extension Service, Tifton, GA 31793.

A study was initiated to evaluate the efficacy of non-tarped applications of methyl bromide (MBr) for the control of Fusarium wilt/root-knot nematode complex in Stoneville 825 cotton (*Gossypium hirsutum*). Tests were conducted in a field naturally infested with *Fusarium oxysporum* f. sp. *vasinfectum* and *Meloidogyne incognita*. Methyl bromide (15, 30, and 60 lbs MBr a.i./A) and 1,3-dichloroproprene (2.82 gals 1,3-D a.i./A) were injected through a subsoil ripper bedder eight days prior to planting. Non-fumigated plots served as a control. Disease symptoms were noted in all treatments. The lowest incidence of Fusarium wilt infected plants, 45%, was observed in the 60 lbs a.i./A MBr-treated plots with the highest incidence, 95%, in the untreated control plots. Incidence of root galling ranged from 20% in the 60 lbs a.i./A MBr-treated plots to 94% in the untreated control plots. Severity of galling in MBr-treated plots decreased as the application rate increased. Plants in all MBr-treated plots produced higher ($P = 0.05$) yields than those in the untreated control plots. Incidence of Fusarium wilted infected plants and root galling was lower ($P = 0.05$) in the 1,3-D-treated plots than in the untreated control plots.

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THE INFLUENCE OF THE TOBACCO CYST NEMATODE AND FUSARIUM WILT-RESISTANT TOBACCO ON FUSARIUM OXYSPORUM DENSITIES IN ROOTS AND SOIL. J. A. LaMondia, The Connecticut Agricultural Experiment Station, P. O. Box 248, Windsor, CT 06095.

Fusarium wilt-susceptible (S) or resistant (R) tobacco was grown in pasteurized soil infested with $4.4x10^3$ F. oxysporum CFU/cm^3 soil and inoculated, or not, with 22 tobacco cyst nematode (TCN) juveniles/cm^3 soil. After 4, 6 and 8 wk, plants were rated for wilt (0=healthy 4=dead) and disinfested roots plated onto Komada's media. Soil dilution plating determined CFU/cm^3 soil. TCN infection increased wilt ratings over 4-8wk (0.8 to 3.9 and 0.8 to 1.3 for S and R tobacco, respectively). TCN infection increased Fusarium colonies/cm root only for S tobacco (4.8 to 9.4 for S and 1.7 to 1.7 for R tobacco, in the absence or presence of TCN, respectively). CFU's of F. oxysporum/cm^3 soil were 44.4, 10.0 and 4.9 after 8wk of S, R and fallow, respectively. Resistant tobacco resulted in F. oxysporum densities intermediate between susceptible and fallow, regardless of nematode infection.

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DISTRIBUTION OF HETERODERA GLYCINES RACES IN ILLINOIS. E. J. Sikora and G. R. Noel. USDA-ARS, Department of Plant Pathology, University of Illinois, Urbana, IL 61801.

Forty-four populations of Heterodera glycines were collected from 23 Illinois counties. Populations were tested using the differential soybean lines Pickett 71, Peking, PI 88788, and PI 90763. Lee 68 was used as the susceptible standard. Seedlings were grown in 7.5-cm-d clay pots and inoculated with 1,000 eggs and second-stage juveniles obtained from H. glycines-infested field soil. Plants were maintained in a greenhouse at 25 C. After 1 month, the number of first-generation white females that developed on each differential was determined and the race of the population was designated. Twenty-eight populations were race 3, twelve were race 1, two were race 5, and there was one each of race 2 and 4. The 28 race 3 populations were found in 18 different counties in all regions of the state. The 12 race 1 populations were found in 10 counties in north, central, and west-central Illinois. Both race 5 populations were found in adjacent counties in east-central Illinois. The race 2 and race 4 populations were found in south-central Illinois.

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FACTORS AFFECTING *HETERODERA GLYCINES* EMERGENCE FROM EGGS. T.C. Walk and T.L. Niblack; Department of Plant Pathology; 108 Waters Hall; University of Missouri; Columbia, MO 65211.

Several factors have been tested for their effects on the emergence of second-stage juveniles (J2) of *Heterodera glycines* from eggs. Eggs mechanically freed from cysts were exposed to a variety of treatments for 12-14 d, and emerged J2 were counted as a measure of hatching. Exposure to 4-64 mM $ZnCl_2$ or 8-128 mM $ZnSO_4$ stimulated emergence. Stimulation peaked at 8-32 mM $ZnCl_2$ or 8-64 mM $ZnSO_4$ and decreased beyond these ranges. Surface disinfestation of eggs by pretreatment in 0.5% chlorhexidine for 15 min did not significantly affect later emergence in either water or 15 mM $ZnSO_4$. Stimulation of emergence by 15 mM $ZnSO_4$ was greater when eggs were extracted from mature females attached to roots of greenhouse-grown soybeans than when eggs were extracted from cysts stored in moist sand at 3-6 C for five months and pretreated in water at 28 C for one week. Root exudates collected from 'Williams 82', 'Fayette', and PI90763 soybeans grown in the greenhouse for 42 d all stimulated emergence, compared with that observed in water. This work confirms that *H. glycines* hatching is sensitive to variations of methods commonly employed in hatching studies.

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ADDITIONAL STUDIES ON THE USE OF BAHIAGRASS FOR THE MANAGEMENT OF ROOT-KNOT AND CYST NEMATODES IN SOYBEAN. R. Rodríguez-Kábana[1], D.B. Weaver[2], D.G. Robertson[1], E.L. Carden[3], and M.D. Pegues[3]. [1]Plant Pathology, [2]Agronomy and Soils, and [3]Gulf Coast Substation, Auburn University, Alabama 36849.

The value of Pensacola bahiagrass (Paspalum notatum) as a rotation crop for the management of nematode problems in soybean (Glycine max) was studied in a field infested with Meloidogyne arenaria and Heterodera glycines. Soybean cultivars Braxton, Kirby, Leflore, Ransom, and Stonewall following bahiagrass in 1988 yielded an average 114% more than the cultivars under monoculture. The superiority in yield of the bahiagrass-soybean rotation over monoculture was maintained for all cultivars through 1989 and 1990; however, there were marked declines in yield with each successive year. For all cropping systems the lowest end-of-season populations of H. glycines juveniles in soil were in plots with Leflore and the highest numbers in those with Braxton or Kirby. End-of-season M. arenaria juvenile populations in soil were generally highest in plots with Leflore in monoculture and in rotation plots.

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THE ETIOLOGY OF GLOBODERA SOLNANCEARUM (OCN) AND GLOBODERA TABACUM (TCN) AND A CRITIQUE OF THEIR TAXONOMIC NOMENCLATURE. W.W. Osborne, IAI, Inc., 1319 Main Street, South Boston, Virginia 24592.

In 1961, the OCN was found on stunted tobacco in Virginia by the author. By 1985, the OCN had spread causing crop failure of tomato and eggplant. A.M. Golden and J. Imbriani verified the OCN identifications. In 1990, OCN was found in tobacco fields in North Carolina (personal communications). B.F. Lowensbury reported in 1955 Connecticut Research that TCN "H. tabacum had not produced marked crop reduction in the field...". Also, "it (TCN) matures on tomato in such small numbers that it appears to be of no consequence as a tomato pathogen". In 1983, Alan Stone published a "Taxonomic note" in Academic Press where he proposed that OCN be a subspecies of TCN based on his statement, "G. tabacum and G. solanacearum are of local importance as tobacco pests in Connecticut and Virginia, respectively". Since 1983, research proves that the OCN and the TCN are definite and distinct species. They are easily separated by their host range, morphology, pathogenicity, host parasite relations, ecology, geographic distribution, and the published illustrated taxonomic key by R.H. Mulvey and A.M. Golden. J. Nematology 15: 1-59.

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RIBOSOMAL DNA (rDNA) POLYMORPHISMS IN *COLLETOTRICHUM GLOEOSPORIOIDES* ISOLATES CAUSING POST BLOOM FRUIT DROP OF CITRUS. H.D. Liyanage, R.T. McMillan, jr., and H.C.Kistler. Plant Pathology Department, University of Florida, Gainesville, FL 32611.

Post bloom fruit drop of citrus is caused by the fungus *Colletotrichum gloeosporioides*. Two major forms of rDNA (form I 9.0 kb and form II 7.0 kb) were detected by the heterologous hybridization of a rDNA clone from *Neurospora crassa* to the *Pst* I digested genomic DNA of the citrus pathogen isolates. Isolates may contain either or both forms. Cloning and mapping of these two rDNA forms revealed a deletion of a 2.0 kb fragment at the 5'end of the cluster giving rise to form II. Separation of chromosome-sized DNA by CHEF-gel electrophoresis and Southern hybridization revealed the presence of rDNA on a single chromosome either 10 mb or 5 mb in size. However, chromosome size is not correlated with the form of rDNA the isolate carries. Further studies suggested that the continuous culture caused the loss of rDNA form II from an isolate of the pathogen that initially contained both rDNA forms.

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ISOLATION AND CHARACTERIZATION OF ß-TUBULIN ALLELES FROM COLLETOTRICHUM GLOEOSPORIOIDES F. SP. AESCHYNOMENE. T. L. Buhr and M. B. Dickman. Department of Plant Pathology, University of Nebraska, Lincoln, NE 68583-0722.

Colletotrichum gloeosporioides f. sp. aeschynomene (C.g.a.) causes anthracnose on northern jointvetch. The fungus is currently marketed as a commercial mycoherbicide for biological control of this weed in both rice and soybeans. A strategy to increase the utility of C.g.a. as a biocontrol agent is to develop strains resistant to common fungicides. As an initial attempt in this direction we have isolated, cloned, and sequenced both wild type and mutant (benomyl resistant) ß-tubulin alleles. Primers were designed from known conserved sequences of ß-tubulin, and using PCR a probe was generated from genomic DNA. Following screening of a wild type lambda library, a strongly hybridizing 3.0 kb Hind III fragment was isolated. Appropriately sized Hind III genomic DNA fragments were purified from a benomyl-resistant strain, ligated into pUC 119, and screened with the PCR probe to obtain the benomyl-resistant ß-tubulin gene. We have sequenced the wild type allele and are comparing this information with the benomyl-resistant allele to identify the molecular lesion. C.g.a. has been successfully transformed with the benomyl-resistant gene to verify is function.

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RELATEDNESS AMONG FORMAE SPECIALES OF *FUSARIUM OXYSPORUM* IN THE CUCURBITACEAE. D. H. Kim, R. D. Martyn, and C. W. Magill, Department of Plant Pathology and Microbiology, Texas A&M University, College Station 77843.

Thirty-nine isolates representative of over 100 isolates of five formae speciales of *Fusarium oxysporum* causing vascular wilt in cucurbits were examined for genetic similarity by RFLP analysis of mtDNA. Total DNA was digested with three enzymes (*Pst*I, *Hin*dIII, and *Eco*RI), Southern blotted, and hybridized with a *F. o. niveum* mtDNA multiprobe (pFON2a - pFON8b). The presence or absence of mtDNA fragments were analyzed by the unweighted paired group method using averages (UPGMA) and Jaccard's association matrix. A total of 14 mtDNA RFLP groups were detected. Within each forma specialis there were unique RFLPs; however, one pattern generally occurred most frequently for each forma specialis. *F. o. niveum, F. o. melonis, F. o. cucumerinum*, and *F. o. lagenaria* shared common RFLP patterns. Matching distances generated by UPGMA suggest *F. o. niveum, F. o. lagenaria, F. o. melonis, and F. o. luffae* are closely related, while *F. o. cucumerinum* is more distant. The RFLP pattern most common in *F. o. niveum* also occurred in one or more isolates from every other formae speciales except *F. o. luffae* and was present in isolates from North America, Europe, and Asia. We speculate that there may have been a common progenitor for formae speciales that cause vascular wilt in cucurbits.

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CLONING AND CHARACTERIZATION OF U-JUN, A HOMOLOG OF C-JUN, FROM A PHYTOPATHOGENIC FUNGUS. H. Luo and M. Perlin, Dept. of Biology, Univ. of Louisville, Louisville, KY 40292.

Ustilago violacea is a heterothallic basidiomycete phytopathogen, commonly known as the anther smut fungus. From a cosmid genomic library a clone was isolated which hybridized strongly with a probe for c-jun, the human proto-oncogene. The DNA from this clone was further subcloned as a 3.5-kb BglII fragment into pBluescript SK(+) (Stratagene). The cloned fragment was restriction mapped and Southern hybridization with the c-jun probe localized the region of greatest homology to a 1.8-kb Pst I fragment. A series of nested deletions of the cloned fragment were constructed using an EXO/METH kit (Stratagene) in order to facilitate sequencing. DNA sequence analysis of one third of the nested deletions have shown that the sequence is approx. 70% G+C (compared to 58% for U. violacea total genomic DNA and 68% for human c-jun). We are currently investigating possible role(s) for the cloned material in regulation of gene expression for this fungus.

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CLONING AND ANALYSIS OF THE HSP70 GENE FROM *PUCCINIA GRAMINIS*. L.J. Szabo[1] and R.C. Staples[2], 1) USDA-ARS Cereal Rust Lab., Dept. of Plant Pathology, Univ. of Minnesota, St. Paul, MN 55108, 2.) Boyce Thompson Institute, Cornell University, Ithaca, NY 14853.

In order to develop transformation vectors for rust fungi, it is important to have homologous regulatory elements from strongly expressed genes. Vectors containing the regulatory elements from the gene encoding the 70-kDa heat shock protein (hsp70) have been used successfully to transform phytopathogenic fungi. Using a heterologous probe from *Ustilago maydis* a hsp70 gene (hss1) was cloned from *Puccina graminis* f.sp. *tritici*. By DNA sequence analysis it was shown that hss1 was incomplete and a second gene (hss2) was obtained by rescreening the library. Southern analysis indicated that hsp70 is a small gene family containing at least 4 members. The level of expression of this gene family shows approximately a five-fold increase after heat shock. The regulatory elements from hss2 are currently being used to construct transformation vectors for *P. graminis*.

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MEIOTIC AND MITOTIC STABILITY OF TRANSFORMING PLASMID DNA IN THE PHYTOPATHOGENIC FUNGUS MAGNAPORTHE GRISEA. P. W. Tooley[1], H. Leung[2], and S. A. Leong[3]. [1]USDA-ARS, Frederick, MD 21702, [2]Dept. of Plant Path., Washington State Univ., Pullman, WA 99164 and [3]USDA-ARS, Univ. of Wisconsin, Madison, WI 53706.

Magnaporthe grisea was transformed with heterologous cosmid pAN7-2 encoding hygromycin B resistance (Leung et al. 1990. Curr. Genet. 17:409-411). In four out of five crosses between hygromycin B resistant transformants and sensitive wild type parents, 1:1 ratios were observed for segregation of hygromycin B resistance, indicating that the character segregated as a single locus. In one of the five crosses rearrangements of integrated DNA were observed among hygromycin B resistant progeny. Transformants produced typical lesions when inoculated onto host plants. Some transformant cultures reisolated from host tissue showed rearrangements of integrated DNA, characterized by excision of one or more copies of the transforming plasmid. Rearrangements were also observed following ten asexual generations on nonselective agar medium.

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ELECTROPHORETIC KARYOTYPE OF LOUISIANA ISOLATES OF THE SUGARCANE SMUT FUNGUS USTILAGO SCITAMINEA. K. E. Damann and D. A. Navarre, Dept. of Plant Pathology & Crop Physiology, Louisiana Agricultural Experiment Station, L.S.U. Agricultural Center, Baton Rouge, La 70803-1720.

Karyotypes of monosporidial isolates of Ustilago scitaminea were determined by transverse-alternating-field electrophoresis (TAFE) and contour-clamped homogeneous electric field (CHEF) electrophoresis. Sample plugs were made using protoplasts and whole cells. A minimum of 17 distinct DNA bands was consistently observed. Chromosome length polymorphism or variation in chromosome number were not detected. The yeast S.

cerevisiae and lambda DNA concatemers were used as size standards. Isolates exhibited two small chromosomal DNA bands of approximately 55 and 95 kilobases (kb). The fifteen other DNA bands ranged from approximately 470 kb to just under 2000 kb. The apparent lack of variability may reflect the relatively recent introduction (1981) of the disease into Louisiana and the homogeneity of the initial inoculum.

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Molecular characterization of *Sporisorium reilianum*. G. Naidoo, C. W. Magill, and R. A. Frederiksen. Department of Plant Pathology & Microbiology, Texas A&M University, College Station, TX 77843.

There is currently little information available on the genetics of *Sporisorium reilianum*, causal agent of head smut of maize and sorghum. This is partly due to the lack of naturally occurring phenotypic mutants and the difficulty in generating auxotrophic mutants. RFLP's and RAPD's are being sought which will be useful as genetic markers. Pulse field gel electrophoresis(PFGE) and Southern hybridizations are being employed to describe the karyotype. Southern hybridizations are being used to localize the *ura3*, *tpi1* and *leu2* genes from *Ustilago maydis* on individual *S. reilianum* chromosome-sized fragments. Nine discrete chromosome-sized fragments were separated using PFGE. The width and densities of the bands suggest that there may be as many as 15 such fragments. Using *Saccharomyces cerevisiae* as a standard, preliminary estimates indicate that the genome size ranges between 18 and 27 Mbs. Flow cytofluorometric measurements of nuclear DNA reveal a genome size of 30 Mbs.

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MOLECULAR CLONING OF A PCR FRAGMENT CONTAINING A PORTION OF THE ARISTOLOCHENE SYNTHASE GENE FROM PENICILLIUM ROQUEFORTI. R. H. Proctor and T. M. Hohn, USDA-ARS, National Center for Agricultural Utilization Research, Peoria, IL 61604.

We are attempting to isolate a gene coding for the sesquiterpene cyclase, aristolochene synthase (AS). The enzyme AS catalyzes the cyclization of farnesyl pyrophosphate to aristolochene. Aristolochene is the probable precursor of PR toxin and several other structurally related sesquiterpenoids produced by the grain and silage mold, Penicillium roqueforti. Degenerate oligonucleotide primers based on the amino acid sequences of peptides derived from purified AS were employed to amplify a fragment of the AS gene by PCR. Amplification with one primer combination yielded a 946 bp fragment (PR1) from a genomic DNA template. Subsequent cloning and sequence analysis of PR1 revealed DNA sequences corresponding to four AS peptides and the presence of two putative introns. Southern blot analysis indicated that PR1 is present as a single copy in the P. roqueforti genome. Characterization of PR1 and progress toward the isolation of the AS gene are discussed.

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CHARACTERIZATION OF DOUBLE STRANDED RNA, AND DNA PLASMIDS OCCURRING IN FLORIDA ISOLATES OF RHIZOCTONIA SOLANI ANASTOMOSIS GROUP 4. J.R. Washington and F.N. Martin. Plant Pathology Department, Univ. of Florida, Gainesville, FL 32611.

Over one half of the 110 isolates of Rhizoctonia solani Anastomosis Group 4 (AG 4) collected from Florida contained double stranded RNA (dsRNA) and DNA plasmids. A wide range of virulence on cucumber, tomato, and sugarbeet was observed in greenhouse trials, with several hypovirulent isolates identified. The low level of virulence was confirmed by tests in autoclaved field soil. The relationship of specific dsRNA's and DNA plasmids to hypovirulence is being investigated. Restriction maps have been constructed for six linear DNA plasmids. Portions of each of the six plasmids have been cloned and utilized as plasmid specific hybridization probes. These will be useful in testing for plasmid transfer among isolates and determining if individual isolates have been cured of the plasmids.

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THE RELATIVE RESISTANCE OF 16 *MALUS* SPP. TO ROOT AND CROWN ROTS CAUSED BY THREE *PHYTOPHTHORA* SPP.. G.T. Browne, S.M. Mircetich, USDA ARS Dept. of Plant Pathology, University of California, Davis 95616; and J.N. Cummins, Dent. of Hort. Sciences, NYS Agric. Exp. Sta., Geneva, NY 14456.

Selections and varieties of 16 *Malus* spp. (*M*spp) (*M. baccata, M. bracteata, M. brevipes, M. coronaria, M. fusca, M. glabrata, M. glaucescens, M. halliana, M. ioensis, M. x magdeburgensis, M. x platycarpa, M. prunifolia, M. pumila, M. rockii, M. sargentii*, and *M. turesi*) were evaluated for resistance to root and crown rots caused by *P. cactorum* (*Pcc*), *P. cambivora* (*Pcm*), and *P. cryptogea* (*Pcr*) in the greenhouse. *M. pumila* 'Delicious'(*Mp*-D), used as a standard for comparison, was among the most susceptible of the *M*spp to *Pcc*, *Pcr*, and *Pcm* (mean root rot of 39%, 60%, and 97%, respectively), but the relative resistance of the other *M*spp varied with *Phytophthora* spp.. For example, compared to *Mp*-D, 13 of the 15 other *M*spp were significantly more resistant to *Pcc*; whereas only 8 of the 15, and 6 of 10 *M*spp evaluated were more resistant against *Pcr* and *Pcm*, respectively. Of 11 *M*spp evaluated against *Pcc*, *Pcm*, and *Pcr*, only *M. x magdeburgensis* and *M. sargentii* were highly resistant (means 1-16% root rot) to all three *Phytophthora* spp.. Thus, *M*spp vary greatly in resistance to *Phytophthora* spp.; however, among *M*spp, assessments of relative resistance to one *Phytophthora* sp. are not necessarily extendable to other *Phytophthora* spp..

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FACTORS AFFECTING GERMINATION OF *PODOSPHAERA CLANDESTINA* CONIDIA ON LEAVES AND FRUIT OF SWEET CHERRY (*PRUNUS AVIUM*). G. G. Grove, WSU-TFREC, 1100 N. Western, Wenatchee, WA 98801.

The effects of temperatures between 10-30 C and moisture vapor pressure deficits of 0-851 Pa on germination of conidia of *Podosphaera clandestina* on sweet cherry foliage (cv. "Bing") were determined under controlled conditions. Germination occurred at all vapor pressure deficits tested and in general increased with decreasing moisture stress. Germination occurred at 15-30 C during 8-hour incubation periods and 10-30 C during 16- and 24-hour incubation periods. The optimum temperature for germination after 16 hr of incubation was 25 C; but after 24 hr, shifted downward to 20 C. After 24 hr, germination exceeded 50% under the optimum conditions of 0 Pa at 20 C. At 20 C, germination decreased from 51.2% at 0 Pa to 29.3% at 466 Pa. Germination on immature (green) fruit (cv. "Bing") increased with incubation time and decreased vapor pressure deficit. At 0 Pa, germination ranged from 8% after 8 hr to 48% after 24 hr. Germination on ripening fruit was lower than on green fruit and was suppressed by increasing soluble solid concentrations. At 0 Pa, germination equaled 26.4% and 2.1% on fruit with soluble solid concentrations of 7-14.9% and ≥15%, respectively. It appears that fruit infection is unlikely immediately prior to harvest when soluble solid concentrations are high.

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ANATOMICAL CHARACTERIZATION OF PECAN SHOOTS WITH TISSUE- AND CULTIVAR-SPECIFIC RESISTANCE TO SCAB. K.M.T. Cason and I.E. Yates. USDA/ARS/MPRU, Richard Russell Research Center, P.O. Box 5677; Athens, GA 30613

The objective of this study was to identify anatomical indices of pecan associated with resistance to infection by the fungus *Cladosporium caryigenum*. Anatomy and surface morphology of current, 1-, 2-, and 3-year-old shoots from scab-immune (Barton, Elliott) and scab-susceptible (Desirable, Schley, Wichita) pecan cultivars were compared with light and scanning electron microscopy. The shoots of resistant cultivars had fewer fissures and significantly fewer lenticels per cm^2 than susceptible cultivars. Lenticel size increased with wood age in all cultivars, but there was no difference in size that correlated with disease resistance. The density and structure of trichomes on 1-year-old shoots varied with cultivar. The absence or low density of complex trichomes on 2- and 3-year-old shoots of all cultivars was associated with age-related resistance to scab.

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PYCNIDIA OF PHYLLOSTICTA AMPELICIDA ON CANES OF EUVITIS GRAPEVINES CONTRIBUTE INOCULUM FOR INITIATING BLACK ROT IN SPRING. C. M. Becker, and R. C. Pearson. Dept. Plant Pathology, Cornell Univ., NYSAES, Geneva, NY 14456.

Cane lesions, caused by Phyllosticta ampelicida, (anamorph of Guignardia bidwellii) are known to produce pycnidia that overwinter with viable conidia, but their function as a source of primary inoculum the year after formation has not been established. Black rot lesions were excised periodically from canes of the Vitis interspecific hybrid cv Aurore and placed in water for 2 hr. The number of conidia detected per lesion (each bearing about 70 pycnidia) declined from 7800 on 9 May (2 to 4 cm shoots) to 1300 on 13 July 1990 (pea-sized berry). Vital stains indicated most conidia were viable. Bundles of 20, 3 to 6-node canes were suspended above unsprayed vines of V. X labruscana 'Concord' prior to budbreak in 1990. The incidence of black rot on clusters and foliage at harvest was 47.5 and 5.5%, respectively, whereas the incidence of black rot on vines without bundles was 2.5 and 0% on clusters and foliage. There was a relationship between proximity of new shoots to one-year-old canes bearing black rot lesions and subsequent disease. Foliar infection was 26.5 and 8.2% on shoots growing beneath and above canes with lesions, respectively. Pycnidia in cane lesions may contribute an important source of primary inoculum in vineyards that are pruned mechanically and where the diseased canes are not selectively removed.

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CONTROL OF ALTERNARIA LATE BLIGHT OF PISTACHIO BY MANIPULATION OF IRRIGATION. Themis J. Michailides, D. P. Morgan, University of California, Berkeley/Kearney Agric. Center, Parlier 93648, and J. Wang, Inst. of Biology, Gansu Academy of Sciences, P. R. China.

Skipping one "critical" irrigation in a flood-irrigated pistachio (cv. Kerman) orchard in early August resulted in a significant ($P < 0.05$) reduction by 60% of fruit naturally infected by *Alternaria alternata* and a trend ($P < 0.10$) towards reduced disease levels on leaves. In blocks irrigated regularly, one application of Kocide 101 in mid August reduced percentage of infected fruit by 31% ($P < 0.05$), infected leaves by 50% ($P < 0.10$) and propagules of *A. alternata* on fruit. In addition, skipping a sprinkler irrigation in early August in another orchard containing both Kerman and Red Aleppo pistachio trees significantly ($P < 0.05$) reduced the levels of infected fruit and rachises by 60-75 and 65-72%, respectively, in both cultivars. The incidence and severity of natural Alternaria late blight were significantly ($P < 0.05$) greater for Red Aleppo than for Kerman trees. In addition, leaves of Red Aleppo inoculated with a suspension of 10^5 conidia of *A. alternata* per ml developed significantly ($P < 0.05$) more lesions than leaves of Kerman or Peters (male) trees. In general, propagules of *A. alternata* on pistachio fruit increased more in blocks irrigated on a regular grower's schedule than in blocks in which one irrigation was skipped in early August.

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IDENTIFICATION OF STRAWBERRY-INFECTING COLLETOTRICHUM SPECIES USING ISOZYMES. M. R. Bonde[1], G. L. Peterson[1], and J. L. Maas[2]. [1]USDA-ARS, Ft. Detrick, Frederick, MD 21702 and [2]USDA-ARS, Fruit Laboratory, BARC-West, Beltsville, MD 20705.

Distinguishing Colletotrichum spp. infecting strawberry or other fruit crops has become critical for import-export concerns, host resistance improvement, and development of disease control strategies. Strawberry isolates of C. acutatum (27), C. fragariae (8), and C. gloeosporioides (13) were distinguished by comparing isozymes of 12 enzymes and 14 putative loci. Intraspecific coefficients of similarity (CS) (maximum possible = 1.00) were 1.00 for both C. acutatum and C. fragariae and 0.80 for C. gloeosporioides. The interspecific CS comparing C. fragariae and C. gloeosporioides was low (0.42), suggesting they are distinct species. The higher CS of 0.77 comparing C. acutatum and C. gloeosporioides suggests they are more closely related. Isolates of C. atramentarium (10) and C. trifoliae (25) showed no intraspecific variation and the species were readily distinguished using isozymes.

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Effects of fruit nutrient management and postharvest yeast application on fungal decay in pear. D. Sugar, R.G. Roberts, R.J. Hilton, E.E. Sanchez, and T.L. Righetti. Oregon State University, Medford OR 97502.

Severity of postharvest decay of Bosc pears by Penicillium expansum and Phialophora malorum was reduced by orchard treatments which minimize fruit nitrogen (N) and enhance fruit calcium (Ca), and postharvest application of yeast species. Fruit from trees receiving no N or soil-applied urea 1 mo preharvest had smaller lesions than fruit from spring-fertilized trees. Summer $CaCl_2$ sprays further reduced decay severity. Postharvest application of Cryptococcus flavus or C. laurentii (10^8 cells/ml) into fruit wounds reduced lesion incidence and size. C. laurentii consistently provided better control of P. expansum and P. malorum than did C. flavus. N, Ca, and yeast treatments appeared to be additive factors reducing decay severity.

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THE EFFECT OF THE COMBINED APPLICATION OF METALAXYL AND OXAMYL UPON PATHOGENIC ORGANISMS, GROWTH, YIELD AND FRUIT QUALITY PARAMETERS IN AN INTEGRATED CITRUS MANAGEMENT TRIAL. J. Menge, J. Pehrson, J. Morse, C. Coggins, T. Embleton, D. Hare, J. Meyer, S. Van Gundy, E. Johnson, E. Pond, and D. Atkin, University of California, Riverside, CA 92521.

A 35-year-old navel orange grove on rough lemon rootstock was treated with a combination of fungicide (i.e. metalaxyl, Ridomil®) and nematicide (i.e. oxamyl, Vydate®) for 7 yrs. This fungicide-nematicide increased ($P=0.05$) root length (91%), root uptake of Rb^{86} (simulates K)/gm root (29%), % foliage K (14%), % foliage Ca (6%), number of new shoots (19%), shoot growth (15%), fruit yield (19%), fruit diameter (3%), % 88 size and larger (9%) and puncture resistance (5%). The fungicide-nematicide reduced ($P=0.05$) *Phytophthora parasitica* population (75%), *P. citrophthora* population (64%), *Tylenchulus semipentrans* larvae (37%), *Fusarium solani* colonized roots (19%), % foliage Mg (8%), fruit soluble solids (5%) and fruit color index (3%).

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CRANBERRY TRASH PILES AS A SOURCE OF INOCULUM FOR FRUIT ROT. F.L. Caruso, University of Massachusetts, Cranberry Experiment Station, P.O. Box 569, East Wareham, MA 02538

After cranberries are water-harvested, trash piles consisting of cranberry leaves, stems, and fruits remain adjacent to the cranberry bed. If left in place, these piles are a likely inoculum source for infections during the subsequent growing season. Thirty-one trash piles were sampled in October 1989 and 1990. Sixty rotted cranberries were selected, cut into halves, and cultured on acidified corn meal agar. Fungi were identified to genus after three weeks. Allantophomopsis, Penicillium, Phomopsis, and Physalospora were detected in all 31 piles; Coleophoma, Glomerella, Godronia, and Pullularia were detected in at least 55% of the piles. Allantophomopsis was isolated most frequently: 37.9% in 1989, 29.8% in 1990. Fifteen pathogenic fungi were detected. Many of the fungi were also detected in the leaves and stems. Fungicide records were compiled from each cranberry bed to determine whether the use of certain fungicides favors the buildup of inoculum of particular fungi.

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A LEAF SPOT OF CRANBERRY CAUSED BY A NEW SPECIES OF PROTOVENTURIA. L. M. Carris, Department of Plant Pathology, Washington State University, Pullman, WA 99164-6430.

An apparently undescribed species of *Protoventuria* (Ascomycetes: Venturiaceae) causing a leaf spot on cranberry was first reported in Oregon in 1990. The fungus has since been found to be relatively common in cranberry bogs in Oregon, Washington and Massachusetts. The leaf spot is characterized macroscopically by a circular reddening of host mesophyll cells; microscopically by laterally flattened dark brown hyphae that form a radiating pattern beneath the host cuticle. Pseudothecia form on dead leaves and ascospores are released in early spring in western Washington. The fungus is characterized by large (up to 33 μm long) ascospores with a dark brown, thickened wall enveloped in a gelatinous sheath. Although the leaf spots caused by the new *Protoventuria* sp. superficially resemble those caused by *Protoventuria myrtilli*, a common fungus in cranberry bogs in Massachusetts and Wisconsin, the two fungi are distinct.

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INFLUENCE OF FLOODING ON PATHOGENICITY OF *PHYTOPHTHORA* SPP. TO CRANBERRY. M. J. Drilias and S. N. Jeffers, Department of Plant Pathology, University of Wisconsin, Madison, WI 53706.

Six distinct morphological types of *Phytophthora* spp. have been isolated from cranberry plants and soil in Wisconsin. Pathogenicity of the most frequently isolated type, which is most similar to *P. cryptogea*, was determined with rooted hardwood cuttings (cv. Searles) planted in pasteurized soil (5 parts sand:1 part peat) that was artificially infested with inoculum (pooled from three isolates) at rates of 0, 2, 5, or 10% (v/v). Plants were grown for 13 wk and received four biweekly flooding periods of 0, 2, 4, or 6 days. Shoot growth was inversely related to both inoculum rate and flooding duration. However, flooding duration had a greater impact on shoot growth than did inoculum rate. Young plants (5-6 wk old) were affected more severely than older plants (19-21 wk old). In addition, relative virulence of four of the six morphological types from Wisconsin and *P. cinnamomi* from cranberry in Massachusetts was compared with 0 and 5% inoculum and 0 and 2 days of flooding. Shoot growth was reduced by three of the four morphological types but only when plants were flooded; however, *P. cinnamomi* reduced shoot growth both with and without flooding. In the absence of inoculum, flooding also reduced shoot growth. This confirms that *Phytophthora* spp. from Wisconsin are pathogenic to cranberry.

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S. T. Lam[1], N. R. Torkewitz[1], C. S. Nautiyal[2], and P. Dion[2]. Impact of the ability to utilize a single substrate on colonization competitiveness. CIBA-

Geigy Biotechnology Research, RTP, NC 27709[1], and Université Laval, Québec, Canada G1K 7P4[2].

We examined whether the ability to utilize a single substrate has an observable impact on the overall colonization competitiveness of a bacterium. Mannopine utilization was used as the model system. Tobacco plants can be transformed to produce mannopine using an appropriate *Agrobacterium* strain. We obtained sibling homozygous mannopine-producing (Mop+) and nonproducing (Mop-) segregants from such a transformation. We also obtained a natural isolate of *Pseudomonas putida* which can utilize mannopine (Mut+) as sole carbon source, and a Tn*5* mutant of it which had lost the ability to do so (Mut-). The relative competitiveness of the two strains was determined. Tobacco seeds were inoculated with a mixture of the two bacterial strains. Bacteria were recovered from some of the seeds and the ratio of the two bacterial strains was determined. The remaining seeds were planted. After 4 weeks, bacteria were recovered from the seedling roots and the ratio of the two bacterial strains were again determined. Our results indicate that the Mut- bacterial strain is as competitive as its Mut+ parent in an environment where mannopine is absent, but is less competitive in an environment where mannopine is present.

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ULTRASTRUCTURE OF FUNGISTATIC *COCHLIOBOLUS VICTORIAE* CONIDIA INCUBATED ON SOIL. T. C. Caesar-TonThat, J. Liebman, and L. Epstein, University of California, Berkeley, CA 94720.

Conidia of the fungus *Cochliobolus victoriae*, made fungistatic by incubation on soil, were examined ultrastructurally for signs of dormancy or metabolic activity. Wild type conidia are heavily melanized and are difficult to fix for electron microscopy. Conidia produced on media containing the melanin biosynthesis inhibitor pyroquilon (1 $\mu g\ ml^{-1}$) were well-fixed, and were not significantly different from conidia produced on media without pyroquilon in all other parameters measured: sensitivity to fungistasis, germination rate of conidia, and growth rate and sporulation of colonies. Germination was low (≤3%) for conidia incubated on soil for up to 96 h, but the conidia remained viable, as indicated by the high germination (83%) of conidia transferred from soil to agarose. When examined ultrastructurally, the fungistatic conidia appeared metabolically active, not dormant. Glycogen-like material was depleted during the 96 h period. Endoplasmic reticulum was well-developed, suggesting active protein synthesis. Mitochondria had well-developed cristae, and were elongated and appeared to be dividing. Nucleoli were prominent, suggesting active ribosome production.

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REDUCTION OF ROOT AND CROWN ROT OF TISSUE-CULTURED ASPARAGUS PLANTLETS, CAUSED BY *FUSARIUM MONILIFORME*, BY PRIOR INOCULATION WITH AN AVIRULENT ISOLATE OF *F. OXYSPORUM*, IN VITRO. Youn Su Lee and W. J. Manning, Dept. of Plant Pathology, Univ. of Massachusetts, Amherst, MA. 01003.

Asparagus plantlets (*Asparagus officinalis* L.) (female clone, NJ 362M) were obtained via meristem tip culture, increased on multiplication medium, and placed on filter paper slants in tubes containing Hoagland's solution. Two agar discs (controls), or two agar discs bearing a *Fusarium* isolate, were placed in contact with roots, just below the crown. In the first test, plantlets were inoculated with an avirulent isolate of *F. oxysporum*, and then challenged with an isolate of *F. moniliforme* at 3,5 and 7-day intervals after inoculation with the avirulent *F. oxysporum*. In test two, only 5 and 7-day intervals were used. In test one, reductions in extent of root and crown rot, caused by *F. moniliforme*, exceeded 50%. In test two, reductions exceeded 40%.

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SOILBORNE PATHOGENS IN VEGETABLES WITH WINTER COVER CROPS AND CONSERVATION TILLAGE. D. R. Sumner[1], S. C. Phatak[1], J. D. Gay[2], R. B. Chalfant[1], K. E. Brunson[1], and R. L. Bugg[3]. [1]University of Georgia, Coastal Plain Experiment Station and [2]Cooperative Extension Service, Tifton, GA 31793, and [3]University of California, Davis, CA 95616

Cucumber was planted in April with in-row subsoiling following 19 different winter cover crops and fallow for two consecutive years without pesticides. Populations of Rhizoctonia solani AG-4 were significantly less following fallow and grasses than following legumes, and crucifers and legume-grass mixtures were intermediate (4,4,19,12, and 12 colony forming units (CFU)/100 g of oven-dry soil). Populations of Pythium spp. were greater following legumes, crucifers, and mixtures than following fallow and grasses (19, 21, 38, 7 and 8 CFU/g, respectively). Cucumber fruit rot was significantly greater following legumes, crucifers, and mixtures than following fallow or grasses (21, 23, 23, 10, and 8% of fruits with lesions, respectively).

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REDUCING APHANOMYCES SEEDLING DISEASE OF SUGAR BEETS BY LIMITED IRRIGATION. K. M. Vaughn and C. M. Rush. Texas Agricultural Experiment Station, P.O. Drawer 10, Bushland, Texas 79012.

One of the most common seedling diseases of sugar beets in the Texas Panhandle is caused by Aphanomyces cochlioides, a zoosporic fungus. A study was conducted to determine if this seedling disease could be reduced by irrigation and/or seed treatments. In a greenhouse study, unprimed, unprimed+Tachigaren, solid matrix primed (SMP), and SMP+fluid seed treatments were planted in boxes that were pre-irrigated and artificially infested with oospores of A. cochlioides. Half the boxes were then irrigated post-plant. Seedlings from SMP and SMP+fluid seed treatments emerged faster than unprimed nontreated seed, but after 6 days there was no significant difference. No seed treatment affected disease incidence, however, irrigation treatments did. In post irrigated boxes, average seedling disease was 56%, but in pre-irrigated boxes only 5%.

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EFFECTS OF FREQUENCY OF FURROW IRRIGATION ON ROOT ROTS OF SQUASH AND PEPPER CAUSED BY PHYTOPHTHORA CAPSICI. A.C. Café-Filho, J.M. Duniway, and R.M. Davis. Dept. of Plant Pathology, University of California, Davis 95616.

Soil adjacent to Early Summer Crookneck squash and Yolo Wonder pepper, in field plots initially free of Phytophthora capsici, was infested 32 and 52 days after direct-seeding squash or pepper, respectively. Furrow irrigation was applied every 7, 14, or 21 days after soil was infested. Disease development was significantly delayed and final severity of symptoms on shoots and roots were reduced with decreasing frequency of irrigation for both crops. Yields in infested soil irrigated every 21 days did not differ from those in controls for either crop. In contrast, squash yields in infested soil irrigated every 7 and 14 days were 41% and 96% of that obtained with irrigation every 21 days in 1990 (33% and 97% in 1989). Corresponding yields for peppers were 45% and 83% in 1990. In controls plots, irrigation schedule had only slight effects on plant water potential and no effect on yield. Results suggest that less frequent irrigation can be used to reduce losses to root rots caused by P. capsici.

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Influence of soil and plant parameters on corky root of tomatoes in organic and conventional production systems. Workneh, F., A. H. C. van Bruggen, L. E. Drinkwater, and C. Shennan. Departments of Plant Pathology and Vegetable Crops, University of California, Davis Ca 95616.

A comparative study was made of five organic and four conventional tomato farms in the central valley of California. Ten soil and three plant parameters were measured at 20 locations within each farm and related to corky root (*Pyrenochaeta lycopersici*). Corky root severity was higher in conventional farms (5.9%) than organic farms(.55%). Microbial activity, measured by hydrolysis of fluorescein diacetate, was higher in soils from organic farms than soils from conventional farms. Corky root severities were grouped into three classes for use in stepwise and canonical discriminant analysis with 10 variables. Six variables (organic carbon, soil ammonium, total plant nitrogen, nitrogen mineralization rate, soil pH and soil nitrate) contributed most to the variation in corky root severity. The first canonical function indicated that organic carbon and nitrogen mineralization rate were negatively associated with disease severity. Soil nitrate and tissue nitrogen were positively associated with disease severity.

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THE SURVIVAL OF THIELAVIOPSIS BASICOLA IN NATURALLY INFESTED SOIL. C. S. Rothrock, Department of Plant Pathology, University of Arkansas, Fayetteville, AR 72701.

The influence of soil temperature, water and texture on natural populations of Thielaviopsis basicola was examined in controlled environmental studies using the selective medium TB-CEN (Specht and Griffin. 1985. Can. J. Plant Pathol. 7:438-441). Initial population levels for experiments ranged from 43 to 85 propagules/g of soil. The percent decrease in survival of T. basicola averaged 2% a week over all treatments, a rate of reduction much lower than those reported for soils artificially infested with chlamydospores. Survival was significantly lower at 24 C and 28 C than 16 C. Soil populations of T. basicola increased as much as 18-fold after planting cotton. Increases in pathogen populations were correlated with disease incidence and severity and the number of infection sites. Disease increased at lower soil temperatures and higher soil matric potentials. Soil texture did not influence T. basicola or black root rot.

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EFFECT OF *PRATYLENCHUS PENETRANS* and *P. CRENATUS* ON INFECTION AND COLONIZATION OF POTATO BY *VERTICILLIUM DAHLIAE*. J. H. Bowers, S. T. Nameth, R. M. Riedel, and R. C. Rowe. Dept. Plant Pathology, Ohio State University, OARDC, Wooster 44691.

Soil was infested in greenhouse studies with known densities of *Verticillium dahliae* and/or two *Pratylenchus* spp. in a factorial design, planted with single-eye potato seedpieces, and destructively sampled after 3, 5, 7, and 9 wk. Six plants per treatment were sampled at each time and four root samples per plant, each 10% of the root system, were excised using a grid method. Root samples were fixed and stained using an immunoenzymatic procedure. Root length and percent colonization were estimated for each sample. Additionally, basal stem sections were assayed to assess colonization by *V. dahliae*. After 5 and 7 wk, 83 and 100%, respectively, of plants growing in soil infested with *V. dahliae* and *P. penetrans* were colonized by *V. dahliae* in basal stem segments. Of those plants growing in soil infested with *V. dahliae* only, or *V. dahliae* and *P. crenatus*, 0 and 50% were colonized after 5 and 7 wk, respectively. Root colonization data will be discussed.

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RELATION OF ROOT ROT SEVERITY TO SUGAR BEET QUALITY PARAMETERS. C. M. Rush and K. M. Vaughn. Texas Agricultural Experiment Station, P.O. Drawer 10, Bushland, Texas 79012.

A study was conducted to evaluate the effects of Aphanomyces and Rhizoctonia root rot on sugar beet quality. Beets were collected from 15 fields during 1990 harvest, and beets from each field were separated into disease severity categories 0-4, with 0=healthy and 4=severely rotted. Multiple subsamples were then taken from each category and evaluated for sucrose content and impurities. From these, loss to molasses was calculated. Sugar loss from beets in each disease category was analyzed using regression analyses. As disease severity increased, percent sugar decreased significantly r=.79 (P=0.05), but impurities and loss to molasses were not correlated with disease rating. The relation between disease severity rating and sugar loss was best described by the equation, percent sugar loss = .43 rating^2, R^2=.89, P=0.0001. Compared to healthy beets, total recoverable sugar was significantly reduced in disease severity categories 2 through 4. The type of root rot did not affect these results.

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INFLUENCE OF IN-ROW FERTILIZER BANDING AND ASSOCIATED SOIL DISTURBANCE ON PERFORMANCE OF SPRING WHEAT AND SPRING BARLEY DIRECT-DRILLED INTO *RHIZOCTONIA*-INFESTED SOIL. R. J. Cook, USDA-ARS, 367 Johnson Hall, WSU, Pullman, WA 99164-6430.

Spring wheat and barley in the Pacific Northwest may be devastated by Rhizoctonia root rot (*R. solani* AG8 and *R. oryzae*) when direct drilled without crop rotation. Yields of spring wheat at Pullman (on a site direct drilled the 3 previous years to winter wheat), and spring barley at Lind, WA (on a site direct drilled the 4 previous years to spring barley) (Rhizoctonia root rot was the dominant yield-limiting factor at both sites) were 62% and 21% higher for wheat and barley, respectively, when shanks banded fertilizer (N,P,S) at planting within each seed row and below the seed compared with the same depth but to one side of each seed row. Yields were uniformly high whether shanks banded the fertilizer in-row or 15 cm to one side of each row in plots treated with methylbromide. Apparently roots of these plants can reach considerable distances to obtain fertilizer if healthy but not if diseased. Disturbing the *Rhizoctonia*-infested soil within each seed row may also provide some control of this disease. Fertilizer placement trials should take root diseases into consideration.

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The influence of low oxygen stress on development of Phytophthora root rot of cork oak. K.A.Jacobs [1], J.D. MacDonald [1], and A. Berry [2]. [1]Dept. of Plant Pathology, [2] Dept. of Ornamental Horticulture, University of California, Davis, CA 95616.

Phytophthora cinnamomi causes a root and canker disease of cork oak that is especially severe in landscaped environments. Excessive irrigation and high soil compaction are characteristic of many such sites, and can lead to hypoxic soils that enhance disease development. Growth chamber experiments with young oak seedlings revealed that 4 % or lower oxygen concentrations inhibit root growth by > 50 %. Point inoculations of root tips with zoospores and mycelium showed that at these same stressful levels, frequency of infection and extent of root colonization by the fungus is greater than at higher aeration levels. Histological comparison of control and infected roots incubated at 0.5 % or 21 % oxygen showed that hypoxic roots had underdeveloped suberized and lignified cell layers (endodermis) and ceased growth after inoculation. Our results indicate that roots stressed by low oxygen tensions are more susceptible to colonization by P. cinnamomi. Quantitative assays of lignin and suberin, and related histopathological results will be discussed.

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SUPPRESSION OF PHYTOPHTHORA ROOT ROT IN SEVERAL SOILS FROM CALIFORNIA AVOCADO GROVES. W. L. Casale and M. K. Rahimian, Department of Plant Pathology, University of California, Riverside, CA 92521.

California avocado grove soils that were suspected of being suppressive to Phytophthora root rot (PRR) in the field were evaluated as part of a project to develop biocontrol for PRR of avocado. Suspected suppressive soils collected from the root zones of avocado trees at various sites were infested with ground millet cultures of *P. cinnamomi* (1 g inoculum/L soil). Several, but not all, soils believed to suppress PRR in the field also suppressed root rot of susceptible avocado (var. Topa Topa) and *Persea indica* in greenhouse experiments. In general, soils that suppressed PRR in the greenhouse had higher organic matter (4.0 - 9.3%) than average, but not all soils with high organic matter were necessarily suppressive. PRR-suppressiveness was eliminated by sterilization and restored by the addition of natural soil to sterilized soil, suggesting a biological component was responsible for suppressiveness.

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MICROBIOLOGY OF CANADIAN SPHAGNUM PEAT SUPPRESSIVE TO PYTHIUM DAMPING-OFF. M. J. Boehm and H. A. J. Hoitink, Dept of Plant Pathology, Ohio State Univ. and Ohio Agricultural Res. and Dev. Center, Wooster, OH 44691.

Microorganisms capable of inducing suppression to damping-off of cucumber (Cucumis sativus cv 'Straight Eight') caused by Pythium ultimum were isolated from both suppressive, slightly decomposed light (H_2 on the von Post decomposition scale), and conducive, decomposed dark (H_4) Canadian Sphagnum peat. Chytrids and Oomycetes were abundant in the light and conspicuously absent from the dark peat. Trichoderma viride, Gliocladium virens, an unidentified actinomycete and fluorescent Pseudomonas spp. were the most efficacious biocontrol agents. Efficacy of isolates was significantly (P=0.05) higher in mixes prepared with the slightly decomposed light (H_2) as compared to those prepared with the more decomposed dark (H_4) peat. In conclusion, suppressiveness was affected by the microflora in and was a function of the decomposition level of the Sphagnum peat.

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CHANGES IN THE POPULATIONS OF MICROORGANISMS ASSOCIATED WITH THE APPLICATION OF SOIL AMENDMENTS TO CONTROL SCLEROTIUM ROLFSII. G. H. Canullo, J. W. Kloepper, and R. Rodríguez-Kábana, Department of Plant Pathology, Auburn University, AL 36849-5409.

Populations of microorganisms from soils treated with guanidine thiocyanate, guanylurea sulfate, thiourea, or furfural were compared with those of untreated soil. The changes they affected in soil microflora varied quantitatively and/or qualitatively depending on the compound. Guanidine thiocyanate (GT) increased total fungal population relative to populations of the other treatments. Populations of Penicillium purpurogenum was markedly higher in GT treated soil. GT also increased total bacterial populations, and was the only compound that increased actinomycete populations. The relative percent of Trichoderma spp. was significantly higher in soils treated with thiourea or furfural than in the other soils. Furfural increased the percent of P. purpurogenum in respect to total fungi, and was as effective as guanylurea sulfate in increasing chitinolytic bacteria and those in the Pseudomonas cepacia-group. Thiourea was most effective in increasing coryneform bacteria.

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A phytoreo-like virus associated with frogskin disease of cassava. L. A. Calvert, M. Cuervo, L. M. Constantino, J. A. Arroyabe, and F. J. Morales. Centro Internacional de Agricultura Tropical, Cali, Colombia.

Frogskin disease (FSD) of cassava is a virus-like disease of unknown etiology that was first reported in 1971 from southern Colombia. Isometric virus-like particles, approximately 80 nm in diameter, were found in thin sections of leaves, petioles, stems, and roots of plants that were affected with FSD using TEM. This disease was transmitted by the whitefly *Bemisia tuberculata* and the plants showed both leaf and root symptoms typical of FSD. Double-stranded RNAs were purified from cassava plants and run on polyacrylamide gels. There were nine species of ds-RNAs that were consistently present in plants affected with FSD. In transmission experiments, these ds-RNAs were not present in the plants prior to inoculation but were present in these plants after they showed symptoms of the disease. Because of the particle morphology, the ds-RNA species associated with this disease and the transmission experiments, it appears that FSD is associated with a virus that is similar to phyto-reoviruses.

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ASSOCIATION OF THE LA FRANCE DISEASE-SPECIFIC DOUBLE-STRANDED RNAS WITH ISOMETRIC VIRUS-LIKE PARTICLES AND DISEASE-SPECIFIC POLYPEPTIDES. M.M. Goodin, B. Schlagnhaufer, and C.P. Romaine, Department of Plant Pathology, The Pennsylvania State University, University Park, PA 16802.

A purification procedure was devised which yielded preparations from La France disease-affected mushrooms (Agaricus bisporus) that were highly enriched in a 35 nm isometric virus-like particle (VLP) and that contained trace amounts of both a 25 nm isometric VLP and the 19 x 50 nm single-stranded RNA bacilliform virus. Analysis of preparations from different mushroom isolates by agarose gel electrophoresis and SDS-PAGE showed the presence of the typical nine disease-specific double-stranded RNAs (dsRNAs) of 0.8 to 3.8 kb and three major polypeptides of MW 63, 66, and 129 kd, respectively. Neither the dsRNAs nor the polypeptides were present in comparable preparations of healthy mushrooms. In one healthy mushroom isolate, 25 nm isometric VLPs were observed, but it is not known if they are identical to those present in diseased tissues. Our data provide the first evidence that the La France disease-specific dsRNAs are encapsidated in 35 nm and possibly 25 nm isometric particles.

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APHID TRANSMISSION FROM AND SEROLOGICAL ANALYSIS OF MIXED INFECTIONS OF SGV AND RMV ISOLATES OF BARLEY YELLOW DWARF VIRUS. D. Hazelwood, and S. M. Gray, USDA-ARS, Cornell University, Ithaca, NY, and T. W. Carroll, Montana State University, Bozeman, MT.

Heterologous encapsidation and phenotypic mixing of capsid protein subunits commonly occur when more than one isolate of barley yellow dwarf virus (BYDV) infects a given plant, and can result in altered vector specific transmission. Field isolates of BYDV from Montana (MT), serologically related to New York (NY) RMV, but efficiently transmitted by both *Rhopalosiphum maidis* and *Schizaphis graminum*, were suspected of being a mixture of RMV- and SGV-like isolates. Transmission assays using single aphids of each species were unable to separate the MT isolates into more than one vector specific isolate. However, single aphids were able to separate the components of a mixed infection of 1) NY RMV and NY SGV, and 2) MT BYDV and NY SGV. *S. graminum* and *R. maidis* transmitted SGV and RMV to 90 and 98% of the test plants respectively. In plants infected with either NY RMV and NY SGV or MT RMV and NY SGV, the two isolates appear to coexist independently, with no evidence of heterologous encapsidation or phenotypic mixing. The MT RMV appear not to be a mixture of RMV- and SGV-like isolates, but rather isolates serologically related to RMV with a broader vector range.

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MOLECULAR PARAMETERS SUGGEST THAT COWPEA APHID-BORNE MOSAIC VIRUS IS A DISTINCT POTYVIRUS AND THAT BEAN COMMON MOSAIC VIRUS CONSISTS OF AT LEAST THREE DISTINCT POTYVIRUSES. D.D Shukla[1], N.M McKern[1], C.W Ward[1], and R.E Ford[2]; [1]CSIRO, Division of Biomolecular Engineering, 343 Royal Parade, Parkville 3052, Australia; [2]University of Illinois, Urbana-Champaign, IL 61801, USA

Bean common mosaic virus (BCMV) consists of a large number of strains which have largely been identified by their characteristic interactions with a selected number of differential bean cultivars. The relationships among these strains and other potyviruses that infect legumes are complex, with indications that BCMV, blackeye cowpea mosaic virus (BlCMV), cowpea aphid-borne mosaic virus (CAMV) and azuki bean mosaic virus (AzMV) may be strains of the one virus. Using high performance liquid chromatographic peptide profiles of coat protein digests, we demonstrate that CAMV-Morocco is a distinct potyvirus; and that BCMV strains belong to at least three distinct potyviruses for which the names BCMV, bean necrosis virus (BNV) and BlCMV are proposed. BCMV-NL1 is a strain of BCMV, BCMV-NL3 is a strain of BNV and BCMV-NY15, AzMV and peanut stripe virus are all strains of BlCMV along with the BlCMV-Type and W strains.

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SIMILARITY OF COAT PROTEIN PEPTIDE PROFILES OF FOURTEEN POTYVIRUS ISOLATES FROM SOYBEAN CONFIRMS THAT THEY ARE STRAINS OF THE ONE VIRUS. R.K. Jain[1], N.M McKern[1], S.A. Tolin[2], O.W. Barnett[3], R.E. Ford[4], C.W. Ward[1], and D.D. Shukla[1]; [1]CSIRO, Division of Biomolecular Engineering, Parkville 3052, Australia; [2]VPI & State University, Blacksburg, VA 24061-0330; [3]Clemson Univeristy, SC 29634-0377; [4]University of Illinois, Urbana-Champaign, IL 61801, USA.

A number of potyvirus isolates identified as strains of soybean mosaic virus (SMV) have been reported in the past on the basis of host range, symptomatology, vector specificity and antigenic properties. Comparison of recently established coat protein gene sequences of two of the strains, SMV-N and SMV-VA, suggested that they belong to two different potyviruses. The taxonomic status of other strains is uncertain at present. To address this question we have compared high performance liquid chromatographic peptide profiles of coat protein digests from 14 such strains. Our results show that these 14 strains belong to the one potyvirus, SMV-N suggesting that it may be the most dominant potyvirus infecting soybeans.

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NATURAL OCCURRENCE OF FIVE COWPEA VIRUSES IN PAKISTAN. M. Bashir and R. O. Hampton. Dept of Botany & Plant Pathology, Oregon State University, Corvallis, OR 97331-2902 U.S.A.

Ninety-one cowpea field samples were collected in July 1990 from two northern Pakistan provinces, Punjab and NWFP. Desiccated samples were tested by DAS-ELISA for the possible presence of seven viruses known to be seed-borne in cowpea: blackeye cowpea mosaic (BlCMV) and cowpea aphid-borne mosaic (CAMV) potyviruses, cowpea mosaic (CPMV) and cowpea severe mosaic (CSMV) comoviruses, cowpea mottle carmovirus (CMoV), cucumber mosaic cucumovirus (CMV), and southern bean mosaic sobemovirus (SBMV). The following viruses previously unreported in Pakistan-grown cowpeas were detected in one or more field samples: BlCMV (6), CAMV (22), CMoV (11), CSMV (5), and SBMV (9). Eleven of 91 samples contained a complex of two or more viruses, with CAMV occurring most frequently. Field collected seeds from a virus-infected local cultivar were tested for seedborne viruses. Wereas the parent plants were infected with BlCMV, CAMV, CSMV, and SBMV, only CAMV was seed-transmitted (7% frequency).

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BLACKEYE COWPEA MOSAIC (BlCMV) AND COWPEA APHID-BORNE MOSAIC (CAMV) POTYVIRUSES: BIOLOGICAL COMPARISONS AND SEROLOGICAL DISTINCTIONS. M. Bashir and R. O. Hampton. Dept of Botany & Plant Pathology, Oregon State University, Corvallis, OR 97331-2902 U.S.A.

Some investigators have proposed that these two potyviruses are indistinguishable and should be called BlCMV. We report biological and serological (ELISA) comparisons of 22 BlCMV isolates and 44 CAMV isolates, each seed-borne in selected Vigna unguiculata seedlots. Antiserum to a tentative CAMV isolate (9-7c, intermediate virulence) yielded IgG that reacted homologously with the Morocco CAMV type isolate and

was completely non-reactive with all BlCMV isolates including the Georgia BlCMV type isolate. This distinction was confirmed against the potyvirus monoclonal panel of R. Jordan. BlCMV and CAMV isolates were not delineated reliably by infectivity/symptomatology on eleven V. unguiculata genotypes previously reported as differential hosts. BlCMV seed-borne isolates tended to be more virulent than CAMV seed-borne isolates. BlCMV and CAMV type isolates were highly virulent.

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IDENTIFICATION OF VIRUSES INFECTING PEANUTS IN ALABAMA. R. T. Gudauskas, K. B. Burch, P. Jin, A. K. Hagan, J. R. Weeks, and J. C. French, Departments of Plant Pathology and Entomology, Auburn University, AL 36849.

Leaf samples collected from 542 peanut plants in 70 fields in 14 counties during July-August, 1990, were tested for peanut mottle (PMV), peanut stripe (PStrV), peanut stunt (PSV), and tomato spotted wilt (TSWV) viruses by enzyme-linked immunosorbent assay and sap inoculations onto indicator plants. Of 387 peanut plants showing virus-like symptoms at time of collection, 69% were infected with PMV, 64% with TSWV, 6% with PSV, and 0% with PStrV. Tests of 155 symptomless peanut plants showed that 21% were infected with TSWV, 19% with PMV, 0.6% with PSV, and 0% with PStrV. PMV and TSWV were identified in at least 3 fields in every county; PSV was identified in 10 of the 14 counties. Overall, TSWV, PMV, and PSV were found in 94%, 91%, and 26%, respectively, of the fields surveyed. These results indicate that the incidence of viruses in peanuts in Alabama is higher than was previously suspected.

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APHID TRANSMISSION OF A NON-APHID-TRANSMISSIBLE ISOLATE OF BEAN YELLOW MOSAIC VIRUS IN MIXED INFECTIONS WITH AN APHID TRANSMISSIBLE ISOLATE. M. S. Patil and M. R. McLaughlin, Department of Plant Pathology and Weed Science, Mississippi State University and USDA-ARS, Crop Science Research Lab., Forage Research Unit, Mississippi State, MS 39762.

Non-transmissibility of a Scott isolate of bean yellow mosaic virus (BYMV-Scott) by Aphis craccivora in Pisum sativum 'Dwarf Gray Sugar', was retested with four other aphid species, Acyrthosiphon pisum, Aphis gossypii, Myzus persicae, and Schizaphis graminum. None of the five species transmitted BYMV-Scott, but all transmitted BYMV-KY204-1 from respective single isolate-infected sources. The most efficient vector of BYMV-KY204-1 was S. graminum. When mixed infections of the two isolates were used as acquisition sources for S. graminum, A. craccivora, and A. pisum, all three species transmitted BYMV-Scott, but always mixed with BYMV-KY204-1 and at a much lower frequency. Transmissibility of BYMV-Scott appears therefore to be determined by properties of the virus which are not specific to one vector species.

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STUDIES ON THE SYNERGISTIC INTERACTIONS BETWEEN SOYBEAN MOSAIC VIRUS (SMV) AND TWO COMOVIRUSES IN MIXED INFECTIONS IN SOYBEANS. J. R. Anjos and S. A. Ghabrial. Department of Plant Pathology, University of Kentucky, Lexington, KY 40546.

Soybean plants dually infected with SMV and either cowpea mosaic virus (CPMV) or bean pod mottle virus (BPMV) developed more severe symptoms than those induced by the individual viruses. Whereas the concentration of CPMV or BPMV in dually infected plants was significantly higher than that in singly infected plants, the SMV titer was unchanged. To determine whether SMV replicase may recognize comovirus RNAs as templates and thus play a role in the enhancement phenomenon, protoplasts from immature soybean embryos were electroporated with a mixture of CPMV B-RNA and M-RNA, the individual CPMV RNAs, SMV RNA alone, or SMV RNA in the presence of CPMV M-RNA. Analysis of protoplast extracts by ELISA indicated that CPMV coat protein was detected only in protoplasts inoculated with CPMV M-RNA plus B-RNA. Similar results were obtained with BPMV RNAs. Transcripts of cloned cDNA representing SMV, BPMV and CPMV genomes are being used in cell free translations and in vivo transient expression assays to determine whether SMV NIa proteinase may process comovirus polyproteins.

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MOVEMENT OF BEET CURLY TOP VIRUS FROM PHLOEM IN PINTO BEANS INDUCED BY TOBACCO MOSAIC VIRUS INFECTION ON PRIMARY LEAVES. P. E. Thomas and Waqar Ahmed, USDA-Agricultural Research Service, and Department of Plant Pathology, Washington State University, WSU-IAREC, Rt. 2 Box 2953A, Prosser, WA 99350-9687.

Pinto bean plants var. Ouray were inoculated with beet curly top virus (BCTV) in the crook-neck seedling stage. Later, the primary leaves of some plants were rub inoculated with the UI strain of tobacco mosaic virus (TMV). Inoculated primary leaves developed local necrotic TMV lesions. BCTV but not TMV infected plants systemically. Systemic BCTV symptoms were more severe and BCTV antigen concentrations much higher in plants inoculated with TMV than in plants infected with BCTV alone. Immunoblot analysis of stems showed that BCTV antigen remained confined in the phloem of plants that were not TMV inoculated but escaped from the phloem tissue of plants that were TMV infected.

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PHLOEM TRANSPORT OF TOBACCO MOSAIC VIRUS IN XANTHI NC TOBACCO INDUCED BY POTATO LEAFROLL VIRUS. Waqar Ahmed and P. E. Thomas, Department of Plant Pathology, Washington State University, and USDA-Agricultural Research Service, WSU-IAREC, Rt. 2 Box 2953A, Prosser, WA 99350-9687.

Before or after aphid inoculation with potato leafroll virus (PLRV) leaves of young *Nicotiana glutinosa* and *N. tabacum* cv. Xanthi nc. plants were rub inoculated with tobacco mosaic virus (TMV). Lesions developed on inoculated leaves. After 3-4 wks, systemic necrotic lesions developed on young leaves and stems of plants inoculated with both TMV and PLRV, but not in plants inoculated with either virus individually. TMV was transmitted from the systemic necrotic lesions of dually infected plants, but not from non-necrotic areas, although these areas contained TMV antigen. Stem necrosis caused collapse of some dually infected plants. Regrowth from the base of such plants was non-necrotic but infectious by graft inoculation. TMV could not be transmitted by rub or by graft from noninoculated leaves and stems of plants inoculated with TMV only.

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REPORT OF A CLOSTERO-LIKE VIRUS FROM TOMATOES IN FLORIDA. R.J. McGovern[1], C.L. Davis[2], R.M. Harveson[1], R.F. Lee[2], H.R. Pappu[3], C.L. Niblett[3] and R.H. Brlansky[2]. Inst. of Food and Agr. Sci., Univ. of Florida, [1]Immokalee, FL 33934-9716, [2]Lake Alfred, FL 33850-2299 and [3] Gainesville, FL 32611.

A clostero-like virus , tentatively named tomato closterovirus (TCV), has been identified in commercially-grown tomatoes in Florida. No consistent symptoms have been associated with TCV, but the newly developing leaves of affected plants are often bright yellow in color, have disrupted phloem, and contain long, flexuous particles. Nucleic acid preparations contain double-stranded RNAs which are RNase sensitive and DNase insensitive and of approximately 20 and 4.3 kilobases, similar in size to those of citrus tristeza virus (CTV), another closterovirus. Radiolabeled cDNA sequences of CTV hybridize to extracts of TCV-affected plants. The economic impact, host range, vector,and relationships with CTV are being investigated.

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OLPIDIUM RADICALE IS THE VECTOR OF CUCUMBER LEAF SPOT VIRUS. R. N. Campbell[1], H. Lecoq[2], C. Wipf-Scheibel[2], S. Sim[1]. [1]University of California, Davis, CA 95616 and [2]INRA, Sta. Patho. Vegetale, B.P.94, 84143 Montfavet Cedex, France.

The ability of 3 cultures of Olpidium radicale and one of O. brassicae to transmit several viruses following in vitro acquisition by zoospores was compared. A bulk culture of O. radicale, from cucumber roots collected near Nantes, France, and a single-sporangial culture derived from it, both transmitted cucumber leaf spot virus (CLSV) and the cucumber fruit streak strain of CLSV (CF-CLSV). A bulk culture of O. radicale obtained from melon roots collected at Montfavet, France, did not transmit CLSV or CF-CLSV. Both bulk cultures and the single-sporangial culture transmitted cucumber necrosis and melon necrotic spot viruses used as positive controls. They did not transmit cucumber soil borne, squash necrosis, petunia asteroid mosaic, or tobacco necrosis viruses. In the same trials a single-sporangial culture of O. brassicae from lettuce in California transmitted only tobacco necrosis virus to cucumber.

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CONFIRMATION OF LEPTOSPHAERIA KORRAE, THE CAUSAL AGENT OF NECROTIC RING SPOT ON KENTUCKY BLUEGRASS IN COLORADO. D. C. Voltz and W. Brown, Jr. Department of Plant Pathology, Colorado State University, Fort Collins, Colorado, 80523.

In northern Colorado, a serious patch disease was prevalent on Kentucky bluegrass turf during the 1980s. An ectotrophic fungus was isolated from root and crown tissue of Kentucky bluegrass. This fungus was identified tentatively as Leptosphaeria korrae by anamorphic characteristics. A 1990-1991 sampling survey showed that L. korrae was associated consistently with plants displaying dark ectotrophic mycelium on roots and crowns. Psuedothecia do not occur naturally on leaf sheaths, stems, or roots but occasionally are produced in culture or on artificial media. Ascospores measure 127 (105-165) by 5.0 um with 7 (5-11) septa. Koch's postulates were completed using a Colorado ascospore isolate of L. korrae. Isolates identified as L. korrae reacted positively to the monoclonal antibody MAb LKc50 in indirect ELISA tests.

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EFFECT OF MELANIN BIOSYNTHESIS INHIBITORS ON GAEUMANNOMYCES SPP. AND MAGNAPORTHE POAE. M. L. Elliott, University of Florida, Fort Lauderdale Research and Education Center, Fort Lauderdale, FL 33314

Isolates of Gaeumannomyces graminis var. graminis, G. g. tritici, G. g. avenae, G. incrustans and Magnaporthe poae were examined for their in vitro response to two groups of compounds that inhibit melanin production. These included DHN melanin-inhibiting compounds (fthalide, chlobenthiazone, pryoquilon and tricyclazole) and DOPA melanin-inhibiting compounds (kojic acid, tropolone, diethydithiocarbamic acid and 2-mercaptobenzothiazole). The compounds were incorporated into potato-dextrose agar at 1, 10 and 100 ug/ml. Plates were inoculated with mycelial plugs. Growth and melanin production were evaluated after 5 and 10 days incubation at 28 C. Initial results for all the fungal species evaluated indicated that only the DHN melanin-inhibiting compounds affected melanin production.

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THE TAXONOMIC STRUCTURE OF *PHYTOPHTHORA CITRICOLA* BASED ON ISOZYME ANALYSIS. P. Oudemans, H. Förster and M.D. Coffey. Department of Botany, Duke University, Durham, N.C. 27706.

Ten electrophoretic types (ETs) from 14 isozyme loci were resolved among a worldwide collection of 122 isolates of *P. citricola*. The ETs clustered into 5 distinct subgroups (CIT1 - CIT5). Of these, CIT1 contained the Sawada type culture, a now defunct species *P. pini*, and an authentic isolate of *P. cactorum* var. *applanata*. Another subgroup, CIT5, was composed only of isolates from avocado. In interspecific comparisons a close relationship was established between *P. citricola*, *P. capsici* and *P. citrophthora* using both isozyme analysis and comparison of mtDNA restriction patterns. In UPGMA cluster analysis isolates of CIT5 clustered with the *P. capsici* and *P. citrophthora* subgroups rather than the other *P. citricola* subgroups. It is proposed that isolates of CIT5 represent a host-specific group from avocado. These may be sufficiently genetically distinct from other subgroups of *P. citricola* to now be regarded as a separate, cryptic, biological species.

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VEGETATIVE COMPATIBILITY GROUPS IN *FUSARIUM GRAMINEARUM* FROM WHEAT HEAD SCAB. R. L. Bowden and J. F. Leslie, Department of Plant Pathology, Kansas State University, Manhattan, KS 66506.

Twenty-four single-spore isolates of *Fusarium graminearum* were obtained from scabby wheat heads or seeds collected from 23 locations in Kansas in 1990. All isolates were sexually fertile and homothallic. Nitrate nonutilizing (*nit*) mutants of each isolate were generated on a medium amended with 1.5% $KClO_3$. Of 378 mutants, 161 were able to utilize nitrite and hypoxanthine (*nit*1), 165 utilized hypoxanthine but not nitrite (*nit*3), 47 utilized nitrite but not hypoxanthine (NitM), and 5 utilized neither nitrite nor hypoxanthine (*nnu*). *nit*1 mutants of each isolate were paired with either a NitM or a *nit*3 mutant of each isolate on media containing nitrate as the sole nitrogen source. Mutant pairs from each isolate were able to complement, but no isolate was able to complement with any other isolate. Therefore, each isolate belonged to a genetically-distinct vegetative compatibility group. This genetic diversity suggests that sexual genetic recombination may be important in the field.

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RELATIONSHIPS AMONG PATHOGENIC AND NONPATHOGENIC STRAINS IN A LOCAL POPULATION OF *FUSARIUM OXYSPORUM*. T. R. Gordon, Department of Plant Pathology, University of California, Berkeley, 94720.

Nonpathogenic (NP) strains of *Fusarium oxysporum* were isolated from an agricultural field soil in the San Joaquin Valley of California, from which *F. oxysporum* f. sp. *melonis* (FOM), cause of Fusarium wilt of muskmelon, also was isolated. One hundred NP strains were comprised of 29 vegetative compatibility (VC) phenotypes. One representative of each VC phenotype was examined for polymorphisms in mitochondrial (mt) DNA. This was accomplished by sequentially probing restriction digests, bound to nylon membranes, with nine *PST* I fragments previously cloned from the mt genome of FOM. Differences between strains included both restriction site changes and changes in length. A total of thirty six changes were treated as characters and scored as present or absent in each strain. Eight unique combinations of characters were identified. Parsimony analysis yielded an unrooted tree which grouped these eight mt DNA haplotypes into three major clusters, one of which included FOM and two NP strains. FOM was separated from the most closely related NP strain by six changes.

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RECYCLING OF SPENT SHIITAKE SUBSTRATE FOR OYSTER MUSHROOM PRODUCTION. D. J. Royse, Dept. of Plant Pathology, Mushroom Research Center, Pennsylvania State University, University Park, PA 16802.

Oyster (Pleurotus sajor-caju) mushrooms were grown on recycled substrate that was used to produce a full crop of shiitake (Lentinula edodes) mushrooms. The substrate used to grow the crop of shiitake mushrooms consisted of red oak sawdust, white millet and wheat bran (8:1:1 ratio; dry wt). Shiitake mushrooms were harvested for three flushes (60 days). At the end of the harvest period, the spent logs were passed through a grinder (9 mm screen) and dried to 48% moisture content. For oyster mushroom production, the spent substrate (basal medium) was supplemented with wheat bran (10% w/w) and millet (10% w/w). Treatments (5) consisted of combinations of 12% (w/w) ground soybean and 1% (w/w) $CaCO_3$. Yield (expressed as percent biological efficiency) were as follows: 1) 12% soybean, 1% $CaCO_3$ = 79.4%; 2) 0% soybean, 1% $CaCO_3$ = 66.4%; 3) 12% soybean, 0% $CaCO_3$ = 61.5%; 4) 0% soybean, 0% $CaCO_3$ = 18.8%; 5) fresh oak sawdust, 0% soybean, 0% $CaCO_3$ = 9.6%. Recycling of spent shiitake substrate for oyster mushroom production could lower materials costs and reduce the amount of solid waste requiring disposal.

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A SEMI-SELECTIVE MEDIUM FOR IDENTIFICATION OF *GAEUMANNOMYCES GRAMINIS* VAR. *TRITICI*. B. K. Duffy[1] and D. M. Weller[2]. [1]Dept. of Plant Pathology, Washington State University and [2]USDA-ARS, Pullman, WA 99164-6430.

Gaeumannomyces graminis var. *tritici* (*Ggt*) causes take-all, an important root disease of wheat and barley worldwide. Isolations from diseased and healthy roots of cereals yield a variety of fungi. Because of the absence of distinctive conidia, selection of putative isolates of *Ggt* depends on variable cultural characteristics such as dark pigmentation and hyphal curling. Ultimately, identification requires host inoculation. A semi-selective medium was developed which expedites the identification of putative isolates of *Ggt*. The medium includes extract from 40g boiled potatoes, 4g dextrose, 150 µg/ml rifampicin, 100 µg/ml rizolex, 18g agar, and 1 liter deionized water (final pH 6.3-6.5). All 81 strains of *G. graminis* var. *tritici* tested grew on the medium and changed the color of the medium from orange to purple. Isolates of *G. graminis* var. *avenae* and *G. graminis* var. *graminis* gave similar reactions. Eighty strains representing 30 different fungal species also were tested and only *Leptosphaeria korrae* strains gave a similar reaction.

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IDENTIFICATION AND GENETIC VARIATIONS AMONG VIRESCENCE-INDUCING MLOS IN CALIFORNIA. M. E. Shaw and B. C. Kirkpatrick. Department of Plant Pathology, University of California, Davis, CA 95616.

Between 1987 and 1990 approximately 250 crop and ornamental plants with symptoms of phyllody, virescence or proliferation were collected throughout California. Mycoplasma-like organisms (MLOs) infecting the plants were identified by leafhopper vector specificity and hybridization analysis using cloned MLO DNAs. Approximately 30% of the diseased plant were infected with the beet leafhopper transmitted virescence agent (BLTVA) and the remainder were infected with aster yellows. The BLTA-MLO isolates were transmitted only by Circulifer tenellus and Macrosteles fascifrons transmitted only AY-MLO isolates. Southern blot analysis showed considerable variation in plasmid profiles, especially among BLTVA-MLO isolates. The genetic diversity of these MLOs was also investigated by restriction fragment length polymorphisms using a cloned fragment of the X-MLO 16S ribosomal gene as probe.

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CLASSIFICATION OF MLOS IN THE ASTER YELLOWS MLO STRAIN CLUSTER ON BASIS OF RFLP ANALYSES. **I.-M. Lee, R.E. Davis, T.-A. Chen, L.N. Chiykowski, J. Fletcher, and C. Hiruki. Microbiol. and Pl. Pathol. Lab., ARS, USDA, Beltsville, MD 20705; Dept. of Pl. Pathol., Cook College, Rutgers Univ., New Brunswick, NJ 08903; Agriculture Canada, Ontario; Dept. Pl. Pathol., Oklahoma State Univ., Stillwater, OK 74078; and Dept. of Pl. Science, Univ. of Alberta, Alberta, Canada.**

Fifteen mycoplasmalike organism (MLO) strains from North America and Europe, including aster yellows (AY), tomato big bud (BB), clover phyllody (CPh), Chrysanthemum yellows (CY), and unknown MLOs, were studied. These MLOs are among those previously identified as members of the AY MLO strain cluster based on dot hybridizations. RFLP analyses revealed that all 15 strains can be classified into 3 genomic types; Type I (typified by eastern AY MLO), Type II (e.g., western AY and CY MLOs), and Type III (e.g., CPh MLO). This classification is consistent with results from monoclonal antibody typing and polymerase chain reaction analysis, but not with classifications based solely on biological properties.

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Isolation and size estimation of entire chromosomes from a phloem-limited bacterialike organism associated with citrus greening diseases and healthy periwinkles. K. H. Chen, Rutgers University, New Brunswick, NJ 08903, H. Neimark, SUNY Health Sciences Center, Brooklyn, NY 11203 and T. A. Chen, Rutgers University, New Brunswick, NJ 08903

Partially purified bacterialike organism (BLO) associated with citrus greening disease and a mitochondria-enriched fraction from infected and healthy periwinkles, respectively, were embedded in agarose blocks. The blocks were treated with SDS, proteinase K and then gamma-irradiated to obtain full size linear BLO and mitochondria chromosomes. Genomic DNA of BLO was readily separated from contaminating host nucleic acids by using pulsed-field gel electrophoresis. The identities of BLO and mitochondria chromosomes were confirmed by southern blot using E. coli 16S rDNA as probe. Chromosome size was determined by comparing the mobility of these full-length chromosomes to that of yeast DNA markers. BLO genomic size was estimated about 1600 kbp while mitochondria chromosome of periwinkle was approximately 90 kbp which was smaller than most of known plant mitochondria DNA.

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PLASMID-LIKE DNAs WITH A MYCOPLASMA-LIKE ORGANISM (MLO) AND THEIR SEASONAL OCCURRENCE. J. Chen and C. J. Chang. Department of Plant Pathology, University of Georgia, Georgia Station, Griffin 30223.

Plasmid-like (PL) DNAs (0.9 to 5 kb) were isolated from walnut tissues infected with walnut witches'-broom (WWB) MLOs. Clones of PL DNA from WWB MLO were used to monitor the presence of WWB MLO in two infected walnut trees during the 1990 growing season from April to late September. Samples were collected in fixed sites (branches) every two to three weeks. Sample DNAs were assayed by Southern-blot without digestion and probed with ^{32}P labeled PL DNA clones from WWB MLO. PL DNA was detected in tissues collected in late June whereas typical WWB symptoms did not develop until mid-August. Those branches that reacted positively with PL DNA clones from WWB MLO developed typical yellowing symptoms, whereas those branches that reacted negatively remained asymptomatic through the season.

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DNA PROBES FOR DETECTION OF MYCOPLASMALIKE ORGANISMS (MLOs) ASSOCIATED WITH LETHAL YELLOWING DISEASE (LY) OF PALMS IN FLORIDA. N. A. Harrison, C. M. Bourne, R. L. Cox, J. H. Tsai and P. A. Richardson. University of Florida, IFAS, Research and Education Center, Ft. Lauderdale, FL 33314.

Preparations enriched with MLOs were obtained from LY-diseased Manila palms (Veitchia merrillii) by differential centrifugation after grinding tissues in an osmotically augmented buffer. DNA from MLO-enriched fractions was subjected to equilibrium centrifugation in cesium chloride-bisbenzimide gradients to resolve an MLO DNA band. Putative MLO DNA was digested with EcoRI. Resulting fragments were ligated with pUC8 and cloned in Escherichia coli DH5-alpha. Seven disease-specific recombinant plasmids were identified by differential hybridizations using ^{32}P-labeled enriched MLO DNA and healthy palm DNA as probes. Cloned MLO DNA inserts used as probes hybridized strongly with extracts of total DNA from LY-diseased representatives of seven palm species. Probes also hybridized with DNA of other MLOs indigenous to Florida.

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PEACH X DISEASE MYCOPLASMALIKE ORGANISM (MLO) GENOMIC STRAIN CLUSTER. **I.-M. Lee, D.E. Gundersen, R.E. Davis, and L.N. Chiykowski. Microbiology and Plant Pathology Laboratory, ARS, USDA, Beltsville, MD 20705; and Agriculture Canada, Plant Research Center, Ontario, Canada K1A 0C6**

Genetic interrelatedness among 13 different MLO strains including aster yellows, clover phyllody, potato witches' broom, ash yellows, elm yellows, beet leafhoopper transmitted virescence, western X (WX), eastern X from Canada (CX), and clover yellow edge (CYE) MLOs, was investigated by dot hybridizations and restriction fragment length polymorphism (RFLP) analyses using 18 CX MLO DNA probes and 3 WX MLO DNA probes. Results from dot hybridizations clearly indicated that CX, WX, and CYE MLOs are closely related and form a separate genomic strain cluster that is only distantly related to other MLOs. Similarity coefficients derived from RFLP analyses indicated that CX, WX, and CYE can be classified into three distinct genomic types.

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EPIDEMIOLOGICAL STUDIES ON THE BLUEBERRY STUNT DISEASE. D. Maeso[1], D. Ramsdell[1], O. Taboada[1], I.M. Lee[2], and R.E. Davis[2], [1]Michigan State University, East Lansing, MI, and [2]USDA/ARS, Beltsville, MD.

Research was conducted on the epidemiology of blueberry stunt disease, caused by a mycoplasma-like organism (MLO). Population dynamics of Scaphytopius spp. suspected of being vectors of the disease were monitored during 1989 and 1990 growing season with yellow sticky traps placed in stunt-diseased bushes at weekly intervals from bud break until leaf drop. Healthy, potted 2-yr-old cv. Bluecrop highbush blueberry trap plants were exposed to leafhoppers under stunt-diseased bushes in the field for 2 wk-periods during the 1989 and 1990 growing seasons. S.frontalis and S.acutus showed two population peaks both seasons; one in June after petal fall stage and a larger second peak in late summer to early fall. S.magdalensis was only trapped in 1989, but showed the same trend. Trap plants and some of the insects trapped were tested with a DNA probe (pAY22) that detects the stunt MLO. The number of MLO-positive trap plants in 1989 was correlated with peaks in Scaphytopius spp. populations.

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MECHANISM OF BIOCONTROL OF *RHIZOCTONIA SOLANI* BY *VERTICILLIUM TRICORPUS* ON HYPOCOTYLS OF COTTON SEEDLINGS. E. J. Paplomatas, and J. E. DeVay. Dept. of Plant Pathology, University of California, Davis, CA 95616.

Verticillium tricorpus exhibits biological control activity against Rhizoctonia damping-off of cotton seedlings under greenhouse and field conditions. Catalase added at 1.1 U/ml, increased virulence of pathogenic isolates of *R. solani* and enabled a non-pathogenic isolate to become a pathogen of cotton, while ascorbate, an inhibitor of catalase activity, reduced virulence. The presence of cell wall-bound and cytoplasmic catalase isozymes of *R. solani* grown in a liquid

medium, was visualized on native PAGE gels. Culture filtrates of *V. tricorpus* inhibited bovine liver catalase activity and did not contain any extracellular catalase. Current evidence is consistent with a key role of catalase in the pathogenicity of *R. solani* on cotton and inhibition of catalase as a unique and critical factor in the mechanism of biocontrol of *R. solani* by *V. tricorpus*.

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STABLE TRANSGENIC PEANUT (ARACHIS HYPOGEAE L.) CALLI PRODUCED BY HIGH VELOCITY MICROPROJECTILE BOMBARDMENT. T. E. Clemente, A. K. Weissinger and M. K. Beute. Departments of Plant Pathology & Crop Science, NC State University, Raleigh, NC.

Transgenic peanut callus lines from 3 genotypes NC-7, Florunner and UPL-PN-4 were generated by high velocity microprojectile bombardment. Embryonic leaves excised from 4 day old peanut were bombarded with approximately 377 ng plasmid DNA (pRT99-GUS) carrying genes for both β-glucuronidase (GUS) and, neomycin phosphotrasferase II (NPT II), providing resistance to kanamycin. Bombarded leaflets were subsequently cultured on a MS based medium amended with 50 ppm kanamycin and subcultured at 2-3 week intervals. Rapidly growing chlorophyllus cell clusters were observed after 2-3 subcultures, within slow growing, white callus. Chlorophyllus masses produced 8 independent transgenic callus lines which were subsequently characterized. Southern blot analysis reveals the presence of multiple integrated copies of both NPT II and GUS within each line. NPT II assays were positive for all 8 lines, while GUS expression occurred in 5 out of the 8 lines.

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CHARACTERIZATION OF TOBACCO PLASMA MEMBRANE (PM) PROTEIN KINASES. X. S. Ye, S. Avdiushko, U. Jarlfors, and J. Kuc. Deptartment of Plant Pathology, University of Kentucky, Lexington, Ky 40546.

Plasma membranes were isolated from leaves of three month-old tobacco plants by aqueous two phase partitioning. Effects of calcium, calmodulin, polyamines, inositol triphosphate(IP3), phorbol esters, inhibitors of protein kinase C (PKC) and calmodulin , organic solvents and nonionic detergents on PM protein phosphorylation were studied. PM protein kinases were calcium but not calmodulin dependent. Spermidine strongly and spermine weakly enhanced phosphorylation of two PM proteins. Putrescine and cadaverine had no effect. The two proteins were highly phosphorylated in the presence of chloroform which broke PM vesicles as observed by electron microscopy. The effects of spermidine and chloroform on phosphorylation of the two proteins were additive. IP3, phorbol esters and various phospholipids had no effect on protein phosphorylation, but PKC inhibitor strongly inhibited phosphorylation at micromolar concentrations. Triton X100, Nonidet P40 and digitonin at 0.125% also inhibited PM protein phosphorylation.

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DETECTION OF PECTINASE ISOZYME POLYMORPHISM IN COLLETOTRICHUM GLOEOSPORIOIDES. B. V. Gantotti and M. J. Davis. University of Florida, IFAS, TREC, 18905 SW 280 St., Homestead, FL 33031.

The pectic enzymes of several isolates of the fungus Colletotrichum gloeosporioides, the cause of post-bloom fruit drop of lime, from different geographic locations in south Florida were examined by pectin-polyacrylamide gel electrophoresis. Pectic zymograms revealed isozymes of polygalacturonases, pectin esterases, and pectin lyases. The strains could be categorized into distinct zymogram groups based on the enzyme profiles. The analysis not only revealed the existence of pectin isozyme polymorphism but also enabled the characterization of strains by their pectic zymogram profiles. The implications of the findings on the epidemiology and structure of pathogen populations will be discussed.

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PLANT-INDUCED NUCLEASE FROM *FUSARIUM SOLANI*: A NOVEL VIRULENCE FACTOR? David Gerhold, Andrew Pettinger, and Lee A. Hadwiger, Department of Plant Pathology, Washington State University, Pullman, WA 99164-6430.

Plant pathogenic microbes produce an arsenal of macromolecule-degrading enzymes for pathogenesis, including cutinase, pectinases, hemicellulases, cellulases, and proteases. During our studies of pathogenicity we have found a nuclease which is secreted by *Fusarium solani* macroconidia during germination on pea pod endocarps. This plant-induced nuclease causes single stranded breaks in both single-stranded and double-stranded RNA and DNA. At calcium concentrations exceeding 5 mM, "Fsp nuclease" becomes highly aggressive, degrading dsDNA rapidly. Fsp nuclease activity is stable to boiling, and trypsin or chymotrypsin treatment, but is inactivated by SDS or proteinase K. Preliminary evidence indicates substantial Fsp nuclease activity exists in preparations of nuclei from fungal-challenged pea pods. Current experiments ask whether endogenous plant RNA or DNA are affected by this enzyme *in vivo*.

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CHARACTERIZATION AND EXPRESSION OF TWO GENES FOR HMG-COA REDUCTASE FROM POTATO. D. Choi, B. L. Ward, and R. M. Bostock, Department of Plant Pathology, University of California, Davis, CA 95616.

HMG-CoA reductase (HMGR) provides mevalonic acid, a precursor for many isoprenoids that are involved in the adaptation of plants to pathological and other environmental stresses. Induction of HMGR activity precedes and is necessary for the wound- and elicitor-induced accumulation of sesquiterpenoid phytoalexins and steroid glycoalkaloids in Solanaceous plants. Activity studies in potato tubers suggest that isozymes of HMGR are compartmented and regulated differently to accomodate cellular requirements for specific pathway endproducts. To better understand regulation of HMGR, we isolated 11 independent cDNAs by screening a tuber library with a probe derived from an *Arabidopsis* HMGR gene. We selected two clones (pHMG17, pHMG47) for further characterization based on their hybridizations with RNA from tuber disks treated with the fungal elicitor arachidonic acid (AA). A region-specific probe from pHMG17 detected an elicitor-induced increase in abundance of HMGR mRNA (approx. 2.6 kb), consistent both in time of appearance and relative abundance with the microsomal enzyme activity profile. A probe from pHMG47 detected a similar size wound-induced transcript but did not detect an AA-induced increase. Both cDNAs have a region with extensive homology (>80% amino acid identity) to tomato *HMG1* (J. Narita & W. Gruissem, The Plant Cell, 1989, 1:181) but also contain regions distinct from each other and from other published HMGR genes. Results of expression studies of these genes in potato tubers following inoculation with *Phytophthora infestans* will also be presented.

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CLONING OF AN ACIDIC PEROXIDASE ASSOCIATED WITH THE INDUCTION OF SYSTEMIC RESISTANCE IN CUCUMBER. J.B. Rasmussen[1], J.A. Smith[1], S. Williams[2], W. Burkhart[2], E. Ward[2], R. Hammerschmidt[1], and J. Ryals[2]. [1]Department of Botany and Plant Pathology, Michigan State University, E. Lansing & [2]Ciba-Geigy Biotechnology, Research Triangle Park, N.C.

The activity of three acidic peroxidase isozymes was enhanced in lf 2 of cucumber 20 hr post-inoculation of lf 1 with *Pseudomonas syringae* pv. *syringae*. The largest and most abundant isozyme was purified and partial amino acid sequence obtained. The data were used to amplify a 452-bp fragment of the gene by PCR. This fragment was cloned, sequenced, and used to isolate a 1.2 kb-cDNA for peroxidase. The deduced amino acid sequences of the PCR and cDNA clones matched observed sequences from isolated protein, and are similar to a tobacco peroxidase thought to be involved in lignification. The 5′ end of the cDNA contains a 23 amino acid signal peptide preceded by 11 bp of the nontranslated leader. Accumulation of peroxidase mRNA began in lf 2 16 hr post-inoculation in lf 1.

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REGULATION OF LACCASE BIOSYNTHESIS OF THE CHESTNUT BLIGHT FUNGUS *CRYPHONECTRIA PARASITICA* BY DOUBLE-STRANDED RNA (dsRNA). Daniel Rigling and Neal K. Van Alfen. Department of Plant Pathology and Microbiology, Texas A&M University, College Station, TX 77843.

Transmissible hypovirulence of the plant pathogenic fungus *Cryphonectria parasitica* is associated with cytoplasmic dsRNAs, of viral origin. Laccase activity was found to be reduced in dsRNA-infected strains suggesting a role for the enzyme in the expression of phenotypes affected by the dsRNA, namely virulence, sporulation, and pigmentation. The extracellular laccase of *C. parasitica* was purified and characterized as a glycoprotein with a molecular mass of approximately 77 kDa. The laccase gene was isolated by screening a cDNA expression library with laccase antiserum. Southern blot analysis indicates the presence of a single copy laccase gene. In liquid culture, laccase activity is reduced by about 75% in a hypovirulent (dsRNA-infected) strain compare to the isogenic virulent (dsRNA-free) one. In contrast, production of biomass is not affected by the dsRNA. Northern analysis showed that dsRNA down-regulates laccase biosynthesis by reducing laccase mRNA accumulation. Experiments to clarify the role of laccase in the biology of *C. parasitica* are in progress.

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RESOURCE PARTITIONING AMONG BACTERIAL EPIPHYTES IN THE PHYLLOSPHERE. M. Wilson and S.E. Lindow. Department of Plant Pathology, 147 Hilgard Hall, University of California, Berkeley, CA 94720.

The nature of resource partitioning among bacterial epiphytes in the phyllosphere was investigated using replacement series experiments, in which bacterial strain-

pairs were inoculated onto leaves in different ratios. Individual populations were enumerated once the population sizes had reached equilibrium. The *Pseudomonas syringae* strain-pairs examined all exhibited competition for a common limiting resource, possibly a specific nutrient or functional group of nutrients. While the isogenic *P. syringae* strain-pair shared the limiting resource according to the initial ratio of the strains, the non-isogenic *P. syringae* strain-pairs, exhibiting either directional or mutual antagonism, shared the limiting resource according to their relative competitive abilities. Other epiphytic bacterial species exhibited higher levels of coexistence with the *P. syringae* reference strain than did the isogenic *P. syringae* strain. High levels of coexistence between *P. syringae* and these species may have been achieved by partitioning of nutritional resources available in the phyllosphere.

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A TEST TUBE ASSAY FOR ESTIMATING POPULATIONS OF LEAF-ASSOCIATED BACTERIA ON INDIVIDUAL LEAVES. E. A. Milus and A. F. Mirlohi, Department of Plant Pathology, University of Arkansas, Fayetteville, AR 72701.

A test tube assay was developed for estimating populations of a rifampicin-resistant mutant (rif mutant) of Xanthomonas campestris pv translucens on individual leaves of wheat grown in the field from seed infested with the rif mutant. Nutrient broth amended with 200 mg cycloheximide and 100 mg rifampicin per liter was dispensed into 16x100 mm test tubes. Leaves were submerged in the broth and incubated on an orbital shaker at 25 C. Tubes were examined 2-4 times/day to establish the time when turbidity from growth of the rif mutant first became visible. A linear regression equation was developed using dilution plating data to convert incubation time to cfu/leaf. Contamination was negligible, and the lower limit of detection was close to 1 cfu/leaf.

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DIVERSITY OF STREPTOMYCES STRAINS PATHOGENIC ON POTATO. C. Doering[1], P. Kampfer[3], S. Manulis[2], G. Kritzman[2], H. Schrempf[4] and I. Barash[1,2]. [1]Tel Aviv Univ. and [2]Volcani Center, P.O.B. 6, Bet Dagan 50250, Israel, [3]Technische Univ., Berlin, and Univ. Osnabruck, D-4500, Osnabruck, Germany.

Streptomyces strains pathogenic on potato were subjected to numerical classification. It was demonstrated that the pathogens were distributed among different cluster groups. The majority of the pathogenic strains belonged to the S. violaceus cluster but strains classified in the other clusters were also detected. RFLPs were determined after digesting the DNA of each strain with either BamHI or BstEII or SalI or PvuII and hybridizing with rRNA operon from S. coelicolor. Depending on the restriction enzyme used, different RFLP similarity groups (SGs) were identified. No correlation was observed between restriction pattern and pathogenicity. No correlation was observed between the clustering obtained by numerical taxonomy and SGs according to RFLPs. Results obtained indicate that host specialization in phytopathogenic Streptomyces strains arisen by convergent evolution.

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DIFFERENTIATION OF *XANTHOMONAS CAMPESTRIS* PV. *VESICATORIA* STRAINS BY THE ELECTROPHORETIC SEPARATION OF THEIR CELLULAR PROTEINS. H. Bouzar, J. B. Jones & R. E. Stall, University of Florida, G.C.R.E.C., 5007 60th Street East, Bradenton, FL 34203.

Xanthomonas campestris pv. *vesicatoria* (Xcv), the etiological agent of bacterial spot of tomato and pepper, can be segregated into two phenotypic groups according to pathogenicity on the tomato genotype 'Hawaii 7998', carbohydrate metabolism, genomic fingerprinting, and pectinolytic activity. To identify antigens useful for epidemiological studies, cells of strains from different origins were SDS-lysed and their proteins were separated by polyacrylamide gel electrophoresis. Silver staining proved superior to Coomassie blue in revealing differential protein profiles. Strains from Taiwan and Florida shared a unique protein band (ca. 30kd), whereas strains from South America and Oceania presented a different protein (ca. 20kd). These data confirm the phenotypic grouping aforementioned and suggest the potential for development of antibodies specific to each Xcv subpopulation.

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SELECTIVE ENHANCEMENT OF *PSEUDOMONAS PUTIDA* CONTAINING THE CATABOLIC PLASMID NAH7 IN SALICYLATE AMENDED SOIL. S. F. Colbert, T. Isakeit, M. Ferri, and M. N. Schroth. Dept. of Plant Pathology, University of California, Berkeley, CA 94720.

Pseudomonas putida strain PpG7, containing the NAH7 plasmid that encodes the degradation of naphthalene and salicylate, was added with 0.17% salicylate to soil microcosms. Population sizes of PpG7 increased from 10^4 to $\approx 10^9$ cfu/g soil and the rate of CO_2 evolution peaked simultaneously, between 48 and 72 hrs, depending on soil type. Indigenous salicylate-utilizing bacteria were rare and did not significantly utilize the added salicylate. Salicylate-utilizing fungi were common to all soils. Fungal competition for salicylate reduced PpG7 growth rate, but did not always affect final population sizes after 7 days growth. These data show that growth rate of added bacteria can be selectively increased in soil by use of specific substrates.

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MOLECULAR CHARACTERIZATION OF A REPETITIVE ELEMENT OF *XANTHOMONAS ORYZAE* PV. *ORYZAE*. C. H. Yun, F. F. White, and J. E. Leach, Department of Plant Pathology, Kansas State University, Manhattan, KS 66506-5502.

The plasmid pJEL101 contains a 2.4 kb DNA fragment with a highly repetitive element that is present in the genome of *Xanthomonas oryzae* pv. *oryzae* (hereafter *Xoo*). Approximately 80 copies of element are dispersed in *Xoo*. Insertion elements of *Xoo* that hybridized with pJEL101 were isolated using the transposon trapping vector (pLSAC). One element, tentatively called IS*203*, had 98% identity with the sequence of pJEL101. Two other elements contained approximately the same size of DNA as IS*203*, although one element has a variant restriction enzyme fragment pattern. DNA sequence analysis has revealed that a sequence within pJEL101 has 52% identity with 670 nucleotides of IS*4351* from *Bacteroides fragilis*.

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GENETIC ANALYSES OF A CLUSTER OF GENES FOR EXPRESSION OF EPITOPES IN *XANTHOMONAS CAMPESTRIS* PV. *VESICATORIA*. J. B. Jones, G. V. Minsavage, R. E. Stall, and R. O. Kelly. GCREC, Univ. of Florida, 5007 60th St. E., Bradenton, FL 34203

Two monoclonal antibodies (Mab's), 2H10 and 5D12, which were produced against *X. c.* pv. *vesicatoria* (Xcv) strain 75-3, reacted with crude lipopolysaccharide. A cosmid library of 75-3 constructed in pLAFR3 was transferred by conjugation into Xcv strain 87-13, which reacted negatively in ELISA with the two Mab's. Transconjugants containing either of two clones, designated EC425 and EC795, out of 400 tested, reacted in ELISA with the two Mab's. By a combination of gusposon mutagenesis and subcloning, a region of about 5 kb within EC795 (23.2 kb) was essential for expression of the epitopes in 87-13. When EC795 was transferred into *X. c.* pv. *pelargonii* strain XCP58, expression of the Xcp pathovar specific Mab was inhibited; however, the epitope which reacted with 5D12 was expressed.

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TRANSFORMING CLAVIBACTER XYLI SSP. CYNODONTIS TO EXPRESS FOREIGN GENES. M.C. Metzler, Y.P. Zhang, & T.A. Chen. Department of Plant Pathology, Martin Hall, Cook College, Rutgers University, New Brunswick, NJ 08903.

Clavibacter xyli ssp. cynodontis is a non-pathogenic endophyte of Bermuda grass which can be transferred to numerous other plant species. We are investigating the basic molecular characteristics of this organism, and are working to develop methods for transforming the bacteria to express foreign genes. About half of the isolates collected from numerous locations in the U.S. and Taiwan contain an indigenous plasmid of about 50 kilodaltons, for which we have a restriction map. Investigations concerning the structure and function of this plasmid are continuing. We have optimized conditions for transforming the bacteria using electroporation, and we have found a broad-host range plasmid which stably replicates in the bacteria after transformation and which can express tetracycline resistance. We are now working to optimize expression from a promotor which will give high levels of expression of foreign genes.

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REGIONAL INCREASE AND SPREAD OF PATHOGENS IN PLANT POPULATIONS OF VARIOUS DENSITIES. D. W. Onstad, Illinois Natural History Survey, 607 E. Peabody Dr., Champaign, Illinois, 61820.

The temporal and spatial dynamics of a hypothetical pathosystem containing one foliar pathogen and one host species in a region of 8,192 host sites were simulated on a Connection Machine supercomputer. The threshold value of iR, potential reproduction per lesion, determines whether the density of infected leaves will increase over a pathogen's generation. This threshold increased as the density of susceptible hosts in the region decreased. If the increase in the density of diseased, including removed, lesions is the basis for prediction, no threshold exists. Theorems for predicting epidemics and asymptotic disease levels were challenged for not considering spatial heterogeneity, temporal scale, and the appropriateness of predictor variable.

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SIMILARITIES OF ERWINIA CAROTOVORA STRAINS RECOVERED FROM NATURAL SOURCES. G. D. Franc, M. D. Harrison and M. L. Powelson. UW Dept. PSIS, Box 3354, Laramie, WY 82071; CSU Dept. Pl. Path., Ft. Collins, CO 80523; and OSU Dept. Botany and Pl. Path. Corvallis, OR 97331.

Survey results identified natural sources of Erwinia carotovora (Ec) inoculum and suggested the bacteria were transported inland and deposited by storms that originated over the Pacific Ocean. Serological tests showed 7 Ec subsp. carotovora (Ecc) serogroups recovered from ocean water were also recovered from rain on the west coast. The single Ecc serogroup recovered from aerosols was also found in rain and ocean water. Inland surveys showed 4 serogroups recovered from snow in Colorado were also found on the west coast and 6 of 7 serogroups recovered from the snow pack were present in surface water in Colorado. Several Ec strains, not previously identified in Colorado, were recovered from snow and from samples collected on the western coast of the United States. Therefore, strain characteristics showed strong similarities and support the theory that long distance transport is occurring.

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POTENTIAL FOR AEROSOL DISPERSAL OF BACTERIAL PATHOGENS OF DRY EDIBLE BEANS. J. R. Venette and R. S. Lamppa. North Dakota State University, Department of Plant Pathology, Fargo, ND 58105-5012.

Viability of airborne Pseudomonas syringae pv. syringae, P. s. pv. phaseolicola, or the nonpathogen Erwinia herbicola was monitored in a stirred-settling chamber filled with air at relative humidities ranging from 95% to 30%. Air samples were collected with buffer-filled impingers and the collection was plated to estimate populations. Plating of co-atomized tracer spores allowed distinction of biological decay. Viability expressed as decimal reduction times (DRT) varied with bacterial strain and relative humidity. DRTs for all strains were longest at the highest relative humidities. In these tests, E. herbicola survived longer than P. s. pv. syringae and P. s. pv. syringae survived longer than P. s. pv. phaseolicola.

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Analysis of foci of infection of Asiatic citrus canker in a Florida citrus orchard. T. R. Gottwald, J. H. Graham, and D. S. Egel. USDA/ARS, Orlando, Florida, 32803.

Multiple foci of infection of Asiatic citrus canker were found in an orchard in south Florida during October 1990. Primary infection was traced to three infected trees on the adjacent property. Restriction endonuclease digest patterns of DNA taken from bacteria from this source were identical to those from a 1986 outbreak on the west coast of Florida confirming a suspected link in inoculum source. Initial infections coincided with a major rainstorm during mid-August 1989. There were three extensive areas of disease, each having a central tree with stem lesions that predated all lesions on other trees. Isopath maps of these areas showed a main focus of infected trees surrounded by secondary foci. Ordinary runs analyses of each diseased area indicated greater within-row than across-row aggregation. No predominant direction of spread was determined by disease gradient analyses. Spatial autocorrelation analyses suggested the occurrence of noncontiguous groups of infected trees. These noncontiguous groups of trees coincided with secondary foci at oblique angles to the primary foci. Apparently, natural spread was confounded by mechanical spread caused by orchard management practices.

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EVALUATION OF HONEY BEES AS VECTORS OF ERWINIA AMYLOVORA AND PSEUDOMONAS FLUORESCENS FROM HIVES TO APPLE BLOSSOMS. K.B. Johnson[1], V.O. Stockwell[2], D.M. Burgett[3], and J.E. Loper[2]. [1]Dept. of Botany & Plant Pathology and [3]Dept. of Entomology, Oregon State Univ., and [2]USDA ARS Hort. Crops Res. Lab., Corvallis, OR 97331.

The ability of honey bees to disperse E. amylovora and a biocontrol strain of P. fluorescens, PfA506, from hives to stigmas of apple blossoms was investigated. Bees walked through a freeze-dried powder of each bacterium as they exited hives to begin foraging activity. PfA506 inoculum contained 10^{10} cfu/g and was dispersed at 10^6 cfu per bee. Inoculum of E. amylovora contained 10^9 cfu/g and was dispersed at 10^5 cfu per bee. Half of the 0.25 ha orchard was treated with copper hydroxide prebloom and with oxytetracycline during bloom. PfA506 was recovered from 23% of non-treated blossoms but E. amylovora was found in < 1% of blossoms sampled. Although PfA506 is resistant to oxytetracycline and copper hydroxide in vitro, blossoms on treated trees supported significantly lower populations of PfA506 compared to non-treated controls. Integration of beneficial bacteria into fire blight management may require modified chemical recommendations.

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MEASURING COMPONENTS OF MORTALITY: ASSESSMENT OF A BIOLOGICAL CONTROL SYSTEM USING PATH ANALYSIS. D.M. Supkoff and J.J. Marois, University of California, Davis, CA 95616.

Path coefficient analysis was used to identify direct and indirect effects of skeletonweed rust (Puccinia chondrillina), gall midge (Cystiphora schmidti), and gall mite (Aceria chondrillae) on change of skeletonweed (Chondrilla juncea) density over a 10 year period at five sites in California. While path analysis has been used to assess components of yield or fecundity, analyses were performed which considered the separation of components of skeletonweed mortality. Skeletonweed rust but not the skeletonweed gall midge was commonly associated with decrease in host density from early spring (rosette state) to late spring (flowering stage). Path coefficients for the direct effect of early spring infection on change in host density from early to late spring ranged from values near zero to values of 0.81 to 0.95, depending on location and year. The importance of early spring infection on yearly change of density was also indirect, through correlation with late spring infection. From March, 1980, to March, 1989 decline in skeletonweed density ranged from 42 to 83 percent at five sites.

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Temporal Variation in Populations of Melampsora lini on wild flax (Linum marginale). A. M. Jarosz[1] and J. J. Burdon[2]. [1] Department of Botany and Plant Pathology, Michigan State University, East Lansing, MI 48824, [2] Division of Plant Industry, CSIRO, GPO Box 1600, Canberra, ACT 2601 AUSTRALIA.

567 isolates of M. lini, collected over a four year period from nine populations of L. marginale in Kosciusko National Park, N.S.W., Australia, were characterized for racial phenotypes using a set of differential hosts. Four races dominated the metapopulation; three being widespread and common, while the fourth (and most virulent) was widespread but rare in all but one population. At five collection sites, there was significant temporal variability which was not correlated with host resistance structure. The data are consistent with the hypothesis that local extinction/colonization events and genetic drift are causing the temporal variability at individual sites.

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POPULATION DIVERSITY AND DISTRIBUTION OF *XANTHOMONAS CAMPESTRIS* PV. *DIEFFENBACHIAE*. A. Alvarez, R. Lipp, D. Norman and A. Benedict. University of Hawaii, Honolulu, HI 96822.

A widespread pathogen of aroids, *Xanthomonas campestris* pv. *dieffenbachiae* (Xcd) consists of diverse subpopulations which were delineated into 12 serological groups by a panel of monoclonal antibodies (mAbs). Strains were biochemically diverse as revealed by starch hydrolysis and oxidation of 96 substrates (BIOLOG), and they carry a variable number of plasmids. Anthurium strains were more virulent to anthurium than strains isolated from other aroids, generally infected a broader host range, and predominantly were in six serological groups. By mapping the distribution and monitoring the spread of serologically distinct subpopulations, infection foci and modes of transmission were determined for Hawaiian anthurium farms. The introduction of new serotypes of *Xcd* on symptomless propagative materials was evidence for interfarm spread of the disease.

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ISOLATION OF TOMATO MOSAIC VIRUS FROM RED SPRUCE IN THE ADIRONDACK MTS. Jacobi, V., Castello, J.D., SUNY College of Environmental Science and Forestry, Syracuse, NY, 13210 and Flachmann, M., Institut fuer Botanik der Universitaet Hohenheim, D-7000 Stuttgart 70, Germany.

In January 1990, needles from seven red spruce (*Picea rubens* Sarg.) trees growing at 1000 m elevation on Whiteface Mt., New York were collected, separated into two 40g subsamples, and concentrated to 1 ml using two virus purification schemes. Tomato mosaic virus (ToMV) was detected in both concentrates by enzyme-linked immunosorbent assay (ELISA) and immunoelectron microscopy (IEM) and was transmitted from one needle concentrate to *Chenopodium quinoa* Willd. The virus was indistinguishable from an isolate of ToMV transmitted in 1989 from a stream draining this forest stand. Five of the seven red spruce trees were resampled in December, 1990 and ToMV was detected in crude needle extracts from all five trees by IEM but not by ELISA. This is the first report of virus infection of red spruce.

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MRI STUDY OF WATER UPTAKE BY LOBLOLLY PINE SEEDLINGS. MacFall, J.S., G.A. Johnson, and P.J. Kramer. Duke University, Durham, NC 27706

High resolution magnetic resonance imaging (MRI) was used to study water uptake from fine moist sand by roots of 9-mo. old loblolly pine seedlings. Container-grown seedlings were root pruned to leave only the taproot and 1-2 first order lateral roots with all attached fine roots, then planted in fine sand and watered to saturation. A reference tube filled with $CuSO_4/D_2O$ was placed vertically in the container, parallel with the taproot. Seedling roots and the surrounding sand were repeatedly imaged by a spin-echo pulse sequence over an 18-hr period. Water content of the depletion zones was measured by comparing the signal acquired from the moist sand with the signal from the reference tube and fitting the ratio to a previously established regression. Water depletion zones could clearly be seen to form during this period. Based on weight and surface area, fine roots were more efficient than lateral or taproots in water uptake, but not based on length. The taproots consistently had a depletion zone form around them, however water uptake by the lateral roots was extremely variable. For seedlings that had lateral roots which were very active in uptake, the fine roots were generally less active in water uptake. It is proposed that water uptake shifts from fine roots to large diameter roots in response to stress factors, such as pathogen challenge.

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EFFECT OF OZONE TREATMENTS AND *FUSARIUM SUBGLUTINANS* INOCULATION ON GROWTH AND DISEASE DEVELOPMENT OF LOBLOLLY PINE SEEDLINGS. W. A. Carey and W. D. Kelley. School of Forestry, 108 M. White Smith Hall, Auburn University, Auburn, AL 36849.

Loblolly pine seedlings (*Pinus taeda*), half of which were wound-inoculated with *Fusarium subglutinans* and half of which were only wounded, were exposed to one of four levels of ozone (< ambient (CF), ambient (NF) and 1.7 or 2.5 ambient) for 105 days before and 50 days after inoculation. Seedling height (H) diameter (D) and volume (V) and canker length (L) width (W) and area (A) were analyzed (SAS ANOVA) for correlation with seedling family, ozone, and inoculation. Family correlated with all variables. Ozone correlated with seedling diameter and the L, W, and A of cankers from inoculated and scars (or periderms) from uninoculated wounds. Inoculation correlated with seedling diameter for NF by 2.5 X comparison. The only interaction indicated was family * inoculation with respect to canker development. Family resistance correlated with canker size but not scarring.

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EFFECT OF METALAXYL ON PHYTOPHTHORA CINNAMOMI ROOT ROT IN PLANTATION-GROWN FRASER FIR. R. I. Bruck, Department of Plant Pathology, North Carolina State University, Box 7616, Raleigh, NC 27695.

Hurricane Hugo (September 1989) deposited some 240 mm of precipitation on the northern mountains of North Carolina. Following the storm's passing, high mortality of plantation-grown (4-8 years) Fraser fir were observed; and confirmation of Phytophthora cinnamomi root rot established. On May 16, 1990, five infected fields were located and 3, 50-tree rows/field, were treated with either SUBDUE 2E at 4, 8, or 12 qts per acre or SUBDUE G at 150, 250 or 350 lbs per acre. During the summer of 1990, all treatment rows were observed for Phytophthora root rot symptomatology and soil removed at 50 cm from the base of each tree and plated onto PCH agar, to monitor Phytophthora cinnamomi propagule levels. All SUBDUE treatments prevented any visual symptomatology during four months of observation on all treated rows. Control rows (non-treated) averaged an increase of 47.4% in symptomatic trees. However, soil propagules of Phytophthora cinnamomi remained high throughout the entire growing season, averaging 61 propagules per gram of soil. Although propagule numbers were reduced in higher treatment levels, sufficient inoculum remains in the soil to potentially reinitiate infection cycles.

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ASH CROWN CONDITION AND THE INCIDENCE OF ASH YELLOWS AND OTHER INSECTS AND DISEASES IN ILLINOIS, IOWA, MISSOURI, AND WISCONSIN. C.J. Luley, M. E. Mielke, J.D. Castello, J. Cummings Carlson, J. Appleby, R. Hatcher. MO Dept. of Cons., Jefferson City, MO, 65102, USDA Forest Service, St. Paul, MN, 55108, SUNY College of Env. Science, Syracuse, NY, 13210, WI Dept. of Nat. Res., Madison, WI, 53711, IL Nat. History Survey, Champaign, IL, 61820, and IA Dept. of Nat. Res., Ames, IA, 50010.

Green and white ash stands in Illinois, Iowa, Missouri, and Wisconsin were surveyed for ash yellows (Ash Y), and other insects and diseases. Twenty-one of 38 white ash and 20 of 41 green ash stands had Ash Y positive trees based on the DAPI DNA staining technique or the presence of witches'-brooms. Ash Y was widely distributed in all the states except Wisconsin, where only 2 of 20 stands were affected. Symptoms associated with Ash Y, other than witches'-brooms, were present in stands that tested both positive and negative for MLOs. More than half of the ash trees had 0-10% crown dieback and 5% were dead. Trees with 50% or greater crown dieback made up about 12% of ash volume (6-7m^3/ha). There was no significant difference in average crown condition rating between Ash Y positive and negative stands. Other insect and disease problems were common but did not appear to be primary factors contributing to crown dieback.

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THE IMPACT OF INSECT AND DISEASE DAMAGE AND HARVEST METHOD ON STAND STOCKING AND GROWTH OF JUVENILE ASPEN. M.E. Ostry and K.T. Ward, USDA Forest Service, North Central Forest Experiment Station, 1992 Folwell Avenue, St. Paul, MN 55108.

The influence of insects, diseases, and harvest methods on aspen (Populus tremuloides) regeneration was studied in three sucker stands in Minnesota and Michigan and analyzed by constructing a life table. Portions of each stand were harvested using either whole-tree or merchantable bole methods. Within these treatment areas, 2400 aspen suckers were permanently tagged the year following harvest and their development was examined at various intervals for up to 11 years. Natural self-thinning due to suppression was responsible for the largest proportion of suckers dying at all study sites and under both harvest systems. The predominant damaging agents directly affecting crown position and subsequent sucker mortality were Venturia macularis, the cause of leaf and shoot blight, and the poplar vagabond aphid Mordwilkoja vagabunda.

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DECOMPOSITION CHARACTERISTICS OF A NONDEGRADATIVE ISOLATE OF THE BROWN-ROT FUNGUS, POSTIA PLACENTA. F. Green III, T. L. Highley, C. A. Clausen, and L. Ferge. U.S. Dept. of Agric., Forest Service, Forest Products Laboratory, One Gifford Pinchot Drive, Madison, WI 53705-2398.

To better understand how brown-rot fungi decompose wood, a nondegradative isolate of Postia placenta (ME-20) was used to study the following: (1) Colonization and ultrastructural changes in wood, (2) effects on strength of wood, and (3) decomposition of isolated hemicellulose and cellulose. Although ME-20 was unable to produce weight loss in wood, cell lumens were heavily colonized and it produced an atypical hyphal sheath that appeared to penetrate the S_3 layer of wood. Cellulose fibers exposed by ME-20 seemed to be undegraded. ME-20 utilized isolated hemicellulose but not cellulose and reduced degree of polymerization of cellulose only slightly.

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ELECTRICAL PROPERTIES, PH AND CATIONIC COMPOSITION OF LIGNOCELLULOSE FROM FIVE TREE SPECIES COLONIZED BY DEGRADATIVE FUNGI. J. Jellison, Botany and Plant Pathology, Univ. of Maine, Orono, ME 04469.

Fungal colonization of wood is accompanied by changes in the pH and electrical resistance of the wood. Acidity and ionic content were seen to

increase progressively during the decay process. This was particularly evident in wood colonized by brown rot fungi, which showed a pH decrease of up to 2.5 units. The electrical resistance of aqueous wood extractions of five wood species infected with four decay fungi was significantly reduced in all cases vs. the control values. The greatest reductions in resistance were seen in wood degraded by brown rot fungi. Cation analysis of degraded woods showed increased levels of water soluble zinc, manganese and iron associated with the increased solubility of these transition metals under acidic conditions.

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TRANSGENIC MUSKMELON WITH THE COAT PROTEIN GENE OF THE WHITE LEAF STRAIN OF CUCUMBER MOSAIC VIRUS. C. V. Gonsalves, B. Xue, S. Namba, K. Ling, J. L. Slightom[1] and D. Gonsalves. Dept. of Plant Pathology, Cornell University, NYSAES, Geneva, New York 14456 and [1]The Upjohn Company, Kalamazoo, Michigan 49007.

Cucumis melo cvs Hales Best Jumbo, Burpee Hybrid and TopMark were transformed by inoculating cotyledons of three-day-old seedlings with Agrobacterium tumefaciens strain C58Z707 containing the binary plasmid pGA482GG, which includes the neomycin phosphotransferase II (NPT II), ß-glucuronidase (GUS), and CMV-WL coat protein genes within its T-DNA region. The 35S CaMV promoter and termination signals, and the CMV leader sequence were used for expression of the coat protein gene. After co-cultivation, explants were regenerated on Murashige & Skoog medium containing 1 mg/L 6-benzylaminopurine (BAP), 150 mg/L kanamycin and 500 mg/L carbenicillin. Shoots were rooted on kanamycin-containing medium and were successfully established in the greenhouse. Transgenic plants were NPT II and GUS positive and some lines expressed the CMV-WL coat protein gene. The same plasmid was also transferred into TopMark melon via the Biolistic process and showed positive transient expression for GUS.

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FIELD EVALUATION OF TRANSGENIC CUCUMBER PLANTS EXPRESSING THE COAT PROTEIN GENE OF CUCUMBER MOSAIC VIRUS. [1]D. Gonsalves, [2]P. Chee, [2]J.L. Slightom, and [1]R. Provvidenti. [1]Dept. of Plant Pathology, Cornell Univ., N.Y.S.A.E.S., Geneva, NY 14456; and [2]Molecular Biology-Unit 7242, The Upjohn Company, Kalamazoo, MI 49007.

Resistance to virus infection can be obtained through transgenic plants that express the virus coat protein (CP)-gene. However, there is little information on the evaluation of CP-gene expressing plants under field conditions. We tested transgenic cucumbers expressing the CP-gene of cucumber mosaic virus (CMV). R_2 seedlings of four transgenic lines of Poinsett 76 were compared against nontransformed plants and the CMV resistant cv. Marketmore 76. Infection was allowed to occur naturally by aphids using a low percentage of infected plants as initial virus sources. Transgenic plants expressing the CMV CP-gene performed much better than susceptible controls and comparably to Marketmore 76. After two months in the field, 8% of transgenic plants showed symptoms versus 98% of the susceptible non-transformed plants and 1% of Marketmore 76 plants.

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EXPRESSION OF COAT PROTEIN AND ANTISENSE RNA OF BEAN YELLOW MOSAIC VIRUS IN TRANSGENIC NICOTIANA BENTHAMIANA. J. Hammond and K. K. Kamo, USDA-ARS, FNCL, Beltsville, MD 20705-2350.

Agrobacterium-mediated transformation was used to introduce the coat protein (CP) gene and a 3' antisense construct of bean yellow mosaic virus (BYMV) into Nicotiana benthamiana. Regenerated plants were analyzed for the presence of BYMV sequences by PCR, and seedling populations further examined. R1 plants with either of two CP constructs expressed up to 20% of the level of CP in an active BYMV infection, as estimated by indirect ELISA. Levels of protection against BYMV and heterologous potyviruses are being determined in homozygous R2 populations. Additional chimeric constructs with the N-terminal portion of BYMV-CP and C-terminal regions from either pepper mottle virus or zucchini yellow mosaic virus CPs are also being examined.

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Transgenic Nicotiana tabacum Plants Expressing Various Forms of the Tobacco Etch Virus (TEV) Coat Protein are Resistant to TEV Infection. J. A. Lindbo and W. G. Dougherty. Department of Microbiology, Oregon State University, Corvallis, OR 97331.

Transgenic tobacco plants (Nicotiana tabacum cv. Burley 49) expressing various forms of the Tobacco Etch Virus (TEV) coat protein (Cp) have been generated. In additions to the complete TEV Cp, three different truncated forms of the TEV Cp have been constructed and tested in this experiment: 1) TEV Cp missing the amino terminal 29 amino acids, 2) TEV Cp missing the carboxy terminal 18 amino acids, 3) TEV Cp missing both the amino terminal 29 and carboxy terminal 18 amino acids. The R1 progeny expressing these different forms of the TEV coat protein have been assayed for their resistance to TEV infection and symptom amelioration. Transgenic plants expressing either truncated or full length forms of the TEV Cp are resistant to TEV infection.

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CONSTRUCTION AND EXPRESSION OF THE VARIABLE HEAVY CHAIN ANTIBODY GENE FROM A POTYVIRUS GROUP CROSS-REACTIVE MONOCLONAL ANTIBODY IN ESCHERICHIA COLI. Ramon Jordan and Liliana Di Nola-Baron. USDA-ARS, PSI, FNCL, Beltsville, MD 20705-2350.

The cloning and expression of the immunoglobulin genes from the hybridoma cell line that produces the potyvirus group cross-reactive monoclonal antibody PTY 1 as Fab fragments in bacteria was initiated with the construction of a variable heavy chain (VH) library. Total or mRNA isolated from hybridoma cells was primed with oligo dT for first-strand cDNA synthesis. Four VH primers (from Stratacyte, La Jolla, CA) and two IgG2a constant heavy region 1 (CH1) primers (of our own design) were then used in the PCR amplification of the VH-CH1 domain sequences. PCR products of ca. 700 bp were digested with primer- and vector-specific restriction enzymes and ligated to a modified λZAPII heavy chain vector (Stratacyte). A VH library of $>2 \times 10^6$ plaque-forming units (with <5% non-recombinant) was obtained. Results from further experiments to construct the variable light chain and combinatorial Fab libraries will also be presented.

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REQUIREMENT OF AN UPSTREAM *CIS*-ELEMENT FOR THE EXPRESSION OF GENES ON THE FULL-LENGTH POLYCISTRONIC TRANSCRIPT OF FIGWORT MOSAIC VIRUS (CAULIMOVIRUS) S. Gowda, F. C. Wu, H. Scholthof and R. J. Shepherd. Department of Plant Pathology, University of Kentucky, Lexington.

The figwort mosaic virus (FMV) genome is transcribed as two major viral RNA's. One transcript is monocistronic and spans only gene VI. The product of this gene transactivates the expression of several closely spaced genes on the second full-length transcript that spans the entire viral genome. When specific portions of the 5'-nontranslated leader were removed from plasmids containing the CAT gene in a downstream position, a sequence coinciding with the small open reading region (designated as gene VII) was found to be required as a *cis*-element for the gene VI response. Further, mutational analysis (frame-shift, in-frame deletions and site specific changes) of this region revealed a 40 base element to be required in *cis* for the transactivation response. Electroporation and assay of other plasmids with CAT in far downstream position on the full length RNA transcript (e.g,. fused in frame near the 5' end of gene IV) showed that high levels of CAT were expressed when the upstream *cis*-element was present but failed to express CAT when it was absent.

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LOCALIZATION OF CAPSID PROTEIN DOMAINS WHICH MAY REGULATE APHID TRANSMISSION OF BARLEY YELLOW DWARF VIRUS. Thomas M. Rizzo and Stewart M. Gray. USDA/ARS, Department of Plant Pathology, Cornell University, Ithaca, NY 14853.

A cDNA clone of the New York PAV isolate of barley yellow dwarf virus (BYDV) containing the entire capsid protein gene has been isolated and sequenced. The ORF of this gene consists of 603 nt (including the stop codon) and encodes a 21993 M_r protein. A defined set of overlapping cDNA fragments specific to the NY-MAV and NY-PAV capsid protein gene has been subcloned into the pGEX expression vectors. *Escherichia coli* cells carrying these plasmids synthesize glutathione *S*-transferase/BYDV capsid fusion proteins which are being used to map epitopes recognized by BYDV-specific monoclonal and polyclonal antibodies by western blot analysis. Some of the monoclonal antibodies inhibit virus transmission when incubated with purified virions and injected into the hemocoel of aphid vectors. Antigenic domains of the capsid protein which bind the transmission-neutralizing monoclonal antibodies may regulate circulative virus transmission in aphid vectors.

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COMPARISON OF REQUIREMENTS FOR REPLICATION AND MOVEMENT OF GENOMIC VERSUS DEFECTIVE INTERFERING RNAs OF TOMATO BUSHY STUNT VIRUS. H.B. Scholthof, Y.-C. Chang, I.T.D. Petty, P.Q. Hearne, D.A. Knorr, T.J. Morris and A.O. Jackson. Dept. of Plant Pathology, Univ. of California, Berkeley CA 94720.

Tomato bushy stunt virus (TBSV) contains a small plus sense RNA genome with five major open reading frames (ORFs 1 through 5). Bioassays showed that *in vitro* transcripts with a deletion in ORF 3 (coat protein) replicated in protoplasts, and movement in plants still occurred albeit to a limited extent. The nested ORFs 4 and 5 were not required for replication in protoplasts but were essential for systemic movement in plants. A recombinant in which the 5' end of ORF 3 was fused in frame with the GUS gene and with the remainder of ORF 3 and the 5' end of ORFs 4 and 5 deleted, induced a high level of GUS activity in protoplasts. Whether TBSV can complement this RNA for movement as it does native defective interfering (DI) RNAs, is being tested. A deletion covering the 3' region of TBSV which is conserved in DIs, destroyed replication. Replication of TBSV and expression of the ORF 3-GUS fusion protein depended on an intact ORF 2, the putative replicase gene. Complementation of ORF 2 mutants by TBSV has been unsuccessful, in contrast to the effective complementation of DIs by the virus.

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IRRIGATION, SALINITY, AND AFLATOXINS. Peter J. Cotty, Southern Regional Research Center, USDA, ARS, New Orleans, LA 70179.

Although irrigation can reduce aflatoxin contamination of some crops exposed to periodic droughts, severe contamination does occur in cottonseed and corn grown under irrigation in arid regions. Soils in such areas in the United States are typically warm, alkaline and tend to accumulate salts. Salt content of soil and irrigation water may influence soil populations of Aspergillus flavus, both qualitatively and quantitatively. Laboratory experiments on influences of varying concentrations of sodium chloride on growth, sporulation, and colonization of crop debris support the speculation that salts associated with prolonged irrigation in arid areas may partially determine regional differences in both the magnitude and toxigenicity of A. flavus populations. This speculation was tested by comparing A. flavus group fungi in regions with high and low incidences of aflatoxin contamination of cottonseed and relating population differences to soil salt content. The results suggest salt may have some influence.

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DEGRADATION OF AFLATOXIN BY LACTOPEROXIDASE AND MYCELIAL EXTRACTS OF ASPERGILLUS PARASITICUS. E.H. Gendloff,[1] F.S. Chu,[2] and T.J. Leonard.[1] [1]Botany Department and [2]Food Research Institute, University of Wisconsin, Madison.

The well-known ability of lactoperoxidase and extracts of A. parasiticus to degrade aflatoxin is being studied using a new assay. Small aliquots (250 ul) of preparations to be tested are put into wells of sterile microtiter plates. Aflatoxin is added and the plate is then covered, sealed, and incubated at various temperatures. Samples (50 ul) are taken after several days. Aflatoxin concentration in the samples are then determined by ELISA. High concentrations of lactoperoxidase (500 u/ml) degraded aflatoxin by >90% in <14 d at 30°C. This degradation was somewhat reduced at 37°C. Soluble mycelial extracts of A. parasiticus were obtained by blending eight day old mycelium in phosphate buffer with glass beads, pelleting the mycelial fragments, dialyzing the supernatant fraction to remove aflatoxin, then filter sterilizing the dialyzed preparation. Fractionation of this preparation with successive additions of ammonium sulphate yielded a fraction at 40%, but not 25% or 50% of saturation that could substantially degrade aflatoxin at both temperatures tested. There was very little peroxidase activity in these fractions, indicating the activity was not due to peroxidase. Purification and characterization of this activity is continuing.

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OCCURRENCE, PATHOGENICITY AND COMPETITION AMONG SPECIES OF *PENICILLIUM* ON PEAR AND APPLE FRUIT. P. G. Sanderson, D. A. Rosenberger, and R. A. Spotts. First and last authors, Oregon State Univ., 3005 Expt. Station Dr., Hood River, OR 97031; second author, Cornell Univ., Box 727, Highland, NY 12528.

Isolates of *Penicillium* spp. were collected from dump tank water and culled pear and apple fruit with blue mold symptoms from packing houses in the Hood River Valley vicinity of OR and WA. Anjou pear and Starking apple fruit were wound-inoculated with isolates of each of ten species of *Penicillium* and incubated at 0, 2.5, 5, 10, and 20 C. At least six species of *Penicillium* caused lesions on pear at all temperatures and on apple at ≥ 5 C. Whereas isolates of all ten species were recovered from dump tank water, *P. expansum* was the predominant species recovered from fruit. *P. expansum* dominated lesions on wounded Anjou fruit that were inoculated with *P. expansum* either combined with, or following 0, 1, 7, or 28 days after inoculation with *P. solitum* or *P. commune*. Consequently, *P. expansum* was able to compete successfully and replace other species of *Penicillium* already established in lesions.

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IDENTIFICATION OF TWO NEW (FORMYL AND DIACETYL) DERIVATIVES OF FUSAROCHROMANONE PRODUCED BY *FUSARIUM EQUISETI*. Weiping Xie, Chester J. Mirocha and Yechun Wen, Department of Plant Pathology, University of Minnesota, St. Paul, MN 55108.

Two fluorescent compounds with trivial names of TDP-2A and TDP-7A, were isolated from a rice culture of *F. equiseti* (Alaska 2-2). Hydrolysis of the two compounds yielded the mycotoxin fusarochromanone (TDP-1). High-resolution electron impact (EI) mass spectrum of TDP-2A yielded a molecular ion at m/z 320.1355 with a molecular formula $C_{16}H_{20}N_2O_5$. The high-resolution EI mass spectrum of TDP-7A yielded a molecular ion at m/z 376.1634 for $C_{19}H_{24}N_2O_6$. Mass spectral and ^{1}H-NMR data analysis indicated that the structure of TDP-2A was 3'-N-formyl fusarochromanone and the structure of TDP-7A was 3'-N-acetyl-4'-O-acetyl fusarochromanone. The structures of the two compounds were confirmed by comparing their spectral data with that of synthetic 3'-N-formyl fusarochromanone and 3'-N-acetyl-4'-O-acetyl fusarochromanone.

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INDUCED RESISTANCE RESPONSE OF SWEETPOTATO TO FUSARIUM ROOT ROT BY UV-HORMESIS C. Stevens[1], V. A. Khan[1], J. L. Lu[1], C. L. Wilson[2], E. Chalutz[3], M. K. Kabwe[1], and Z. Haung[1]. [1]George Washington Carver Agricultural Experiment Station Tuskegee University, Tuskegee Institute, AL, 36088. [2]USDA/ARS Appalachian Fruit Research Station, Kearneyville, WV., [3]ARO, Volcani Center P.O. Box 6, Bet Dagan, Israel, 50250.

Jewel sweetpotato storage roots previously treated with ultraviolet (UV-C) light and then stored for 30 days before inoculation with Fusarium solani showed increased resistance to Fusarium root rot as indicated by reduced lesion size, the rate and decay of rotted tissues. There was a hormetic relationship between the incidence of Fusarium root rot and UV-C doses. The optimum dose of UV which reduced Fusarium root rot was 3.6×10^4 ergs/mm^2. Exposure of sweetpotato to UV-C doses promoted phenylalanine ammonia-lyase (PAL) production with the maximum PAL activity occurring at 3.6×10^4 ergs/ mm^2. Crude extracts from UV-C treated sweetpotatoes reduced germination, germ tube elongation and growth of F. solani when compared to untreated extracts.

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WOUND GUM OR LIGNIN? MECHANISM OF WOUND HEALING IN CITRUS FRUIT. R. Stange, Jr., and J. W. Eckert. Department of Plant Pathology, University of California, Riverside, CA 92521.

Material deposited in wounds of citrus exocarp resistant to *Penicillium* matched the histochemical reactions of wound gum (Wg) rather than of lignin. Wg is Mäule(-); vascular lignin is Maule(+). Both Wg and lignin turned red in phloroglucinol/HCl [pg/HCl(+)]; however only Wg remains colored after 7 days in pg/HCl. The pg/HCl(+) reaction of lignin is due to CHO groups within the lignin polymer, whereas the reaction of Wg is attributed to presence of aromatic aldehydes trapped in a carbohydrate matrix. Experiments showed that extracts (EtOH or EtOAc) of healed, but not freshly wounded tissue, contained four induced pg/HCl(+) compounds separable by TLC. Further, appearance of these compounds correlated with development of a pg/HCl(+) reaction of material in fresh sections of injury sites. Inhibitors that blocked development of resistance also prevented appearance of pg/HCl(+) material and of extractable pg/HCl(+) compounds. Both healed and control tissue contained vascular lignin.

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NUTRITIONAL ENHANCEMENT OF BIOCONTROL OF POSTHARVEST DISEASES OF POME FRUITS. W. Janisiewicz, B. Bors, and M. Carpenter. USDA, ARS, AFRS, Kearneysville, WV 25430.

Thirty-six organic carbon sources and twenty organic and three inorganic nitrogen sources were evaluated for their effect on mycelial growth and condia germination of Botrytis cinerea (gray-mold) and Penicillium expansum (blue-mold), and on the growth of an antagonist Pseudomonas syringae (L-59-66 or K-1). The compounds, which stimulated growth of the antagonist and least affected mycelial growth and spore germination of the

pathogens, were used as additives in suspension of the antagonist. Apples and pears were treated with the antagonist as a postharvest treatment against blue-mold and gray-mold. Antagonist suspensions containing L-asparagine and L-proline exhibited the most effective biocontrol. As concentration of L-asparagine increased from 5 to 80 mM, effectivesness of the biocontrol increased on mature pears. Average lesion diameter and percentage infected wounds declined from 8 to 0 mm and from 45 to 0 percent, respectively.

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THE EFFECT OF MODIFYING THE ENVIRONMENT IN GREENHOUSES CONTAINING GERANIUM STOCK PLANTS ON SPORULATION OF *BOTRYTIS CINEREA* AND BOTRYTIS BLIGHT. M.K. Hausbeck, S.P. Pennypacker, and R.E. Stevenson. Dept. of Plant Pathology, Penn. State University, University Park, PA 16802.

White plastic mulch and/or intervals of forced heated air in a research greenhouse containing geranium (*Pelargonium* x *hortorum*) stock plants significantly reduced incidence of sporulating *Botrytis cinerea* on necrotic leaves in comparison to a control for all treatments according to the area under the disease progress curve (AUDPC) data. The combination of mulch and heated air was significantly more effective in reducing the incidence of sporulating *B. cinerea* on necrotic leaves than the treatments individually. AUDPC data indicated that continuous, forced heated air used in a commercial greenhouse containing geranium stock plants significantly reduced incidence of stem blight and incidence of sporulating *B. cinerea* on blighted stems and necrotic geranium leaves. Airborne concentrations of *B. cinerea* conidia occurring during grower activity were lower within the treated area than within the control area.

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TRANSMISSION OF XANTHOMONAS CAMPESTRIS PV. BEGONIA IN AN EBB AND FLOW IRRIGATION SYSTEM. V. P. Atmatjidou, R. P. Fynn and H. A. J. Hoitink, Dept of Plant Pathology, Ohio State Univ., Wooster, OH 44691

Low populations of a rifampin resistant mutant of Xanthomonas campestris pv. begoniae were disseminated from infected roots of Begonia hiemalis into the recycled ebb and flow irrigation solution. Plants irrigated with an infested solution developed low levels (2.5% incidence) of the disease. Higher disease levels (up to 7.5%) were detected after post-harvest incubation of these plants. A high death rate of the pathogen in the irrigation solution explained the low level of disease transmission. It was concluded that transmission of this pathogen through the ebb and flow irrigation solution does not pose a great threat to producers of flowered begonias if appropriate sanitation procedures are practiced. This rate of transmission would be important in the production of pathogen-free stock plants.

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DAMPING-OFF AND LEAF SPOT OF ICELAND POPPY CAUSED BY A SEED-BORNE *DENDRYPHION* SPECIES. R. L. Wick and R. Shrier, Dept. of Plant Path., Univ. of Massachusetts, Amherst, MA 01003.

Several occurrences of damping-off and leaf spot of plug-grown Iceland poppy, *Papaver nudicaule* L. were observed. A species of *Dendryphion* Wallroth. was cultured from symptomatic plants and determined to be pathogenic by completion of Koch's postulates. *Dendryphion* sporulated on lesions 4 days after inoculation. Only the older leaves were susceptible. Inoculation of seed resulted in 100% damping-off (mostly post-emergence). Fourteen different commercial seed lots of Iceland poppy were assayed (100 to 200 seeds/lot). *Dendryphion* was recovered from 7 of the lots at rates of 4 to 41%. In two trials, where naturally contaminated seed was either not treated or treated with 0.5% NaOCl, *Dendryphion* was recovered from 9 and 6% of the untreated seed and 1% from each of the NaOCl treatments. Germination was 52 and 66% in the untreated seed and 72 and 77% in the NaOCl treatments.

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IN VITRO SENSITIVITY OF *MAGNAPORTHE POAE* AND OTHER ROOT AND CROWN-INFECTING FUNGI CAUSING PATCH DISEASES OF TURFGRASS TO SELECTED FUNGICIDES. D. C. Thompson and B. B. Clarke, Department of Plant Pathology, Rutgers University, New Brunswick, NJ 08903.

Four isolates of *Magnaporthe poae* and single isolates of *Leptosphaeria korrae*, *Gaeumannomyces incrustans*, *G. graminis* var. *avenae*, *Fusarium culmorum* and *Trichoderma* sp. were exposed in vitro, to 15 fungicides at 0, 0.0016, 0.008, 0.04 0.2, 1.0, 5.0, 25.0, 125.0, 500.0 mg a.i./L. EC_{50} values were determined for each fungus-fungicide combination by measuring radial growth on 1/2 strength fungicide amended potato dextrose agar. EC_{50} values of *M. poae* isolates were 0.04-0.2 mg a.i./L for propiconazole, terbuconazole, fenarimol, myclobutanil and four experimental fungicides; 1.0-5.0 for triadimefon, benomyl, thiophanate-methyl and two experimentals; and 25.0-125.0 for iprodione. Values for the other five fungi were determined and will be discussed.

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INOCULATION OF KENTUCKY BLUEGRASS WITH *MAGNAPORTHE POAE* TO ASSESS ITS DISTRIBUTION IN THE SOIL PROFILE USING A WHEAT SEEDLING BIOASSAY. K.A. Plumley and B.B. Clarke, Rutgers University, New Brunswick, NJ 08903.

Baron Kentucky bluegrass was artificially inoculated with *Magnaporthe poae* (Landschoot and Jackson), the causal agent of summer patch, to determine the distribution and spread of the pathogen in the soil profile under field conditions. The turf was inoculated by placing 0.1g of colonized oat grains at a 2 cm depth. Soil cores (1.3 x 15 cm) were extracted 5, 15, 25, 35 and 45 cm from infection foci at weekly intervals from April to September 1990. Cores were sectioned into 5 cm pieces and the organic and mineral fractions were separated by flotation. Each fraction was assayed separately for the presence of *M. poae* using a wheat seedling bioassay. *M. poae* was found to be associated with the organic (root) fraction and not the mineral fraction. *M. poae* was evenly distributed in the soil profile to a depth of 15 cm. The pathogen was detected in root sections prior to visual foliar (patch) symptom development and up to 30 cm beyond patch margins. Movement in the soil profile was rapid and *M. poae* was present on bluegrass roots 6-8 weeks prior to foliar symptom expression (mid to late July).

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PHYTOPHTHORA ROOT ROT OF ITALIAN STONE PINE. C. M. Sandlin and D. M. Ferrin, Department of Plant Pathology, University of California, Riverside 92521.

Root rot, caused by *Phytophthora parasitica* Dastur, significantly reduces growth of containerized Italian stone pine (*Pinus pinea* L.) grown in UC bark mix (50% bark, 50% sand). Eleven months after inoculation of 4-wk-old seedlings, shoot height of inoculated plants was 63%, dry shoot mass was 49% and dry root mass was 68% of noninoculated controls. On average, 19.3% of the total root length of 10-wk-old seedlings was infected 5 wk after inoculation (as determined by plating entire root systems). Average lesion length increased with time. Histological examination of infected roots revealed mycelium throughout the cortex, but not within the stele; growth of the fungus was apparently limited by the endodermis. Disease appears to be more severe in fumigated potting mix than in nonfumigated mix. Inoculated plants grown in fumigated mix had a significantly (P=.064) higher percentage of infected roots (19%) than did plants grown in nonfumigated mix (10%).

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CANKER OF GRAFTED JAPANESE MAPLE CAUSED BY COLLETOTRICHUM ACUTATUM. V. L. Smith, The Connecticut Agricultural Experiment Station, P. O. Box 1106, New Haven, CT 06504.

Grafts of Japanese maple (Acer palmatum Thunb.) onto a seedling rootstock at a commercial nursery in Connecticut failed due to infection by Colletotrichum acutatum Simmonds. The fungus was consistently isolated from cankers at the graft union and from pruning wounds on the rootstock. Cankers with black acervuli first appeared 1-2 months after grafting, and resulted in a high incidence of tree mortality during winter storage. Koch's postulates were completed on Japanese maple seedlings in the greenhouse. The ability of the fungus to cause bitter rot on Granny Smith apple fruit also was confirmed. Loss of grafted Japanese maple has been reduced by 20-40% by application of mancozeb during storage, and mancozeb will be used prophylactically on scion wood this season.

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EVALUATION OF 10 DOGWOOD CULTIVARS FOR DISEASE AND FREEZE DAMAGE RESISTANCE. M. T. Windham and A. S. Windham. P.O. Box 1071, Knoxville, TN 37901 and P.O. Box 110019, Nashville, TN 37222.

Trees of 10 Cornus florida cultivars ('Barton', 'Cherokee Princess', 'Cloud 9', 'First Lady', 'Fragrant Cloud', 'Plena', 'Purple Glory', 'Springtime', 'Rubra' and 'Welch's Jr. Miss') were arranged in a randomized complete block design with 5 replications (5 trees per rep) in 1986. Trees were evaluated in 1989 and 1990 for spot anthracnose, dogwood canker and freeze damage resistance. 'Plena' was highly resistant to spot anthracnose, whereas 'Cloud 9' and 'Barton' were very susceptible. 'Cherokee Princess', 'Springtime', and 'Rubra' did not develop dogwood canker, whereas incidence in 'Purple Glory' was over 80% after 4 yr. 'Welch's Jr. Miss' was rated highly susceptible for freeze damage and 'Barton' and 'Cloud 9' were rated susceptible. Other cultivars were rated resistant to freeze damage.

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EVALUATION OF NEW FUNGICIDES FOR THE CONTROL OF ENTOMOSPORIUM LEAF SPOT ON PHOTINIA. A. K. Hagan, Department of Plant Pathology, Auburn University, AL 36849, J. Olive, and W. J. Foster, Ornamental Horticulture Substation, Mobile AL 36608.

Several rates of tebuconazole, diniconazole, myclobutanil, and flusilazole, applied at various rates and spray schedules in 1987 to 1989, were compared with chlorothalonil for the control of Entomosporium leaf spot caused by E. mespili on photinia and evaluated for their effects on plant growth. Although all fungicides reduced disease severity compared with the non-sprayed control, differences in leaf spot control were observed. Myclobutanil, the most effective of the new fungicides, was as efficacious as chlorothalonil. In 1987, the flusilazole-treated plants were disease-free but some twisting and discoloration of new leaves was seen. Tebuconazole and diniconazole did not control leaf spot as effectively as myclobutanil and chlorothalonil. Growth of photinia was reduced with all rates of diniconazole as well as the highest rates of myclobutanil and tebuconazole.

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USE OF TWO NATURAL COMPOUNDS EXTRACTED FROM NEEM SEED TO CONTROL RUST ON GREENHOUSE GROWN SNAPDRAGON. J. C. Locke and J. F. Walter, USDA, ARS, Beltsville, MD 20705-2350 and W. R. Grace & Co.-Conn, WRC, Columbia, MD 21044.

Foliar spray applications of aqueous emulsions of two hydrophobic, solvent-extracted compounds from neem seed (Azadirachta indica) were shown to be effective in preventing rust (Puccinia antirrhini) development on greenhouse grown snapdragons. Rate studies demonstrated that applications of as low as 0.5% (w/w) extraction product could effectively control rust infection. Although the oil and wax compounds provided an equivalent level of control, the wax compound appeared to have a longer residual activity. In comparison with two commercially registered fungicides and a petroleum-based horticultural spray oil, the compounds derived from neem seed had equivalent or superior activity. The efficacy of these "soft" pesticide alternatives gives promise in the search for replacements to currently available synthetic pesticides.

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BROWNLINE DISEASE OF PLATANUS X ACERIFOLIA. A. H. McCain[1], S. M. Mircetich[2], R. H. Molinar[3] and J. C.Schwegemann[3]. [1]Dept. of Plant Pathology, Univ. of California Berkeley, [2]USDA ARS, Dept.of Plant Pathology, Univ. of California, Davis, and [3]Univ. of California Cooperative Extension, Hayward, CA.

In the spring of 1989 we were asked to look at a planting of *Platanus X acerifolia* trees in an industrial park in Newark, CA. The trees were transplanted in 1985 and had been growing vigorously in previous years. Some trees were declining as evidenced by sparse foliage, while other trees were growing normally. The rootstock (*P. racemosa*) of affected trees was suckering. By the end of summer the scions of some trees were dead. A narrow strip of necrotic cambial phloem tissues at the graft union of the scion and rootstock was present in all affected trees. Because of the similarity of the symptoms to prune brownline and walnut blackline, phloem tissues from above and from below the necrotic zone of four affected trees was sampled and evaluated by ELISA for the presence of tomato ringspot virus and cherry leaf roll virus. Neither virus was detected.

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THE SYMPTOMOLOGY AND CYTOPATHOLOGY OF A ROSE MOSAIC DISEASE CAUSED BY TOMATO RINGSPOT VIRUS IN TEXAS. C. Edward McClelen, and R. W. Toler, Texas A&M University, 77843.

Hybrid-tea and multi-flora roses exhibiting winter kill, vein clearing, mosaic, fine line, and ring patterns on leaves were found in Robertson County, Texas. Symptoms were only seen early and late season, becoming strongest at 16 C and abaiting at 24 C. ISEM was performed using apple mosaic, prunus necrotic ringspot, tobaco ringspot, and tomato ringspot viral antiserum. Only tomato ringspot antiserum trapped virus particles at levels signifigantly above controls. Virus particles were strongly polyhedral and 30nm in diameter. TEM revealed virus containing tubules and virus-induced vesicles filled with strands similar to dsRNA. The virus-induced vesicles differed from those reportedly caused by other Nepoviruses in their origin being distinctly through budding into a vacuole by the tonoplast membrane. Vesicles initially contained a dense matrix of strands which diffused and divided into numerous minute vesicles inside the original primary vesicle, resulting in minute vesicles containing fully assembled virions. This produces a virus assembly pathway giving rise to membrane coated virions which could fuse to produce virus-containing tubules or fuse with the tonoplast to enter the vacuole.

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A MOSAIC OF COLEUS CAUSED BY CUCUMBER MOSAIC VIRUS. G. E. Holcomb and R. A. Valverde. Department of Plant Pathology and Crop Physiology, LA Agr. Exp. Sta., LA State University Agr. Center, Baton Rouge, LA 70803.

Landscape plantings and garden center stocks of coleus cultivar Alabama were found showing virus-like symptoms of mosaic, oak leaf line patterns and ringspots. A virus was transmitted mechanically from coleus plants with symptoms to cowpea, Turkish tobacco and Chenopodium quinoa. Transmissions back to coleus were positive. The virus was identified as a strain of cucumber mosaic virus based on gel electrophoresis patterns of double-stranded ribonucleic acid, serology and particle morphology. Leaf samples from stock plants of cv. Alabama from Bellingrath Gardens, where the cultivar originated, were virus free.

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THE CURRENT USE OF PLANT GROWTH-PROMOTING RHIZOBACTERIA (PGPR) IN CHINESE AGRICULTURE. L. Liu[1,2], R. Mei[2], Y. Chen[2], and J.W. Kloepper[1]. [1]Auburn University, Alabama and [2]Beijing Agricultural University, Beijing, China.

In China, PGPR have been used in fields to promote plant growth and provide biological control of several diseases since 1979. PGPR currently used include strains of Bacillus cereus and B. spp. Since 1979, PGPR have been applied to 48 crops over an area of 13 million ha in 29 provinces and cities. Multiple-year average yield increases vary with crop and range from 10% with cotton to 22% with sweet potato. The incidence of 9 soil-borne or seedborne diseases has been reduced with PGPR. The % reductions in disease incidence range from 50% with cotton wilt caused by Fusarium oxysporum to 85% with seedling blight of rice caused by Xanthomonas campestris pv. oryzae. Production, delivery, and extension activities related to PGPR application are accomplished through a central organization which has pathologists, extension agents, industrialists, and Ministry of Agriculture staff.

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Commercial potential of rhizobacteria for the suppression of crown and root rot (CRR) of tomato caused by Fusarium oxysporum f.sp. radicis-lycopersici (FORL). M. S. Reddy, S. E. Campbell, S. E. Young, and G. L. Brown. Esso Chemical Canada, Ag Biologicals, 402-15 Innovation Blvd., Saskatoon, SK Canada 27N 2X8

A disease optimization assay was developed by screening several isolates of FORL for their effect on tomato seedling germination, stand development and CRR severity in peat-based growing mix. The concentrated inoculum (microconidia) of FORL was diluted in peat to give log 0, 3, 5, 7 conidia/g peat. Tomato seedlings were rated for CRR severity 6 weeks after planting. Log 5 conidia/g peat produced a consistent level of CRR (3.0 on a numerical scale of 0-5). A total of 45 bacterial strains isolated from peat, peat bogs and plant sources were introduced into the assay as seed or peat treatments for ability to suppress CRR. Four strains significantly reduced CRR when applied as seed treatment and 2 strains as peat treatment. Results indicate that mode of application of bacteria has a major role on the efficacy of introduced strains. Many of the strains showed antagonistic activity against several fungal pathogens. Survival in peat, chemical compatibility, colonization potential, cyanide production and bacterial derived plant growth regulators of the potential strains will be discussed.

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STUDIES ON THE ANTAGONISM OF PSEUDOMONAS CEPACIA. Luisa Visintin, Ram S. Upadhyay and R. K. Jayaswal, Department of Biological Sciences, Illinois State University, Normal, IL 61761.

Two strains of Pseudomonas cepacia, RJ3 and ATCC 52796, have been identified as potential antagonists. In the present study, we have compared their antagonistic activity against various phytopathogenic fungi. Isolation of a transposon induced mutant, deficient in the antagonistic activity and the anti-fungal compound, suggests that the antagonistic activity is due to the production of an antifungal compound. The antifungal compound was purified from growth medium by HPLC and characterized with NMR and GC-mass spectroscopy as pyrrolnitrin. The antagonism was highly influenced by nutritional and environmental conditions such as carbon and nitrogen sources, pH and temperature. The effect of these conditions on the antagonistic activity of P. cepacia will be discussed.

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BIOCONTROL OF SEPTORIA TRITICI BY FLUORESCENT PSEUDOMONADS. Edna Levy and F. J. Gough. Department of Plant Pathology, Oklahoma State University, Stillwater, OK 74078; and USDA-ARS, 1301 N. Western Street, Stillwater, OK 74075.

Fluorescent pseudomonads antagonistic to Septoria tritici secreted a few inhibitory compounds. Pseudomonas fluorescens (strain PFM2) produced three compounds in culture which inhibited growth of S. tritici. The most abundantly produced compound, identified as 2,4-diacetylphloroglucinol, also inhibited growth of other phytopathogenic fungi and bacteria. Pseudomonas sp. (strain LEC 1) produced two phenazine antibiotics: 1-hydroxyphenazine and chlororaphin. There is a combined effect of antibiotics and other agents produced by pseudomonads.

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BIOCONTROL PERFORMANCE CHARACTERIZATION OF PSEUDOMONAS CORRUGATA ISOLATES PS. 2140 AND PS. 2161. P. A. Kovacevich and M. Ryder. Monsanto Agricultural Company, St. Louis, Missouri 63198, CSIRO Division of Soils, Waite Road, Urrbrae, PMB 2, Glen Osmond, Adelaide, S.A. 5064, Australia.

Pseudomonas corrugata strains Ps. 2140 and Ps. 2161 isolated from wheat field soils in Australia, showed superior activity characteristics as biocontrol agents in growth chamber tests, when compared to other take-all suppressive Pseudomonas isolates. Ps. 2140 and to a lesser degree Ps. 2161, suppressed wheat take-all lesion development in raw field soils when applied at 10^9 CFU per seed and effective take-all lesion suppression was achieved with application rates as low as 10^3 CFU per seed in pasteurized potting medium. Treatment with either Ps. 2140 or Ps. 2161 increased wheat emergence in soil infested with Pythium ultimum var. sproangiferum, however seedling vigor was not consistently improved. Combinations of either bacterial strain with the commercial fungicide Baytan® did not improve take-all control, although no detrimental effect on bacterial viability was observed as a result of Baytan® treatment. Tests conducted with varying soil matric potential indicated Ps. 2140 was less effective against take-all under dry conditions (less than -1.0 bar), and was more effective under wet soil conditions (-0.1 bar to field capacity). Results will be discussed in relation to biological control agents for wheat take-all disease.

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BIOLOGICAL CONTROL OF RING NEMATODES BY PSEUDOMONAS AUREOFACIENS. D. A. Kluepfel[1] and T. M. McInnis[2], [1]Dept. of Plant Pathology and Physiology, and [2]Dept. of Biological Sciences, Clemson University, Clemson, SC. 29634.

Soils suppressive to Criconemella xenoplax (ring nematode) multiplication have been identified in several peach orchards in South Carolina. Field populations of the ring nematode were consistently lower than 1% of populations found in adjacent non-suppressive sites. Pasteurization of suppressive soils destroyed all suppression of ring nematode multiplication. Several strains of fluorescent pseudomonads have been isolated from suppressive soils which inhibit ring nematode multiplication on peach seedling roots. Final populations of ring nematodes introduced onto peach roots inoculated with these pseudomonads where reduced by more than 50% as compared with those from trees not receiving a bacterial treatment. Bacterial populations on peach roots increased to 1 X 10^8 CFU/g fresh root weight and then fell to 5 X 10^2 CFU/g root 12 weeks after inoculation. Over the same period, nematode levels increased from 200/plant to 620/plant on control plants while trees treated with the suppressive pseudomonad isolate supported populations of approximately 250 nematodes/plant.

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Identification, Cloning, and DNA Sequence of a Promoter Region Involved in Phenazine Antibiotic Production by *Pseudomonas aureofaciens* Strain 30-84.
L. S. Pierson III[1] and L. S. Thomashow[2]. [1]Dept. of Plant Pathology, University of Arizona, Tucson, AZ 85721 and [2]USDA-ARS, Pullman, WA. 99164.

We previously reported a cosmid carrying a 22 kb insert from a genomic library of *P. aureofaciens* (Pa.) strain 30-84 that contained the genes involved in phenazine biosynthesis. Although an *E. coli* strain containing this cosmid did not produce phenazines, insertion of a 9.2 kb subclone of this cosmid downstream of either of two *E. coli* promoters resulted in production of all three phenazine antibiotics in this heterologous host. We have cloned the region of the Pa. chromosome immediately upstream of the phenazine structural genes. This 1.1 kb fragment, when inserted upstream of two promoterless reporter genes, resulted in the expression of these genes in strain 30-84. Unexpectedly, these reporter genes were also expressed in *E. coli*. The promoter region has no significant DNA homology to other known promoters. The sequence has an A/T rich region containing two 8 bp direct repeat sequences which are flanked by a palindromic sequence and a sequence that resembles the *E. coli* consensus ribosome binding sequence. Direct repeat sequences have also been reported in both the eta and plc promoter regions of *P. aeruginosa*.

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USE OF PHYLLOPLANE BACTERIA TO ENHANCE THE PERFORMANCE OF THE MYCOHERBICIDE, COLLETOTRICHUM TRUNCATUM. D.A. Schisler, K.M. Howard, and R.J. Bothast, USDA-ARS, NCAUR, Peoria, IL 61604.

Studies were conducted to determine whether phylloplane microorganisms could be employed to increase disease incited by C. truncatum in the weed Sesbania exaltata. S. exaltata was grown in soil collected from 22 field sites and appressoria formation by C. truncatum was examined on seedling leaves using epifluorescence microscopy. Over 200 phyllosphere microorganisms were isolated from plants which supported superior appressoria formation. Fifteen of seventy-three microbial isolates assayed stimulated appressoria formation on cellophane membranes. Five of eight superior isolates from in vitro assays also enhanced disease symptoms induced by C. truncatum on S. exaltata compared to seedlings treated with conidia only. Superior isolates initiated no apparent symptoms and rarely decreased seedling growth parameters in the absence of C. truncatum. This is the first reported utilization of phylloplane microorganisms to increase disease incited by a mycoherbicide agent.

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METABOLISM OF *PYTHIUM* SPORANGIUM GERMINATION STIMULANTS BY *ENTEROBACTER CLOACAE* AND OTHER SOIL BACTERIA. Eric B. Nelson and Cheryl M. Craft, Department of Plant Pathology, Cornell University, Ithaca, NY 14853.

Strains of *Enterobacter cloacae*, effective in suppressing seed rot caused by *Pythium ultimum*, reduced the stimulatory activity of cotton seed exudate when grown on in vitro-collected exudate solutions for 24 hr. The stimulatory activity of seed exudates released into and extracted from natural soils and sand was reduced when seeds were treated with strains of *E. cloacae* prior to planting. When combined with untreated exudate, concentrates prepared from exudate on which *E. cloacae* strain EcCT-501 had grown for 24 hr had no effect on the stimulatory activity of untreated exudate. This suggests that metabolism of stimulants, and not the production of inhibitors, by *E. cloacae* was responsible for the observed reductions in the stimulatory activity of cotton seed exudate to sporangia of *P. ultimum*. Other bacterial genera isolated from a *Pythium*-conducive soil were incapable of reducing the stimulatory activity of cotton seed exudate while the majority of *Pythium*-suppressive bacteria isolated from various non-agricultural soils were capable of reducing the stimulatory activity of seed exudate. Our results suggest that bacterial metabolism of propagule germination stimulants may be an important trait in the biological control of *Pythium* diseases.

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N. R. Torkewitz and S. T. Lam. The effect of the population level of biocontrol bacteria on disease control efficacy. CIBA-Geigy Biotechnology Research, P.O. Box 12257, RTP, NC 27709.

Rhizoctonia solani causes damping-off in cotton seedlings. Under gnotobiotic conditions, the susceptibility of cotton seedlings to *Rhizoctonia* infection was shown to be much reduced three days after germination. Similar results were obtained whether healthy seedlings were transplanted into infected soil or fungal inocula were added to healthy seedlings in uninfected soil. Our studies showed that, during this critical time period, the population size of a desired biocontrol bacterial strain on plant roots could be modulated by mix-inoculation, at various ratios, with a competing bacterial strain. Disease control experiments were carried out under the same mix-inoculation conditions, with the inoculated seeds then planted into *Rhizoctonia* infected soil. Our results indicate that the biocontrol strain must achieve a threshold population level to obtain effective disease control.

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POPULATION DYNAMICS OF BACILLUS MEGATERIUM B153-2-2 IN SOYBEAN RHIZOSPHERE SOIL. Zonglin Liu, and J. B. Sinclair, Dept. of Plant Pathology, Univ. of Illinois at Urbana-Champaign, 1102 S. Goodwin Ave., Urbana, IL 61801-4709.

Field population dynamics of Bacillus megaterium B153-2-2 without or with amended Rhizoctonia solani in an extended soybean rhizosphere were studied in Catlin silt loam (pH 6) for 2 yr under nontilled conditions. Soil samples were taken at depths of 10, 20, and 30 cm and 10 cm away from tap roots. A cubic relationship described the bacterial population (log cfu/g soil) dynamics in rhizosphere soil as a function of days after introducing the bacterium for the initial year and a quadratic model for the subsequent yr. In general, models described the bacterial population dynamics better in the absence than presence of R. solani. A threshold of 10^6 cfu/g soil for B153-2-2 was effective in reducing the population of R. solani ($p=0.05$). B153-2-2 survived in fallow soil in the field and was recoverable during the subsequent growing season. In December, the population of B153-2-2 was recorded as 10^4 cfu/g soil, which could be considered as a survival population.

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HOST RANGES OF FLORIDA ISOLATES OF BACTERIOPHAGES OF ERWINIA CAROTOVORA. Eayre, C.G., Concelmo, D.E., and Bartz, J.A. 1991. Plant Pathology Dept., University of Florida, Gainesville, FL 32611.

Nineteen of 22 isolates of bacteriophages from lake water had distinct host ranges in tests with 62 strains of Erwinia carotovora. Six phage isolates had similar, but not identical, host ranges and five of these failed to survive in storage for 7 wks at -70 C. All other phages were viable after such storage. In tests with representatives of 24 serotypes of E. carotovora, 12 of the 15 phages caused plaques in lawns of 16 serotypes and the number of hosts susceptible to a given phage ranged from 1 to 10. Four of the five serotypes most often associated with major outbreaks of bacterial soft rot were hosts for at least one phage isolate. Phages with wide host ranges and those with complimentary host ranges are potentially useful for biological control of soft rot.

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BACTERIAL BIOCONTROL OF SOYBEAN CYST NEMATODE. R.M. Zablotowicz , C.D. Bierle, M.L. Matheney, C. Faye Rudolph, J.W. Kloepper, R. Rodriguez-Kabana, B. Herzfeldt, R. Heier and M. Yorke. Plant Science Research Inc, BioTechnica Int'l.Inc.and Auburn Univ.

Our goal is to identify bacterial strains to reduce the severity of the soybean cyst nematode (SCN), Heterodera glycinea. Six selected bacterial strains were evaluated as soybean inoculants in greenhouse studies using a conetainer based assay and SCN-infested soil. All strains reduced the level of SCN infestation in at least one assay. The most consistent strain (Bacillus thurengiensis, UZ404-8d) significantly reduced SCN infestation significantly (20 to 52%) in five of seven studies. Similar reductions were observed in field microplot studies. Although not as effective as the nemacide, Nemacur, bacterial inoculants may have potential in biorational approaches to reduce SCN disease losses.

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GENETIC ANALYSIS OF TRAITS IMPORTANT FOR THE BIOLOGICAL CONTROL OF PYTHIUM SEED ROTS BY *ENTEROBACTER CLOACAE*. Alan P. Maloney and Eric B. Nelson, Department of Plant Pathology, Cornell University, Ithaca NY 14853.

To begin to understand in detail the traits and genes that are important in suppression of soilborne Pythium seed rot, *E. cloacae* strain EcCT 501 was subjected to transposon mutagenesis with the mini-Tn*5*/*pho*A suicide plasmid. One model for *Pythium* suppression by EcCT501 postulates that lectin-dependent adherence to fungal mycelium is a first step in the interaction. Mutants were screened for over-expression of alkaline phosphatase and loss or reduction in suppression of seed rot on creeping bentgrass in an *in vitro* bioassay. Southern hybridizations demonstrated that more than 80% of the mutants arose from unique transposon insertions. Approximately 2% of the bacterial mutants were *pho*A-positive, indicating that successful translational fusions of *pho*A occurred in secreted, periplasmic, or cell-surface protein genes. Three *pho*A$^+$ mutants failed to suppress *Pythium* in the bioassay. Identification and isolation of the mutant and wild type genes is underway. In other enterobacteria, fimbriae and associated adhesin proteins have been implicated in pathogenicity. A heterologous probe synthesized from an *E. coli* fimbrial gene cluster detected several restriction fragments in EcCT501 genomic DNA; putative fimbrial genes are being isolated for study of their role in adherence and fungal disease suppression.

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EXPOSURE OF TEXAS MALE STERILE CYTOPLASM MAIZE LEAVES TO HIGH TEMPERATURE STRESS OR INFILTRATION WITH AMINO ACIDS IS ACCOMPANIED BY INCREASED SENSITIVITY TO TOXIN FROM BIPOLARIS MAYDIS RACE T. M. O. Garraway, Dept. of Plant Path., The Ohio State University, Columbus, OH 43210.

The effect of high temperature stress (HTS) on the sensitivity of maize to BMT-toxin was determined by infiltrating leaves from Normal (N) and Texas male sterile (T) cytoplasm isolines for 6 hr in the light at 28 C with BMT-toxin followed by 20 minutes in the dark at 42 C. Sensitivity to BMT-toxin was evaluated by comparing the rates of electrolyte leakage from stressed and non-stressed infiltrated leaves. Both isolines had increased sensitivity to BMT-toxin when exposed to HTS but the response was significantly greater with T than with N cytoplasm maize. Also, leaves of T cytoplasm maize not exposed to HTS had increased sensitivity to BMT-toxin when infiltrated with 2-4 µg/ml of either an amino acid extract from maize or 1-2 µg/ml of KH_2PO_4. Therefore, increased leakage of ions from T maize leaves may contribute to the HTS-induced increase in susceptibility to BMT-toxin.

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RAPID ACCUMULATION OF PEROXIDASES AND PHENOLIC POLYMERS IN SOYBEAN COTYLEDON TISSUES FOLLOWING PHYTOPHTHORA MEGASPERMA F. SP. GLYCINEA (PMG) WALL GLUCAN TREATMENT. M. Y. Graham and T. L. Graham, Dept. of Plant Pathology, The Ohio State University, Columbus, OH 43210.

PMG wall glucan has been extensively characterized in soybeans as an elicitor of the pterocarpan phytoalexins, the glyceollins, and of conjugates of the isoflavones, daidzein and genistein. Here we report that PMG wall glucan also induces the accumulation of phenolic polymers in soybean cell walls immediately adjacent to the point of elicitor application. Phenolic polymers are ten times those in wounded controls in just 4 hr. By comparison, isoflavone and glyceollin accumulation begin at 8 and 12 hr respectively. Phenolic polymer deposition is also several times greater than the isoflavone and glyceollin accumulations combined. The wall phenolics include both lignin and suberin like polyers as well as simple esterified coumaric and ferulic acid monomers. The accumulation of wall bound phenolics is accompanied by an equally rapid and massive induction of specific anionic peroxidases.

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RELATIONSHIP BETWEEN FIELD AND GREENHOUSE REACTION OF SOYBEAN TO FUSARIUM SOLANI. P.A. Stephens, C.D. Nickell, Department of Agronomy, S.M. Lim, USDA-ARS, Department of Plant Pathology, Univ. of Illinois, Urbana, IL 61801, and C.K. Moots, Asgrow Seed Co., Stonington, IL 62567.

Reaction of soybean to Fusarium solani, the causal agent of sudden death syndrome (SDS) was evaluated on a set of 12 soybean cultivars in micro-plots. Micro-plots were established by transferring soil from F. solani infested field sites to 122cm x 112cm x 20 cm containers at Urbana. Significant differences in SDS symptom development was found among the 12 cultivars. For greenhouse evaluation, isolates of F. solani pathogenic to soybeans were cultured on sterilized oat grains for 21 days at 24 C. Seeds were planted in sterilized soil and seedlings were inoculated at V1 by placing 3 oat culture grains per plant 5mm below the soil surface adjacent to the root.

Temperature was maintained at 24 C and chlorotic leaf symptoms appeared within 10 d. Correlation between AUDPC for the microplots and greenhouse leaf severity at 3 weeks after inoculation was highly significant ($r = 0.73^{**}$). These results indicate that inoculation of soybean seedlings with the oat grain culture in the greenhouse is a reliable method for evaluating reactions of soybeans to F. solani.

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VARIABILITY AMONG ISOLATES OF RHIZOCTONIA SOLANI ASSOCIATED WITH DRY BEAN AND SOYBEAN IN OHIO, USA AND ZAIRE, AFRICA. G. Muyolo, P. E. Lipps, and L. J. Herr, Dept of Plant Pathology, Ohio State Univ., Wooster, OH 44691

Nine morphological groups, based on cultural characteristics, were distinguished among 290 isolates of Rhizoctonia solani. Twelve foliar isolates from each of soybean and dry bean, representing anastomosis group 1 (AG-1), caused foliar blight, hypocotyl lesions, and root rot in the pathogenicity tests. AG-1 isolates from soybean grew faster (3.9 cm/day) in culture than those from dry bean (Phaseolus spp.) (3.5 cm/day) ($P=0.05$). Of the 36 isolates tested from hypocotyls and roots (AG-2 and AG-4), 35 failed to cause foliar symptoms under 100% relative humidity for 5 days. Isolates obtained from soybean roots in Ohio (AG-2) were significantly more virulent on roots and hypocotyls of soybean and dry bean than isolates obtained from roots of dry bean in Ohio (AG-2) or Zaire (AG-4). These isolates grew slower (1.9 cm/day) than isolates from dry bean in Ohio (2.3 cm/day) and Zaire (2.8 cm/day).

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FIELD INOCULATIONS OF SORGHUM WITH SCLEROTIA AND CONIDIA OF THE SOOTY STRIPE FUNGUS RAMULISPORA SORGHI IN WEST AFRICA. Melville D. THOMAS and F. Bocoum, International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), B.P. 320, Bamako, Mali, West Africa.

In studies on screening techniques for sooty stripe of sorghum various inoculation methods were assessed in the field on several sorghum genotypes. In 1989, at 30 days after sowing (DAS), a suspension of powdered sorghum leaves containing 2×10^4 ml^{-1} of 11 month old sclerotia of R. sorghi was either sprayed on plants to runoff or 1 ml was placed in the whorls. In addition, either 1g powder or 1 ml suspension was placed at the base of plants. In 1990, seeds were sown with 1g of the leaf sclerotium powder per hill. The inoculum sprayed at 30 DAS in 1990 contained 7×10^4 sclerotia ml^{-1}. Plants were also sprayed with a suspension containing 6×10^6 conidia ml^{-1} obtained the same day from leaf lesions. Noninoculated plants served as controls in both years. In 1989, at 93 DAS, disease severity was highest for plants sprayed with sclerotia. In 1990, at 105 DAS, disease severity was highest for plants sprayed with conidia. In both years, those two treatments were significantly different ($P \leq 0.01$) from the control on susceptible genotypes. The increase in disease over the control from the other treatments was not always significant.

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EFFECT OF FUSARIUM MONILIFORME ON KERNEL INFECTION BY ASPERGILLUS FLAVUS IN INOCULATED MAIZE EARS IN MISSISSIPPI. N. Zummo and G.E. Scott, USDA-ARS, Mississippi State Univ., P.O. Drawer PG, Mississippi State, MS 39762

Fusarium moniliforme Sheld. and Aspergillus flavus Link ex Fr. are frequently recovered from symptomless maize (Zea mays L.) ears and kernels in Mississippi. When maize ears were inoculated simultaneously with F. moniliforme and A. flavus using needle inoculation in 1988, 1989, and 1990, significantly fewer kernels were infected by A. flavus than when ears were inoculated with A. flavus alone. Kernel infection by F. moniliforme in ears inoculated with F. moniliforme alone or in ears inoculated with both fungi did not differ in 1988 and 1989 but differences were found in 1990. Inoculation of ears with A. flavus alone using the pinbar in 1990 or needle in 1989 and 1990 resulted in significantly more natural infection of kernels by F. moniliforme. In contrast, natural infection of kernels by A. flavus in ears inoculated with F. moniliforme alone did not differ significantly from uninoculated ears.

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Cercosporin lesions resemble *Cercospora zeae-maydis* lesions in maize seedlings. M. R. Carter, E. L. Stromberg, H. L. Warren, D. M. Orcutt, and D. N. Radin. Department of PPWS and CSES, VPI&SU, Blacksburg, VA 24061-0331.

Cercospora zeae-madis (CZM), causal agent of gray leaf spot (GLS) of maize, produces cercosporin, a red-colored, light-activated toxin, that may play a role in GLS development. Toxin was applied at varying concentrations in 1-5% EtOH:water to excized shoots of maize seedlings. Treated seedlings varying in GLS resistance were exposed to a 12 h night/day cycle. By 39 h, leaf tip tissue appeared watersoaked and became necrotic with chlorotic borders. These lesions are similar to those produced by CZM in the field. Lesion size increased with greater cercosporin concentrations. Chromatographic analysis using HPTLC of leaf lesion extracts revealed that the toxin was present in lesion from cercosporin-treated seedlings and quantitative differences were observed. These data support a role for cercosporin in GLS disease development.

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ANALYSIS OF A LOCAL POPULATION OF FUSARIUM OXYSPORUM F. SP. LYCOPERSICI FOR VIRULENCE, RACE, AND VEGETATIVE COMPATIBILITY. R. W. Schneider, K. S. Elias, R. M. Davis, and P. Tsaraboulidou. Dept. Plant Pathology & Crop Phys., Louisiana State University Agric. Center, Baton Rouge, LA 70803.

A collection of Fusarium oxysporum f. sp. lycopersici was made from a tomato field in California in which race 3 was first isolated during the previous two years. Isolates were obtained from tomato cultivars that were susceptible to all three races (A), races 2 and 3 (B), and race 3 (C). The collection was assessed for race and virulence (290 isolates) as well as vegetative compatibility group (VCG) (96 isolates). Race 2 isolates belonged to three multiple-member and five single-member VCGs. All race 3 isolates were members of one VCG (0030). The percentages of race 1, 2, and 3 isolates were 3.6, 81.1, and 15.3 from A cultivars; 0, 66.1, and 33.9 from B cultivars; and 0, 9.3, and 90.7 from C cultivars, respectively. Race 2 and 3 isolates from C cultivars were more virulent than those obtained from A and B cultivars.

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RELATIONSHIP BETWEEN VEGETATIVE COMPATIBILITY AND COMPETITIVE INFECTION ABILITY IN NONPATHOGENIC STRAINS OF FUSARIUM OXYSPORUM IN TOMATO. M. M. Lear and R. W. Schneider. Dept. Pl. Pathology & Crop Phys., Louisiana State University Agric. Center, Baton Rouge, LA 70803

Nonpathogenic isolates of Fusarium oxysporum were collected from symptomless tomato roots obtained from five locations in Louisiana. The isolates were grouped into vegetative compatibility groups (VCG) that consisted of 31 multiple member and a large number of single member VCGs. Multiple member VCG tester isolates were used in initial competitive infection tests versus a pathogenic orange mutant (OM) of F. o. f. sp. lycopersici. Four VCGs were chosen for further study, and all members of these VCGs were tested against the OM in competitive infection tests and used for isozyme analysis. There was a definite relationship between VCG and competitive infection ability.

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INFLUENCE OF TEMPERATURE AND MOISTURE ON GROWTH OF GLOEOTINIA TEMULENTA AND INFECTION OF ANNUAL RYEGRASS. S. C. Alderman, USDA-ARS NFSPRC, 3450 SW Campus Way, Corvallis, OR 97331.

Germination, growth, and sporulation of Gloeotinia temulenta on PDA increased from 5 through 20 C, then declined. No germination or growth was observed at 30 C. Radial growth on PDA amended with NaCl, KCl, or sucrose declined with decreasing water potentials through -9 to -10 MPa. Spore production on sucrose-amended PDA increased through -4 MPa then declined through -10 MPa. No spores were produced on KCl- or NaCl-amended media. Infection of annual ryegrass occurred at 15, 20 and 25 C but not at 30 C. A dew period was not required for infection when a drop of conidial suspension was placed in open flowers of annual ryegrass or when a conidia-laden exudate from infected seed was transferred to ovaries in open flowers. Percentage infection increased with increasing inoculum concentration from 10^2 to 10^4 conidia/ml. Numbers of conidia on infected seed at 20 C increased from six through 14 days after inoculation.

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PHYTOPHTHORA INFESTANS IN EASTERN GERMANY FROM 1976-1990: ANALYSIS OF MATING TYPE, ALLOZYME PHENOTYPE, AND METALAXYL SENSITIVITY. S. S. Daggett and E. Gotz[1], Dept. of Biology, The Pennsylvania State University, University Park, PA 16802 and [1]Institute for Potato Research, Gross Lusewitz 2551, Germany.

One hundred and six isolates of P. infestans collected in eastern Germany (formerly the GDR) from 1976 to 1990 have been analyzed for mating type, allozyme phenotype and response to metalaxyl. The A2 mating type was first isolated in 1980. The percentage of isolates of this mating type was 9.1% in 1987. This increased to 34.7% in 1988, but decreased in 1990 to 12.5%. There does not, therefore, seem to have been an overall increase in the frequency of the A2 mating type since its initial isolation. The percentage of isolates resistant to metalaxyl increased from 0% in 1976 to 40% in 1990. The frequency of isolates which demonstrated an intermediate sensitivity to metalaxyl has remained constant at approximately 20% during this period. Prior to 1984 all A1 isolates had the phenotypes 86/100 and 92/100 for the allozymes Gpi-1 and Pep, respectively. A2 mating type isolates during this period were either 100/100, 100/100 for Gpi-1 and 100/100, 92/100 for Pep. Since 1985 isolates of both mating types have been either 100/100 or 90/100 for Gpi-1. During the period 1987-1990 two new alleles, Gpi-1 90 and Pep 83, appeared in the German isolates. These observations suggest that sexual recombination may be occurring in Eastern Europe.

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COMPARISON OF DIAPORTHE PHASEOLORUM ISOLATES IN SOUTH AFRICA. W.A. Smit[1] and M.J. Wingfield[2]. [1]Fruit and Fruit Technology Research Institute, Private Bag X5013, Stellenbosch 7600, South Africa and [2]Department of Microbiology and Biochemistry, University of the Orange Free State, Bloemfontein 9300, South Africa.

Diaporthe phaseolorum is an important pathogen of perennial trees and shrubs in South Africa. It is, however, not known on soybean which is its best known host in other parts of the world. Anamorph and teleomorph characters of D. phaseolorum in South Africa were identical to those of reference cultures from soybean. Local isolates could be separated into groups based on differences in pathogenicity and growth temperature optima as well as on vegetative incompatibility. Morphological similarity between South African isolates of D. phaseolorum suggests that they represent a single species. Differences in their biology, however, demand that they be compared more thoroughly in the future.

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LOCAL AND TRANS-CANADIAN DISTRIBUTION OF CLONES OF SCLEROTINIA SCLEROTIORUM ON CANOLA AS IDENTIFIED BY MYCELIAL INCOMPATIBILITY AND MOLECULAR FINGERPRINTS. Y. Kohli and L.M. Kohn, Dept. of Botany, University of Toronto, Erindale College, Mississauga, Ontario, Canada L5L 1C6.

The clonal variability among field populations of Sclerotinia sclerotiorum in canola growing areas of Western Canada was examined by two independent markers, mycelial incompatibility and RFLP analysis (DNA fingerprinting). Thirty-seven clones were identified among 66 isolates from seven locations in Alberta, Saskatchewan and Manitoba. Every field population was genetically heterogenous, i.e. composed of several clones. The most widely distributed clone was found in all three provinces and represented 18% of the 66 isolates. In addition six other clones were found in at least two provinces. A comparison with clones from two fields in Ontario (Kohn et al. Phytopathology 81: 480-485) showed that one of the predominant clones from the Ontario study was also represented in Manitoba and Saskatchewan. In another experiment, sibling monosporous isolates from homothallic fruiting showed no meiotic segregation for either mycelial compatibility or the DNA fingerprints.

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CONIDIAL GERMINATION IN EUTYPA ARMENIACAE. T. -M. Ju[1], D. A. Glawe[2], and J. D. Rogers[1]. [1]Department of Plant Pathology, Washington State University, Pullman 99164 and [2]Department of Plant Pathology, University of Illinois, Urbana 61801.

In Eutypa armeniacae Hansf. & Carter [=Eutypa lata (Pers.: Fr.) Tul & C. Tul. fide Rappaz], and other species of Diatrypaceae, ascospores have been considered the only source of inoculum because conidia were thought not to germinate. Consequently, conidia were not investigated in research on the epidemiology of diseases caused by diatrypaceous fungi. Recently we germinated conidia of E. armeniacae isolated from Vitis labrusca in Washington State. Conidia were placed on potato dextrose agar amended with 5 g/L yeast extract. In four trials involving 9,400,000 conidia, approximately 0.0015% germinated. Previously, germinating conidia may have been overlooked because they occurred at low rates, or because it is frequently difficult to distinguish the filiform conidia from the mycelia they produce. These results suggest that conidia of diatrypaceous plant pathogens may serve as an effective source of inoculum.

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BENOMYL TOLERANT ISOLATES OF *PHOMOPSIS* FROM SOYBEAN SEEDS. R.S. Ferriss and J.M. Baker. University of Kentucky, Lexington 40546.

Benomyl tolerant isolates of *Phomopsis* sp. were recovered from soybean seeds (cv Century) produced in a research plot that had received seven foliar sprays of the fungicide. Seed infection by *Phomopsis* spp. was 75%, and all isolates recovered from the seed lot were benomyl tolerant. For nine tolerant isolates and six non-tolerant isolates, the ranges of linear growth rates on potato dextrose agar were similar. On PDA amended with benomyl, linear growth of the non-tolerant isolates was inhibited 50% by 0.05 to 0.11 ppm benomyl; linear growth of the tolerant isolates was not diminished significantly by 10 ppm. Although benomyl tolerant soybean *Phomopsis* isolates have been found previously in work that was aimed at generating tolerance, this is to our knowledge the first report of their unexpected development.

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RELATIONSHIP BETWEEN POD DEVELOPMENT STAGE, TEMPERATURE, POD WETNESS DURATION AND INCIDENCE OF PURPLE SEED STAIN OF SOYBEANS. Wolfgang Schuh, Dept. of Plant Pathology, The Pennsylvania State University, University Park, PA 16802.

The incidence of soybean seed infected by Cercospora kikuchii was influenced by temperature, length of pod wetness period and developmental stage of the soybean pods at the time of inoculation. The statistical significance was determined using logistic regression. Infected seeds were observed when small pods (<0.5 cm length) and large pods (0.5-2.0 cm length) were inoculated. No infection was observed when flowers in full bloom or post bloom (dessicated petals) were inoculated. The optimal temperature for infection was 25 C whereas no infection was observed at 15 C and 35 C. Pod wetness periods of 24 hr were required for disease development at all temperatures. In most instances, disease incidence increased with increasing pod wetness periods up to 30 hr; however, extending this period to 36 hr resulted in decreased disease incidence in some temperature/pod wetness period combinations. Predictive models developed for the two pod developmental stages performed satisfactorily when comparing observed and predicted disease incidence. There was no significant relationship between seed infection and seed germination.

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INCIDENCE OF SCLEROTINIA MINOR IN PEANUT SEED FROM GROWER FIELDS. H. A. Melouk, K. E. Jackson, J. P. Damicone and R. J. Sholar. USDA-ARS and Departments of Plant Pathology and Agronomy, Oklahoma State University, Stillwater 74078.

Six fields of runner-type and six of spanish-type peanut were selected in Caddo Co., OK. Two fields of low, moderate or high severity of sclerotinia blight per market type were sampled. One week before harvest, plants from four random 2m-row-lengths in each field were hand-dug. Samples were threshed, pods dried at 28C and machine shelled. Seeds were sized by metal screens. Those retained on the 17/64 screen were agitated for 1 min in 0.2% liquid detergent, soaked 1 min in 1.0% sodium hypochlorite. Seeds (1,000/field) were plated on potato-dextrose-agar containing 100 ug/ml streptomycin sulfate. About half of seed samples of runners from moderate and high blight fields were positive for S. minor, whereas, 25% of samples of spanish from high blight fields were positive. Incidence of S. minor in seeds of positive samples ranged from 0.4 to 0.6%.

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PLANT DISEASE PROBLEMS IN TURKEY AND STRATEGIC CHANGES OF THEIR CONTROL. A.Çıtır, Cumhuriyet University, Tokat 60110, Turkey.

With her 777 000 square kilometers land Turkey is the second largest country in Europe. Because of the great variation in its climate and topography at least 60 cultivated plant species and their hundreds of cultivars are grown economically. These plants however are always become a target of new infections. Enormous human activities and trasportation have put all valid quarantine regulations into an inefficient trade bariers in Turkey. Beside a number of abiotic plant diseases, more than 1100 host-pathogen relations have been determined since 1900. So 78 kinds of disease control pratices supported by government are in service for growers. Extensive usage of high quality certified seed increased agricultural yield for export in last dacade. In order to reduce pesticide usages for disease control and to prevent residue on yield, integrated pest management projects put into investigation in 1991.

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MACROPHOMINA PHASEOLINA, HETERODERA GLYCINES, AND *PHYTOPHTHORA MEGASPERMA* F.SP. *GLYCINEA* AS PATHOGENS OF SOYBEAN. M.A. Chapman, T.D. Wyllie, J.D. Mihail, T.L. Niblack, and J.T. English; Department of Plant Pathology; 108 Waters Hall; University of Missouri; Columbia, MO 65211.

The interaction among *Macrophomina phaseolina, Heterodera glycines,* and *Phytophthora megasperma* f.sp. *glycinea* as pathogens of soybean was studies in 60 1M x 1M microplots. The design consisted of five blocks, three soybean cultivars (Williams, Williams 82, and Fayette), two levels each of *M. phaseolina* and *H. glycines*. Amendment of the soil with laboratory-produced inoculum of *M. phaseolina* and *H. glycines* was unsuccessful. The background rhizosphere populations of *M. phaseolina* remained constant throughout the season, while those of *H. glycines* increased with the susceptible Williams and Williams 82 and decreased with the resistant Fayette. Other data obtained were plant germination, final stand count, early infection of roots by *M. phaseolina* and *H. glycines*, pod count, seed number and weight, node count, and plant height.

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EFFECT OF VISUAL, REMOTE SENSING, AND IMAGE ANALYSIS ASSESSMENT METHODS ON INTRA-RATER AND INTER-RATER RELIABILITY ESTIMATES IN THE DOLLAR SPOT-BENTGRASS PATHOSYSTEM. F. W. Nutter, Jr., M. L. Gleason, J. H. Jenco, and N. C. Christians[1]. Departments of Plant Pathology and Horticulture[1], Iowa State University, Ames, 50011.

Different amounts of Sclerotinia-infested ryegrass grain were used to inoculate a bentgrass green to establish a wide range of disease severities. Plots 1 m^2 were visually assessed by four raters using a 0 to 100 percentage scale. Reflectance values at 600 and 800 nm were also recorded from each plot using a hand-held radiometer. Color photographs were digitized and analyzed by the ISU Image Analysis Facility. Visual and remote sensing evaluations were repeated 24 hr later to obtain intra-rater reliability estimates. Correlations among raters were performed to obtain inter-rater reliability estimates. Intra-rater reliability estimates ranged from 98.0 to 99.5 % using the radiometric method as compared to 91.2 to 99.0 % using the visual scale. The reliability estimate for the image analysis method was only 80.1 %. Inter-rater reliabilities were highest for the radiometric method (99.2 to 99.8 %) and were only slightly lower for the visual method (88.7 to 94.5 %). However, the radiometric method had lower standard errors and less bias (slopes closer to 1.0) than the visual method.

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EPIDEMIOLOGY AND CONTROL OF *EXOBASIDIUM OXYCOCCI* (ROSE BLOOM) ON CRANBERRY. P. R. Bristow and G. E. Windom. Washington State University, 7612 Pioneer Way E., Puyallup 98371.

Abnormal fleshy lateral branches (rose bloom) of cranberry began to grow from infected axillary buds in April in western Washington. Basidiospores produced on the surface of these branches were discharged from early May through mid-June. The number of spores trapped with a Burkard volumetric spore trap was correlated with the number produced. Spore discharge exhibited a diurnal pattern with the fewest number of spores trapped during mid-day. Spore discharge occurred at temperatures between 5 and 22C; germination between 10 and 22C; and optimum growth at ca. 20C (germination and growth on potato dextrose agar). Three fungicide applications made at 2-week intervals starting in mid-May protected buds and reduced the incidence of rose bloom the following spring. Mancozeb, benomyl and propiconazol were the most effective compounds.

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C.M. Liddell, J.A. Woodard, C.A. Fields, and J.E. Newberry. Integration of plant disease forecast models into a regional weather interpolation system. New Mexico State University, Las Cruces.

Integration of plant disease forecast models into a regional weather interpolation system. C.M. Liddell, J.A. Woodard, (Department of Entomology, Plant Pathology and Weed Science), C.A. Fields, and J.E. Newberry (Computing Research Laboratory). New Mexico State University, Las Cruces, NM 88003.
Most existing disease forecast models, developed from microclimatic data, require intensive monitoring of conditions at each site where predictions are required. Regional disease prediction system (RDPS) can be developed using a terrain-sensitive weather interpolation system that requires a low density of monitoring stations. Hence, existing site-specific models can be easily implemented on a regional grid. The RDPS receives live hourly surface weather data from National Weather Service stations, additional data from testing sites, and hourly satellite images of cloud cover. An RDPS has been developed for the web blotch disease of Valencia peanuts, and is being tested in Roosevelt county, New Mexico. The RDPS allows the incorporation of multiple models, such as crop, disease, pest, and economic models into a single prediction system.

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EFFECTS OF PRODUCTION ENVIRONMENT ON THE SUSCEPTIBILITY OF ROSE FLOWERS TO INFECTION BY *BOTRYTIS CINEREA*. P.E. Hammer and K.B. Evensen, Department of Horticulture, Pennsylvania State University, University Park, PA 16802.

The relationship between environmental conditions during production and susceptibility of rose flowers to postharvest infection by *B. cinerea* was studied. Environmental conditions in a rose production greenhouse were recorded using a micrologger. Rose flowers were harvested periodically and inoculated with *B. cinerea* conidia under standardized laboratory conditions. The slopes of the inoculation concentration - disease severity relationships were used to quantify the susceptibility of the flowers to infection. Susceptibility was linearly correlated (r^2 = 0.94) with the mean air velocity during the 5 week period before harvest. Susceptibility also was correlated with the mean air to leaf temperature gradient (r^2 = 0.69) and inversely correlated with wetness measured by an electronic leaf (r^2= 0.88), but these correlations are interpreted as secondary effects of air movement. There were no significant correlations between susceptibility and temperature, relative humidity, or the other factors measured. Greenhouse experiments are in progress to verify the effect of air movement on susceptibility.

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POPULATION STRUCTURE AND OUTCROSSING IN THE CHESTNUT BLIGHT FUNGUS, *CRYPHONECTRIA PARASITICA*. M. G. Milgroom and S. E. Lipari, Cornell University, Ithaca, NY 14853-5908

DNA fingerprinting and single-copy RFLP probes were used to determine the extent of clonality and to estimate outcrossing rates in a population of *Cryphonectria (Endothia) parasitica* on chestnut sprouts in Virginia. Among 39 isolates collected from a 25 X 25 m forest plot, there were 33 haplotypes; 6 of these haplotypes each occurred twice. Three pairs of isolates sharing the same haplotype were from cankers on the same trees. Outcrossing rates were estimated from ascospore progeny collected in the field. Progeny from approximately two-thirds the perithecia showed segregation for at least one genetic marker, indicating an outcross event. Estimates of gametic disequilibrium from single-copy RFLP markers were not significantly different from zero. These results are consistent with the hypothesis that most reproduction is sexual (selfing and outcrossing), rather than clonal, in this population of *C. parasitica*.

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RELATIONSHIP BETWEEN TOPOGRAPHIC FEATURES, STAND COMPOSITION, AND DOGWOOD ANTHRACNOSE IN THE SOUTHERN APPALACHIANS. [1]D.O. Chellemi, [2]K.O. Britton, and [3]W.T. Swank. [1]North Florida Research and Education Center, Quincy, FL 32351, [2]USDA Forest Service, Athens, GA 30602, and [3]USDA Forest Service, Otto, NC 28763.

Sixty seven undisturbed 0.08 ha plots within the Nantahala Mountain Range of the southern Appalachians were surveyed for dogwood anthracnose. The extent of foliar symptoms (disease severity) and limb dieback within canopies was determined for all trees (Cornus florida L) with a basal diameter of 1.5 cm or greater. Incidence of dogwood anthracnose ranged from 53% to 100% and severity ranged from 3% to 65%. No relationship between disease incidence and host density, aspect, slope, or elevation was observed. Disease severity and limb dieback decreased with increasing host density.

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UNCINULA NECATOR ASCOSPORE RELEASE, VIABILITY AND INFECTION IN FIELD CONDITIONS IN CALIFORNIA. C. S. Thomas, W. G. Gubler and L. Bettiga. Department of Plant Pathology and Cooperative Extension, University of California, Davis CA 96516 and Salinas, CA 93901.

In some California vineyards, Uncinula necator (Schw.) Burr. overwinters as cleistothecia lodged in the bark. Cleistothecia development, maturity, movement during rains, ascospore release, and primary infection were studied in two coastal vineyards in California from 1989 to 1991. Cleistothecia formed in July in both vineyards. By July 27, some

cleistothecia were capable of releasing viable ascospores. Movement of cleistothecia during fall and winter rains was determined by trapping into filter paper funnels. During fall rains, cleistothecia (up to 1400 / funnel) were washed off leaves and lodged in bark or fell to the soil below the canopy but most did not move more than a few inches from the drip line. Each year, ascospores were trapped with a volumetric spore trap. Ascospores were released in waves which were associated with rain, fog or dew. The waves occurred throughout the trapping period (February - June). However, the magnitude of these waves of release decreased after prolonged periods of maximum daily temperatures greater than 25 C. Disease symptoms were observed in the vineyards 5 to 10 days after the first wave release following budbreak. This is the first report of July ascospore viability and spring ascospore release due to fog or dew in the United States.

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SPATIAL PATTERNS OF EUTYPA DIEBACK IN CALIFORNIA VINEYARDS. G.P. Munkvold, J.A. Duthie, and J.J. Marois. Department of Plant Pathology, University of California, Davis, CA 95616.

Spatial and temporal patterns of vines with symptoms of Eutypa dieback, caused by *Eutypa lata* (syn. *E. armeniacae*), were characterized in 8 vineyards from 4 wine grape growing areas in northern California in each of 3 years. In each vineyard, presence or absence of symptoms was recorded for every vine in a contiguous block of 1,250 or more vines. Proportion of vines with symptoms ranged from .046 to .363 in 1989, and from .055 to .655 in 1990. Mean disease increase between 1989 and 1990 was .087. Based on ordinary runs, geostatistical, and spatial autocorrelation analyses, spatial patterns of diseased vines in all vineyards contained a random component. This suggested the existence of airborne inoculum from distant sources. Some vineyards also contained nonrandom patterns, such as disease gradients or first-order spatial autocorrelations, which suggested some spread from vine to vine. The degree of autocorrelation, but not disease incidence, was related in general to mean annual rainfall, and the presence or absence of perithecia in the vineyard.

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INFECTION OF GRAPE BY *BOTRYTIS CINEREA* AND PATHOGEN REPRODUCTION AS INFLUENCED BY MICROCLIMATE. J.T. English[1] and J.J. Marois[2]. [1]Department of Plant Pathology; 108 Waters Hall; University of Missouri, Columbia, MO 65211; and [2]Department of Plant Pathology; University of California; Davis, CA 95616.

Infection of grape berries by *Botrytis cinerea* was influenced by temperature and duration of berry wetness. At least four hours of wetness were required for infection at temperatures between 12 and 28 C. Infection did not occur at 32 C. Infection occurred most rapidly between 16 and 24 C. At all temperatures, maximum spore production by *B. cinerea* on berries over an eight-day period occurred at relative humidities of 86% and 96%. Regardless of temperature, a latent period of two or three days was observed at these relative humidities. At relative humidities between 76% and 43%, the latent period ranged between three and eight days. The quantification of the relationships of these environmental factors and infection and reproduction by *B. cinerea* are discussed.

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RELATIONSHIP BETWEEN AGE OF VINEYARD AND INCIDENCE OF EUTYPA DIEBACK. J.A Duthie, G.P. Munkvold, J.J. Marois, S. Grant, and D.O. Chellemi. Dept. of Plant Pathology, University of California, Davis, CA 95616.

Dieback of grapevine, caused by *Eutypa lata*, is more frequent in old vineyards but effects of vineyard age have not been evaluated critically. In April 1990, we surveyed, near Livingston, CA, 11 spur-pruned vineyards (cv. French Colombard) aged 5 to 34 yr. In each vineyard, we recorded proportion of spurs with symptoms of dieback (y_S) on every fifth vine in every fifth row. Both y_V (proportion of vines with symptoms) and y_S increased sigmoidally with age (t). A logistic equation, $y = A/[1+Bexp(-Ct)]$, in which C gauged the relative rate of increase of y toward the upper asymptote A, was fitted to data. The integration constant $B = (A-y_O)/y_O$ was fixed by assuming that y_O (y at t=0) was 0.001 and A and C were estimated iteratively. In old vineyards, most vines but less than half of the spurs were diseased. For y_V (A_V=0.92, C_V=0.55, R^2=0.98) and y_S (A_S=0.40, C_S=0.39, R^2=0.91), respectively, 90% of asymptotic levels were reached by ages 17 and 21 yr. Pruning diseased spurs may provide some degree of disease control.

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SURVIVAL OF THE TALL FESCUE ENDOPHYTE IN THE DIGESTIVE TRACTS OF CATTLE AND HORSES. R.A. Shelby and S.P. Schmidt. Depts. of Plant Pathology and Animal and Dairy Sciences, Auburn University, AL.

As an obligate symbiont, the tall fescue endophyte (Acremonium coenophialum) is spread only in seed of its host. To evaluate the potential spread of this fungus by herbivores, the viability and chronology of fescue seed and associated endophyte were measured as it was ingested and passed through the digestive tract of grazing livestock. When fed as a single dose, viable fescue seed and live endophyte were recovered from feces of a steer from 10 to 38 hr after feeding. Twelve percent of the seed remained viable, and 12% of surviving plants were endophyte infected. When steers were allowed to graze seed-bearing stands ad libitum, the chronology of passage was similar. Horses were also found to pass live infected seed, although at a reduced level when compared to steers. Though survival is low, these animals are a potential source of endophyte infestation into endophyte-free pastures, however, a three-day quarantine period is sufficient to prevent infestation of endophyte-free pastures.

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MOLECULAR ANALYSIS OF THE dsRNA ASSOCIATED WITH NB58, A HYPOVIRULENT STRAIN OF THE CHESTNUT BLIGHT FUNGUS FROM NEW JERSEY. M.P.Brown and B.I.Hillman, Dept. of Plant Pathology, Rutgers University, New Brunswick, NJ 08903.

Hypovirulent strain NB58 of the chestnut blight fungus contains a virus with one 12.5 kbp segment of double-stranded (ds) RNA as its genome. We previously reported the mapping of cDNA clones representing the entire dsRNA, and the sequencing of the dsRNA termini. Here we report the nucleotide sequence of the 5'proximal 4.5 kb. The first long open reading frame, ORFA, has the potential to encode a polypeptide of only 438 amino acids, compared to the 622 aa coding potential for ORFA of EP713 dsRNA, which shares approximately 50% sequence similarity with NB58 dsRNA. Unlike ORFA of EP713, ORFA of NB58 does not appear to encode a Cys proteinase as its N-terminal product. Although different in position to the UAA terminator of ORFA of EP713, surrounding residues are similar, suggesting a similar mechanism for initiation of ORFB translation. Like ORFA, the N-terminal portion of ORFB shares little similarity between the two viruses.

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THE ROLE OF THE RED CLOVER NECROTIC MOSAIC VIRUS CAPSID AND CELL-TO-CELL MOVEMENT PROTEINS IN VIRAL INFECTION. Z. Xiong, K. H. Kim, and S. A. Lommel. Dept. of Plant Pathology, N. C. State Univ., Raleigh, NC.

The genome of the red clover necrotic mosaic dianthovirus (RCNMV) consists of two non-homologous RNAs. RNA-1 encodes replicase and capsid protein (CP), and RNA-2 encodes a 35 kDa cell-to-cell movement protein. A series of deletion and insertion mutations were constructed within the capsid and cell-to-cell movement protein genes. Viruses containing CP mutations replicated as free RNA and moved cell-to-cell throughout the inoculated leaves of *Nicotiana benthamiana* forming wild-type symptoms. Systemic infection also occurred but was delayed and limited to a few leaves, forming an unusual necrotic "oak-leaf" symptom. All deletions within the CP cistron rendered the CP unstable and undetectable *in vivo*. As many as 39 amino acid residues can be deleted from the carboxyl-terminus of the cell-to-cell movement protein without reduction in RCNMV ability to move from cell-to-cell. Larger deletions within the movement protein prevented cell-to-cell movement. These data suggest that the successful infection of RCNMV in a systemic host depends on two distinct events: cell-to-cell movement and long distant movement. For RCNMV cell-to-cell movement, the RNA-2 encoded movement protein is necessary but the CP is dispensable. For rapid long distance movement, both the movement protein and the CP are required.

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IDENTIFICATION OF DEFECTIVE INTERFERING-LIKE RNAs IN BROAD BEAN MOTTLE VIRUS. J. Romero and J. J. Bujarski. Plant Molecular Biology Center, Northern Illinois University, DeKalb, IL, 60115.

Studying the differences among genomic RNAs of various BBMV isolates we have found that two strains designated Mo and Tu, contained electrophoretically distinguishable additional components. By using the RNA-2 specific primers and the RT-PCR reactions, we have obtained, in addition to the full-length cDNA-2 two smaller cDNA products of 2.4 and 1.0 kb for the Mo strain and one 1.4 kb product for the Tu strain. Sequencing analysis of the cloned cDNAs revealed that they contained both 5' and 3' domains from RNA-2 and internal deletions. This report represents the first description of a defective interfering-like RNA molecule in tricornaviruses.

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Possible Emergence of a New Group within the Geminivirus Group-Tomato Yellow Leaf Curl Virus (Thailand), a Virus With both Mono- and Bipartite Characteristics..D.E.Rochester, C.M.Fauquet, J.J.DePaulo and R.N.Beachy. Dept. of Biology,Washington University, St. Louis, Missouri 63130.

Analysis of the geminivirus, Tomato yellow leaf curl virus (Th), indicates that this virus has characteristics which are generally restricted to either the

bipartite or the monopartite groups. Complete sequence analysis indicates the genomic organization is clearly similar to other whitefly transmitted geminiviruses including African cassava mosaic virus (ACMV) and tomato golden mosaic virus (TGMV). There are five potential open reading frames on the A-DNA and two such cistrons on the B-DNA. Comparison at the protein sequence level indicates that TYLCV-Th maintains the highest degree of similarity with ACMV. Unlike other whitefly transmitted geminiviruses, TYLCV-Th is capable of a systemic infection when only the A-DNA is present. Severity and symptomology of infection is increased dramatically when the B-DNA is also present. Possible functions of the open reading frames of TYLCV-Th will be presented.

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INFECTIOUS DNA CLONES OF A NEW GEMINIVIRUS ASSOCIATED WITH TOMATOES IN FLORIDA. R. L. Gilbertson[1], S. H. Hidayat[2], M. R. Rojas[2], and D. P. Maxwell[2]. [1]Dept. of Plant Pathology, University of California, Davis, CA 95616, [2]Dept. of Plant Pathology, University of Wisconsin, Madison, WI 53706.

Samples of tomatoes from Florida with virus symptoms hybridized strongly with a general DNA probe for bipartite geminiviruses. Using isolate-specific DNA probes, it was determined that the geminivirus(es) infecting tomatoes in Florida were different from the bean-infecting geminiviruses prevalent in the Caribbean Basin and South America. A Florida tomato geminivirus (TGV-FL) was readily sap-transmitted to *Nicotiana benthamiana*. Geminiviral double-stranded DNA was extracted from infected tomatoes and putative full-length clones of DNA-A and DNA-B obtained. Full-length linear monomers (2.6 kb) of these clones were infectious on *N. benthamiana* after mechanical inoculation. Restriction maps and partial nucleotide sequence data for these clones indicates that the TGV-FL is different than previously characterized geminiviruses from tomato. DNA sequence analysis revealed similarities between the TGV-FL and bean dwarf mosaic geminivirus.

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TOMATO GOLDEN MOSAIC VIRUS GENE FUNCTION. G. Sunter, M. D. Hartitz, S. G. Hormuzdi, D. C. Stenger, and D. M. Bisaro, Biotechnology Center, Ohio State University, Columbus, Ohio, 43210.

Tomato golden mosaic virus is a whitefly-borne agent belonging to the geminivirus group. The viral genome is divided between two DNA components (A and B) which are both required for infectivity. Mutants carrying lesions within the six TGMV ORFs have been constructed, and their replication in protoplasts has been examined. We have found that coat protein (AR1) mutants, which are infectious, accumulate reduced amounts of ssDNA. AL2 mutants display a similar phenotype in protoplasts, although AL2 mutants are not infectious. Further analysis has revealed that the AL2 gene product transactivates expression of the coat protein gene and BR1, a gene necessary for movement of the virus between cells. AL1 mutants do not replicate DNA, while AL3 mutants accumulate reduced amounts of all viral DNA forms. Mutations in B component ORFs (BL1 and BR1) have no effect on the synthesis or accumulation of viral ssDNA or dsDNA, although they cannot infect plants. Therefore, BR1, BL1 and AL2 proteins are involved either directly or indirectly in virus movement.

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REPLICATIONAL RELEASE OF GEMINIVIRUS GENOMES FROM TANDEMLY REPEATED COPIES: EVIDENCE FOR ROLLING CIRCLE REPLICATION. D. C. Stenger, G. N. Revington, M. C. Stevenson, and D. M. Bisaro, Ohio State Biotechnology Center, Ohio State University, Columbus, Ohio, 43210.

Agro-inoculation of *Nicotiana benthamiana* with Ti plasmids bearing tandem genome repeats derived from different strains of the geminivirus beet curly top resulted in the production of unit-length, recombinant progeny genomes in systemically infected plants. When two putative plus strand origins of replication were present in constructs used as inocula, a replicational release mechanism was favored which resulted in progeny genomes of a single predominant genotype. Sequencing across the junction between parental strains in the recombinant progeny allowed mapping of the plus strand origin of replication to a 20 bp sequence within the geminivirus conserved hairpin. In contrast, several progeny genotypes were observed when the inoculum contained a partial tandem repeat with only a single conserved hairpin, a result expected if progeny genomes were generated by random intramolecular recombination events. These results and other considerations suggest that geminivirus DNA replication occurs by a rolling circle mechanism.

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CHARACTERIZATION OF A FLORIDA TOMATO GEMINIVIRUS (FTGV). Abouzid, A.M. and Hiebert, E. Department of Plant Pathology, University of Florida, Gainesville FL 32611

A whitefly-transmitted geminivirus has become a serious problem in Florida's tomato production. Affected plants displayed systemic chlorosis or characteristic mosaic as well as leaf distortion. The new virus was mechanically transmitted to *Nicotiana x edwardsonii*. Geminivirus replicative form (RF) DNA was isolated from infected plants and digested with restriction endonucleases. Hybridization of the digested FTGV RF with probes prepared to the Florida bean golden mosaic virus indicated that FTGV has a divided genome consisting of two DNA molecules of about 2600 bp. Full-length clones of both DNA species were prepared in the pGEMEX vector. A comparison of the FTGV nucleotide sequences with known whitefly-transmitted geminiviruses indicates that it is a distinct geminivirus closely related to abutilon mosaic virus.

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THE MOLECULAR CLONING AND NUCLEOTIDE SEQUENCE OF THE COAT PROTEIN GENE OF THE NL-3 STRAIN OF BEAN COMMON MOSAIC POTYVIRUS. R. L. Gilbertson[1], E. M. Zambolim[2], S. H. Hidayat[2], E. P. Rybicki[3], and D. P. Maxwell[2]. [1]Dept. of Plant Pathology, University of California, Davis, CA 95616, [2]Dept. of Plant Pathology, University of Wisconsin, Madison, WI 53706, and [3]Dept. of Microbiology, University of Cape Town, Private Bag, Rondebosch 7700, South Africa.

The NL-3 strain of bean common mosaic potyvirus (BCMV) is a necrosis-inducing BCMV strain, which is distinguished from other strains by induction of a systemic hypersensitive reaction in bean cultivars possessing the dominant inhibitor gene I. cDNA clones of the NL-3 strain were prepared from purified viral RNA using an oligo dT primer. A clone of approximately 4.5 kb was selected for characterization and the 3'-terminal 1500 nucleotides were determined. The putative NL-3 coat protein sequence and 3' non-coding region were identified. Comparison of the deduced amino acid sequence of the NL-3 strain coat protein to that of another necrosis-inducing strain, NL-8, showed 97% similarity; whereas comparison to that of a non-necrosis inducing strain, NL-4, showed only 67% similarity. The NL-3 strain of BCMV was only distantly related to bean yellow mosaic and clover yellow vein potyviruses.

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COMPARISON OF THE CAPSID PROTEIN CISTRON (CP) FROM TWO SEROLOGICALLY DISTINCT STRAINS OF SWEETPOTATO FEATHERY MOTTLE VIRUS (SPFMV). J. A. Abad and J.W. Moyer. Dept. Plant Pathology, N.C. State Univ., Raleigh, NC 27695-7616.

Complementary DNA to the CP cistrons of SPFMV strains RC and C were cloned, sequenced and compared with published CP sequences of other potyviruses. A single open reading frame followed by a noncoding region and a nearly 100 residues terminal Poly A was identified downstream from a putative potyviral consensus cleavage sequence in both strains. Overall amino acids (aa) alignment of SPFMV-RC and C CP was 84 %. However, the carboxy terminal 117 residues were identical consequently the remaining (amino) region showed less than 66 % identity for both strains. A decreasing tendency of mismatches (49 to 0) was found every 120 nucleotides (nt) in the alignment of both strains. Mismatch tendencies between strains of individual viruses usually occur at low frequency and are uniformly distributed. The 3' noncoding regions of both strains were 98 % homologous confirming they belong to the same virus. The CP cistron sequence of SPFMV is longer than most potyviruses being similar in size to plum pox virus CP, which is 70 % homologous to SPFMV.

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AMPLIFICATION OF MITE- AND FUNGUS-TRANSMITTED POTYVIRAL 3'-TERMINAL FRAGMENTS BY PCR. Nancy L. Robertson and Roy French. USDA, ARS, Department of Plant Pathology, University of Nebraska, Lincoln, Nebraska 68583

From the limited sequence data available it appears that several regions of the polyprotein encoded by potyviruses are highly conserved. A fully-degenerate primer (a 32-mer, including a 5'-Bam HI site) was synthesized based on one such conserved amino acid motif (VCVDDFN) within the putative viral polymerase genes (NIb) of tobacco etch virus, tobacco vein mottle virus, potato virus Y, and plum pox virus. When used in the polymerase chain reaction (PCR) in conjunction with a primer specific for 3'-polyadenylated RNAs, ca. 2 kbp cDNA fragments were amplified from the RNA genomes of several grass-infecting potyviruses. These included hordeum mosaic virus, two strains of the mite-transmitted agropyron mosaic virus, and the fungus-transmitted wheat spindle streak mosaic virus. The PCR products allow rapid cloning of a portion of the NIb and the entire coat protein gene and 3'-noncoding region of these, and likely other, potyviruses for which sequence information is unavailable.

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THE STABILITY OF FOREIGN GENE INSERTIONS IN TOBACCO MOSAIC VIRUS. C. Kearney, J. Donson, and W. Dawson, Dept. Plant Pathology, University of California, Riverside, CA 92521.

The bacterial gene dihydrofolate reductase (DHFR) has been inserted into a modified sequence of tobacco mosaic virus between the 30K and coat protein open reading frames. In vitro transcripts of this sequence were inoculated to Nicotiana benthamiana and then transferred through 10 passages (170 days in planta). cDNA was made to the viral RNA population of the final transfer, amplified by PCR, and cloned into E. coli. Sequencing these clones showed that the DHFR sequence was conserved with only a single point mutation fixed in the DHFR ORF of the viral population (24 of 25 clones) and only 7 of 25 clones having any additional mutations. Other foreign gene inserts are now being examined and their stability compared to native TMV sequence.

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FIGWORT MOSAIC VIRUS (CAULIMOVIRUS) RNA CONTAINS *CIS*-ACTING SEQUENCES WITHIN GENE VI INVOLVED IN *TRANS*ACTIVATION OF UPSTREAM GENES. H.B. Scholthof, F.C. Wu, S. Gowda, and R.J. Shepherd. Dept. of Plant Pathology, Univ. of Kentucky, Lexington KY 40546.

Figwort mosaic virus (FMV) DNA is transcribed into two RNAs. A monocistronic RNA produces the gene VI protein which is a *trans*activator for gene expression of the full-length polycistronic RNA. For these studies a reporter gene (CAT) was fused in frame to the 5' end of the coat protein gene in a partially redundant clone of FMV. Following electroporation into protoplasts, a high level of CAT activity was observed only when gene VI was available to provide the *trans*-acting protein. However, efficient *trans*activation also required *cis*-acting sequences coinciding with gene VI, downstream of the CAT gene. Insertion of a transcriptional terminator between the CAT gene and gene VI strongly reduced the response to *trans*activation. Hence, the *cis*-acting sequences are contained within the RNA of gene VI of the polycistronic transcript. A positive interaction of the gene VI protein with this *cis*-element was also observed for plasmids expressing the CAT gene from the monocistronic gene VI transcript. Apparently the combination of *trans*-acting gene VI protein and the *cis* element enhances the expression of viral transcripts.

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STRUCTURE AND RELATIONSHIPS OF THE SATELLITES OF PANICUM MOSAIC VIRUS. J. Monis, D. S. Sopher, and A. O. Jackson. Department of Plant Pathology, University of California, Berkeley, CA 94720.

Two strains of panicum mosaic virus (PMV) naturally found infecting centipede and Saint Augustine grass, PMV-C and PMV-S, respectively were investigated. These PMV strains are associated with two distinct classes of satellite agents: a satellite virus (826 nt) that encodes its own capsid protein and satellite RNAs (ca. 380 - 440 nt). We have initiated studies to determine the structure-relationships between the helper, the satellite virus and the satellite RNAs. For these investigations, the satellite RNAs from the PMV-S strain (ca. 380 nt) and the PMV-C strain (ca. 380 nt and 440 nt) have been cloned and sequenced. The sequence data indicates that the these satellite RNAs have extensive relatedness at the 5' end of their genomes but no significant relatedness with the satellite virus. Full length transcription cDNA clones of PMV-C (ca. 440 nt) and PMV-S (ca. 380 nt) satellite RNAs were produced. These plasmids produced transcripts of the expected size for PMV-C and PMV-S satellite RNAs. The biological activity of these RNA transcripts are presently being tested. Work is in progress to determine the *cis* acting elements required for the replication and packaging of viral RNAs associated with PMV.

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DETECTION OF PRUNUS NECROTIC RINGSPOT VIRUS IN HERBACEOUS PLANTS AND PRUNUS SPECIES USING A cRNA PROBE. J.M. Crosslin, R.W. Hammond, Microbiology and Plant Pathology Laboratory, and F.A. Hammerschlag, Plant Molecular Biology Laboratory, USDA-ARS, BARC-W, Beltsville, MD 20705

Complementary DNAs (cDNAs) were prepared by random priming of total RNA extracts from nucleoproteins of a peach isolate (PE5) of Prunus necrotic ringspot virus (PNRSV). Recombinant pUC19 plasmids were identified and the inserts were subcloned into pGEM-7fz(+). Labeled cRNA transcripts of a 900 bp fragment designated pJC5-20, were transcribed using SP6 and T7 promoters. The SP6 transcripts hybridized to crude extracts from PNRSV-infected Chenopodium quinoa at dilutions of 10^{-1} to 10^{-2} and in nucleic acid extracts at 10^{-2} to 10^{-3}. Extracts from dormant buds of cherry and peach showed moderate or poor hybridizations, respectively, at 10^{-1}. pJC5-20 readily detected PNRSV serotypes CH9 and CH30. ELISA tests, however, showed very low or negative results with CH30 serotype-infected tissues.

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USE OF ELISA KITS FOR DETECTION OF METALAXYL IN PLANTS. J.W. Pscheidt, J.Z. Burket, and P.B. Hamm*, Dept. of Bot. & Pl. Path., O.S.U., Corvallis, OR 97331-2903, *Box 105, Hermiston, OR 97838.

The effectiveness of new ELISA kits (Millipore) for detection of metalaxyl in plant tissues was evaluated on potted azalea, juniper, and port-orford-cedar. Plants were drenched with labeled rates of metalaxyl. Kits were also tested on detached holly leaves with petioles submerged in metalaxyl for 24 hrs. Leaves and roots of potted plants were collected, weighed, and extracted with methanol before testing with the kits. The concentration of metalaxyl in holly leaves was calculated based on the absorbance at 450 nm. Qualitative differences between drenched and undrenched potted plants were detected. Holly leaves soaked in 0, 80, and 300 ppm of metalaxyl averaged 0.1 (below the 0.3 threshold), 5.2, and 14.4 μg metalaxyl/g fresh wt, respectively. This procedure has the potential to monitor metalaxyl levels in plants and aid retreatment decisions. More information is needed on the level of metalaxyl required for control of Phytophthora diseases in various plant species before kits such as these can be incorporated into an IPM program.

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PHYSICAL CHARACTERISTICS OF SPRAY-APPLIED POLYMER MULCHES AS RELATED TO POTENTIAL MANAGEMENT OF SOILBORNE PLANT DISEASES IN THE SAN JOAQUIN VALLEY. J. J. Stapleton, Statewide IPM Project, University of California, Kearney Ag Center, Parlier, CA 93648.

The versatility of sprayable polymer mulches may facilitate numerous applications in integrated crop management strategies, including control of plant diseases. Microplot experiments were conducted in different soil types during summer 1990 to evaluate physical characteristics of several latex and starch/resin spray mulches in comparison to those of conventional polyethylene films. Under test conditions, some spray mulches were nearly as effective as 4 mil polyethylene film for elevating soil temperature, retaining soil moisture, and reducing numbers of soilborne Pythium spp. by solarization. Performance of spray mulches on sandy loam soil were superior to those on clay loam, due to cracking of the heavier soil which destroyed mulch integrity. Development of biodegradable spray mulches could eliminate disposal problems encountered with polyethylene film.

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EFFECTS OF TILLAGE AND ROW SPACING ON SOYBEAN FOLIAR DISEASES, AND HETERODERA GLYCINES POPULATION DYNAMICS. J.A. Wrather, S.H. Anderson, and N.C. Wollenhaupt, University of Missouri-Delta Center, Portageville, MO. 63873.

Experiments were conducted to determine the effects of two tillage systems and two row spacings on double crop soybean foliar diseases, and H. glycines population dynamics. The two tillage treatments were no-till and subsoiling with a parabolic shank to a depth of 45-cm followed by disking. The two row spacings were 38-cm and 75-cm. At planting, H. glycines cyst populations were similar among all treatments the first year. At the end of the first growing season, H. glycines cyst populations were lower in no-till soybeans. Tillage treatments did not affect H. glycines cyst populations during the second year. Bacterial blight and Septoria brown spot incidence was lower in no-till soybeans. Row spacing did not affect H. glycines populations or the incidence of bacterial blight or Septoria brown spot.

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EFFECTS OF O_3, CO_2, AND VA MYCORRHIZAL FUNGI ON GROWTH OF SUBTERRANEAN CLOVER. M. S. Bamford and S. R. Shafer, Dept. of Plant Pathology, North Carolina State University, Raleigh 27695-7616.

Seeds of Trifolium subterraneum were inoculated with rhizobia and spores of VA mycorrhizal fungi (Glomus etunicatum, Glomus intraradix, or Gigaspora margarita) or microorganisms in spore washings and transplanted into pots of low-P soil. Three-wk-old plants were exposed in greenhouse chambers to factorial combinations of low or high O_3 concentrations (30 or 100 nL L^{-1}; 6 h day^{-1}, 5 days wk^{-1}) and ambient or high CO_2 concentrations (350 or 700 uL L^{-1}; 24 h day^{-1}, 7 days wk^{-1}). Plant dry weights were determined after 8 wk of exposure. G. margarita or G. etunicatum stimulated root and shoot growth, and total dry weights of plants inoculated with these fungi were 51% or 37% greater than controls, respectively. G. intraradix, CO_2, and the O_3 X CO_2 interaction had no effect on dry weights or shoot/root ratios. Ozone increased the shoot/root ratio of plants inoculated with G. margarita or G. etunicatum (1.28 in high O_3, 1.17 in low O_3) but

suppressed shoot/root ratio of plants that were inoculated with G. intraradix or spore washings (1.21 in high O_3, 1.08 in low O_3). Results indicate that O_3 altered biomass allocation of plants that had been inoculated with growth-stimulating VA mycorrhizal fungi.

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PHYTOTOXICITY OF Fusarium ISOLATES FROM JIMSONWEED AND THEIR PHYTOTOXINS FUMONISIN, FUSARIC ACID, AND MONILIFORMIN. Hamed K. Abbas*, R.F. Vesonder; USDA-ARS, Peoria, IL 61604, C.D. Boyette, USDA-ARS-SWSL, Stoneville, MS 38776 and Paul Nelson, Pennsylvania State University, Univ. Park, PA 16802.

Eleven isolates of Fusarium moniliforme were obtained from infected jimsonweed and various crops. These fungi were cultured on autoclaved rice. Phytotoxicity of the isolates was monitored by the ability of the isolate and its phytotoxins to produce necrotic and chlorotic tissue. All isolates produced fumonisin B_1 as the major phytotoxin and caused infection and death to plants. A F. oxysporum isolate from jimsonweed caused necrosis and chlorosis and produced moniliformin at a rate of 2.5 mg/g. Three F. semitectum isolates, one Cephalosporium spp. and one Alternaria crassa isolate produced no phytotoxic effects and none of these phytotoxins were detected in the extracts. Pure fumonisin, fusaric acid, and moniliformin caused similar symptomology to the fungal isolates which produced these compounds.

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EARLY-SEASON ISOLATION OF *CEPHALOSPORIUM GRAMINEUM* FROM ROOTS AND STEMS OF FIELD-GROWN WINTER WHEAT PLANTS. C. M. Stiles and T. D. Murray. Dept. of Plant Pathology, Washington State University, Pullman, WA 99164-6430.

Winter wheat plants removed from field plots between Sep and Mar in 1989 and 1990 were divided into roots and shoots, surface-sterilized and placed on a medium semi-selective for *C. gramineum* (CG). In 1989, CG was first isolated from roots and stems of asymptomatic plants on 30 Oct. Symptoms were apparent by 16 Jan 1990 and CG was isolated from all plants sampled on 20 Feb. In 1990, CG was first detected in roots and stems on 4 Oct and was isolated from all of the plants sampled on 8 Nov. Symptoms were first observed on 19 Nov. Soil population density of CG was greatest in Dec 1989 and Jan 1991 (6.0 and 6.3 log cfu/g, respectively). In both years, a high proportion of plants (47% in 1989 and 100% in 1990) were colonized in Nov, before soil freezing occurred and before pathogen soil populations were greatest. These results suggest that frozen soil is not a prerequisite for infection of plants by *C. gramineum*.

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SEASONAL VARIATION IN SOIL POPULATION DENSITY OF *CEPHALOSPORIUM GRAMINEUM* IN RESPONSE TO SOIL pH. T. D. Murray, Dept. of Plant Pathology, Washington State University, Pullman, WA 99164-6430.

In 1989 and 1990, the population density of *C. gramineum* (CG) was determined from autumn through winter in beds containing soil adjusted to pH 4.5, 5.5, 6.5, and 7.5 (0.01 M $CaCl_2$) and covered with straw naturally-infested by CG. The population density was greatest in Dec. averaging 5.8 and 5.5 log cfu/g dry soil in 1989 and 1990, respectively. Soil pH had a small but significant effect in both years, with population densities 1.5- to 2-fold greater at pH 4.5 than at all other pH values. In 1990, the population density of CG was determined in field plots adjusted to the same pH values, but inoculated with oat kernels colonized by CG. Population density increased from 3.8 to 6.3 log cfu/g between 15 Oct. 1990 and 17 Jan. 1991, then declined. Soil pH had a significant effect, with population densities 8- to 14-fold greater at pH 4.5 than all other pH levels. Increased soil population density may contribute to increased Cephalosporium stripe in low pH soils.

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FORMATION AND SURVIVAL OF SCLEROTIA OF AG-2-2 OF *RHIZOCTONIA SOLANI* ON SUGAR BEET ROOTS. Cheryl A. Engelkes and Carol E. Windels, University of Minnesota, Department of Plant Pathology, St. Paul, 55108 and Northwest Experiment Station, Crookston, 56716.

Sclerotia often form on sugar beet roots infected by *Rhizoctonia solani* AG-2-2, which causes root and crown rot. Factors affecting sclerotial formation and survival were evaluated in the field. Three sugar beet cultivars (≥ 8-wk-old) were inoculated with 17 AG-2-2 cultures isolated from sugar beet and bean crops. Sclerotia formed on 50% of roots of 'Maribo Ultramono' (root rot susceptible) compared to 37% of roots of 'ACH 184' and 24% of 'FC 712' (both root rot tolerant). Sclerotia formed on 50% (n=1536) of beet roots inoculated with AG-2-2 cultures isolated from sugar beet and 29% (n=1728) of beet roots inoculated with AG-2-2 cultures isolated from bean crops. Of 600 buried sclerotia representing three AG-2-2 cultures (isolated from sugar beet), only one sclerotium germinated after 2 yr. Thus, while sclerotia produced on sugar beet are a source of inoculum, they survive for less than 2 yr in soil.

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OCCURRENCE OF *THANATEPHORUS CUCUMERIS* ON TABLE BEETS IN NEW YORK STATE. G. Olaya and G. S. Abawi, Dept. of Plant Pathology, Cornell University, Geneva, NY 14456

Foliar infections incited by *Rhizoctonia solani* Kuhn in table beets resulting in the "pocket rot" disease syndrome have become increasingly important in recent years throughout the table beet production areas. *Thanatephorus cucumeris* (Frank) Donk, teleomorph of *R. solani*, was observed for the first time occurring on infected beets in several commercial fields in 1990. The hymenial layers of *T. cucumeris* appeared as a thin dusty growth on the crown and lower parts of petioles and was white to light brown in color. Basidia and basidiospores typical of *T. cucumeris* were observed under the microscope. Several isolates of *T. cucumeris* were obtained from hymenial layers and from small circular lesions obtained from leaves of infected plants. The existence of *T. cucumeris* under field conditions may explain the recent increase of "pocket rot" epidemics on table beets and the observations of foliar infections of *R. solani* on beans and cabbage in New York State.

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CROWN ROT, A SERIOUS DISEASE OF CANOLA IN THE MIDWEST. D.M. Huber, L.J. Herr, E.P. Christmas and T. S. Roseman. Purdue University, W. Lafayette, IN and Ohio State Univ., Wooster.

A late winter and early spring survey of canola (winter rape) fields in Indiana in 1990 and 1991 showed a plant kill of 0 to 90% depending on cultivar and seeding date. Dead and declining plants had a severe crown rot with varying degrees of cortical necrosis caused by *Rhizoctonia* (cardinal temperatures of 4/16-24/30 C). Root tissues below the crown generally were not infected. The base of leaves frequently had a dark brown to black necrotic lesion that resulted in leaf death. Early seeded plants, which made extensive growth in the fall, were more severely infected than smaller plants. Infection of crown tissues appeared to be by direct penetration near the soil surface as well as through leaf "scars". The winter and spring of 1990 and 1991 were cool with abundant moisture. Crown rot was generally more prevalent in wetter areas of fields but also was severe on early seeded canola on sandy soils. Seeding date and cultivar selection may be important management considerations to reduce severity of this disease.

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EFFECT OF SOIL MATRIC POTENTIAL ON INFECTION OF SWEETPOTATO FIBROUS ROOTS BY *STREPTOMYCES IPOMOEA*. J. B. Ristaino, Department of Plant Pathology, North Carolina State University, Raleigh, NC, 27695.

Sweetpotato plants (cultivar Jewel) were grown in tension funnels in a 3:1 sand-soil mix that was infested with either vermiculite media containing *S. ipomoea* or uninfested media. Soil matric potentials (ψ_m) were adjusted to levels of 0, -1.0, -2.5, -5.0, -7.5, -10.0, or -20.0 kPa. Soil ψ_m had a significant linear effect ($r^2 = 0.76$) on disease severity on fibrous roots. Greatest disease occurred on roots held in soil at ψ_m values < -5.0 kPa. Root and shoot dry weights were reduced by 48-69% and 27-53%, respectively, in comparison to uninfested controls, in soil infested with *S. ipomoea and* held at ψ_m values between -5.0 and -20.0 kPa. In growth chamber experiments, drip irrigations were applied at three frequencies either daily to maintain soil ψ_m above -5.0 kPa, every 4 days, or every 6 days to plants in uninfested or *S. ipomoea*-infested soil. Disease severity increased in fibrous roots in plants given less frequent irrigations. Fibrous root growth was significantly increased with more frequent irrigations and disease severity was reduced, thus suggesting that irrigation may provide a useful management strategy for Streptomyces soil rot in sweetpotato.

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ROOT COLONIZATION OF ALFALFA SEEDLINGS BY *PYTHIUM* SPP. AND ITS EFFECT ON ROOT SYSTEM MORPHOLOGY. R.P. Larkin, J.T. English, and J.D. Mihail; Dept. of Plant Pathology; 108 Waters Hall; University of Missouri; Coloumbia, MO 65211.

Infection of alfalfa seedlings by *Pythium* and *Phytophthora* spp. was monitored in relation to seed or soil treatment with fungicide in the field and in greenhouse experiments. Over 1000 isolates of *Pythium* spp. and related fungi collected from alfalfa roots are being identified to species and representative isolates tested for their effect on root growth and mortality. Root system morphology was characterized by the abundance and length of root orders using the morphometric system. Numerous *Pythium* spp., including *P. ultimum, P. irregulare, P. sylvaticum, P. torulosum, P. spinosum,* and others, were isolated from most plants, regardless of chemical treatment. In preliminary analyses, root system morphology was not influenced significantly by fungicide treatments. Relationships between treatments, root growth, and the temporal succession of *Pythium* spp. will be discussed.

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CHARACTERIZATION OF wts GENES FROM ERWINIA STEWARTII AND THEIR HOMOLOGY WITH THE hrp GENE CLUSTER FROM PSEUDOMONAS SYRINGAE PV. PHASEOLICOLA. R. D. Frederick, D. R. Majerczak, and D. L. Coplin. Dept. of Plant Pathology, The Ohio State University, Columbus OH 43210-1087.

Watersoaking (wts) mutants of Erwinia stewartii are unable to cause lesions and wilting in corn plants. Two overlapping cosmid clones from an E. stewartii genomic library, which complemented ten wts mutants, were genetically mapped by gene replacement mutagenesis using transposons Tn*5* and Tn*3*HoHo*1*. The wts region spanned more than 28 kb and contained many complementation groups. Hybridization of the cloned wts genes to seven subclones from the Pseudomonas syringae pv. phaseolicola hrp region revealed that the two gene clusters contain homologous genetic loci but are arranged differently. wtsA shares nucleotide sequence identity with hrpS, a positive regulatory gene. Expression of a wtsB::lacZ fusion was shown to be dependent upon wtsA function in E. stewartii and rpoN in E. coli. These results suggest that wts and hrp genes have a common function and mode of regulation even though E. stewartii cannot efficiently induce an HR in tobacco.

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

E. c. carotovora strain W3C105, which causes soft rot of potato, produced a catechol siderophore that is similar to enterochelin (Ent), a siderophore produced by many enteric bacteria. In crossfeeding bioassays, the catechol produced by E. c. carotovora provided iron to entA, entC, and entE mutants of E. coli; and enb1 and enb7 mutants of Salmonella typhimurium. These mutants are deficient in various steps of enterochelin biosynthesis but can utilize ferric enterochelin and certain precursors as a source of iron. A cloned 5.6 kb fragment of genomic DNA of E. c. carotovora complemented entA, entC, entD and entE mutants, but not entB mutants of E. coli. The cloned fragment hybridized to the entE gene of E. coli, but not to entA, or entC. A transposon insertion into the 5.6 kb region of the genome resulted in loss of catechol siderophore production of W3C105, as assessed colorimetrically and in crossfeeding bioassays. A second unlinked genomic region of W3C105 complemented an entB mutant, but not entA, entC, entD, or entE mutants of E. coli. At least two genomic regions of strain W3C105 are involved in catechol production.

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ACTIVATION OF TRANSCRIPTION OF *pel-1* WHICH ENCODES AN EXTRACELLULAR PECTATE LYASE IN *ERWINIA CAROTOVORA* SUBSP. *CAROTOVORA* (Ecc) STRAIN 71. H. Murata, T. Souissi, and A.K. Chatterjee; Department of Plant Pathology; 108 Waters Hall; University of Missouri; Columbia, MO 65211.

The *pel-1* gene in the chromosome of a LacZ⁻ derivative of Ecc71 was exchanged with a *pel1-lacZ* operon fusion. The resulting strain, AC5030, did not produce Pel-1, but produced Pel-2, Pel-3, and other extracellular enzymes. While the known inducers of Pel activated *pel-1* transcription, maximal stimulation of transcription occurred with a hot water extract of celery petioles. Also, *pel1-lacZ* expression was higher at later growth stages than during the early exponential growth. In addition, AepA, a gene product known to stimulate the production of secreted enzymes in Ecc71 (Murata *et al*., Molec. Plant-Microbe Interac., in press), activated transcription of *pel-1*. These findings support the hypothesis that stimulation of the production of secreted proteins by AepA results from the activation of transcription of the target genes.

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DNA-MEDIATED TRANSFORMATION OF FRANKIA. B.P. Steele, B. Nielsen, D.B. Steele and J.J.Shaw, Department of Botany and Microbiology, Auburn University, Auburn AL 36849.

Frankia is a soil actinomycete capable of fixing atomospheric nitrogen in the nodules of diverse plants. Transformation of Frankia with DNA has not been previously reported and the lack thereof has constituted a serious block in understanding the molecular biology of the Frankia-plant symbiosis. We report here the successful transformation of Frankia with exogenous DNA. Portions of a Frankia plasmid were cloned into pUC18 and used to transform Frankia strain NPI0136010 by electroporation. Transformants were selected on ampicillin and DNA isolated by mini-screen. The recombinant plasmid DNA from Frankia was rescued by introduction into E. coli.

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E. COLI GALU MUTANT BLOCKS AVIRULENCE GENE D ELICITOR PRODUCTION IN CULTURE. D. Kobayashi[1], M. Stayton[2] and N. Keen[3]. [1]Rutgers U, Dept. Plant Path., New Brunswick, NJ 08903; [2]Dept. Mol. Biol., U of Wyoming, Laramie, WY 82071; [3]Dept. Plant Path., UC Riverside, CA 92521.

The avirulene gene D, originally cloned from Pseudomonas syringae pv. tomato, elicits the defensive hypersensitive response (HR) on soybean cultivars carrying the Rpg4 resistance gene. Overexpression of avrD in Escherichia coli has resulted in the purification of a low molecular weight compound that has been identified as the active elicitor. Elicitor production was tested in E. coli mutant strains that can no longer produce membrane-derived oligosaccharides (MDO), which are involved in osmotic adaptation. Elicitor production, assayed by HR induction on soybean leaves, was not observed by an E. coli galU mutant strain, defective in UDP-glucose production. Elicitor production was observed by an E. coli mdoA mutant strain; however, culture medium color associated with elicitor production was different from the wild-type parent strain. These results suggest UDP-glucose functions in the production of the active elicitor and may also be associated with the MDO pathway in E. coli.

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ANALYSIS OF PLASMID PROFILES OF PLANT GROWTH-PROMOTING RHIZOBACTERIA (PGPR) STRAINS. C.M. Press[1], J.W. Kloepper[1], S. Tuzun[1], and J.J. Shaw[2]. [1]Dept. of Plant Pathology and [2]Dept. of Botany and Microbiology, Auburn University, Alabama 36849.

Previous reports have suggested that plant growth regulators, produced by PGPR, and increased nutrient availability, may be involved in direct growth promotion. Plasmids have frequently been shown to carry genetic determinants necessary for successful plant-bacteria interactions. Plasmid profiles were obtained for nine PGPR strains including the following species: Pseudomonas fluorescens, P. putida, Serratia proteamaculans, Clavibacter sp., and Enterobacter cloacae. All strains previously showed direct growth promotion activity in the absence of visible pathogens. Megaplasmids of at least 200 kb were detected in 3 of the strains. Studies are underway to determine the restriction digest profiles of the megaplasmids and hybridization patterns between the megaplasmids, the chromosomes of various PGPR strains, and other plasmids known to be involved in plant-bacteria interactions (eg. pTi).

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Identification of a Cluster of Genes Involved in Phaseolotoxin Production in Pseudomonas syringae pv. phaseolicola. Y. X. Zhang, K. Rowley, S. S. Patil. Department of Plant Pathology and Biotechnology Program, University of Hawaii, 3050 Maile Way, Honolulu, HI. 96822.

Phaseolotoxin is produced by the bacterium Pseudomonas Syringae pv. phaseolicola, the causal agent of soybean halo blight. Previously, we showed that three heterologous groups of clones isolated from a wild-type genomic library were able to supress 80 EMS-induced mutants and one UV-induced mutant(Tox$^-$). Among them, one clone, pHK120, containing a 23 kb insert, also supressed Tn5 Tox$^-$ mutants, and harbors structural genes for phaseolotoxin production. Restriction mapping and Tn5 mutagenesis resulted in the isolation of over 200 pHK120::Tn5 derivative plasmids which had Tn5 within the insert. Fifty of these, each with the Tn5 located at a unique position, were marker exchanged into the chromosome of the wild-type strain by homologous recombination. The resulting homogenotes were assayed for toxin production. Homogenotes with a Tox$^-$ phenotype were mated with selected pHK120::Tn5 plasmids in a paired-complementation analysis. From these studies, four regions containing genes involved in phaseolotoxin production were identified and subcloned.

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IDENTIFICATION OF PROTEINASE GENES FROM *XANTHOMONAS CAMPESTRIS* PV. *PHASEOLI*. D. K. Fujimoto, J. E. Leach[1], and A. K. Vidaver. Dept. of Plant Pathology, University of Nebraska, Lincoln, NE 68583 and [1]Dept. of Plant Pathology, Kansas State University, Manhattan, KS 66506.

Common blight on dry bean is caused by the bacterium *Xanthomonas campestris* pv. *phaseoli* (*Xcp*). Six putative proteinase-positive clones were identified from a genomic library of *Xcp* through colony blot hybridization using a heterologous serine proteinase gene from *X. c.* pv. *campestris* as a probe. The presence of a proteinase gene on a 7.5 kb fragment contained in all clones was confirmed by DNA blot analysis of plasmids digested with *Bam*HI. By screening for proteinase activity on media amended with 1% skim milk, several library clones that did not hybridize to the serine proteinase gene probe were found to exhibit proteolytic activity. This indicated the presence of at least one other proteinase gene. *Xcp* Tn5 mutants deficient in protease production were created by marker exchange. The growth of, and symptom expression elicited by, the Tn5 mutants were compared to the parental strain *in planta*.

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PARTIAL CHARACTERIZATION OF TWO PATHOGENICITY GENES OF *XANTHOMONAS CAMPESTRIS* PV. *GLYCINES*. I. Hwang, *S. M. Lim, and P. D. Shaw. Department of Plant Pathology and *USDA-ARS, University of Illinois, Urbana, IL 61801.

A genomic clone of *Xanthomonas campestris* pv. *glycines* complemented a nonpathogenic mutant, NP1. Three regions responsible for restoring pathogenicity have been identified by Tn*3*-HoHo1 mutagenesis. Two were in a 2.7 kb *Cla*I fragment and one in a 2.1 kb *Xba*I/*Bam*HI fragment. The 2.7 kb *Cla*I fragment was sequenced, and two possible open reading frames that could encode proteins of about 19 kd (ORF1) and 23 kd (ORF2) were found. A promoterless CAT cassette and *lacZ* fusions in ORF1 and ORF2 indicated that ORF2 but not ORF1 may be expressed in *E. coli* and in *X. campestris* pv. *glycines*. Southern hybridization analysis indicated that DNA sequences in the 10 kb *Hin*dIII fragment are conserved among other *X. campestris* pathovars tested but not in *Pseudomonas syringae* pvs. *glycinea* and *tabaci*. The functions of the two putative polypeptides are unknown; however, the carboxy terminus of the potential polypeptide encoded by ORF2 has an amino acid sequence similar to the gamma subunit of oxaloacetate decarboxylase which is involved in sodium ion transport in *Klebsiella pneumoniae*.

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HOST SYMPTOM AND PATHOGEN POPULATION DEVELOPMENT IN SOYBEAN INOCULATED WITH COMPATIBLE AND INCOMPATIBLE BACTERIA AT DIFFERENT TIMES IN THE CIRCADIAN CYCLE. B. W. Kennedy and R. L. Denny, Department of Plant Pathology, University of Minnesota, St. Paul, MN 55108.

Compatible (*Pseudomonas syringae* pv *glycinea*, Young, Dye & Wilkie) and incompatible (*P. syringae* pv *pisi*) bacteria were introduced into leaves of soybean (*Glycine max* L. cv Sibley) at 6 h intervals in chambers at 25 C and a 12 h light/dark schedule. Symptoms of typical susceptible and resistant reactions, as well as bacterial population counts, were recorded every 6 or 12 h for 0-96 h. In susceptible combinations occurrence of watersoak was faster and population increase was significantly greater during dark than light periods. Results were similar if plants were placed in continuous light following inoculation, indicating a free-running rhythm in disease development. Incompatible reactions were not free-running under similar circumstances and both symptoms of hypersensitivity and bacterial population increases were significantly more pronounced in light.

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TOXINS PRODUCED BY THE DOGWOOD ANTHRACNOSE FUNGUS *Discula* sp. P. Venkatasubbaiah and W. S. Chilton, North Carolina State University, Raleigh, NC 27695.

Several isolates of the dogwood anthracnose fungus *Discula* sp. were found to produce phytotoxins in culture. Four phytotoxic phenols, a novel toxin, 4-hydroxy-3-(3'-methyl-2'-butenyl) benzoic acid (prenylated hydroxybenzoic acid), 4-hydroxybenzoic acid, (+)-6-hydroxymellein and (-)-isosclerone were isolated from the cell-free culture filtrate. Several dogwood collections and nine weed species were used to test the phytotoxicity of the metabolites by leaf bioassay. Prenylated hydroxybenzoic acid and its acetate derivative were consistently the most toxic compounds to dogwoods and different weed species. There was considerable variation in reaction to different toxins among dogwood collections. However, all four toxins and derivatives were non-host specific. All four toxins and derivatives were also tested for their antimicrobial properties by *in vitro* disk assay. Only prenylated hydroxybenzoic acid and its acetate derivative showed antimicrobial properties against most of the bacteria and fungi tested.

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PHYTOTOXIC AND ANTIMICROBIAL PROPERTIES OF THE SECONDARY METABOLITES PRODUCED BY *Ascochyta hyalospora*. P. Venkatasubbaiah and W. S. Chilton, North Carolina State University, Raleigh, NC.

Five isolates of *Ascochyta hyalospora* (Cooke and Ell.) Boerema, Mathur and Neergaard, causal agent of leaf spot and stem necrosis of lambsquaters (*Chenopodium album* L.) were found to produce phytotoxins in culture. Ascochytine was the most abundant toxin isolated from culture filtrate. Other toxins isolated were pyrenolide A, ascochytilide and a novel γ-pyrone. Nine different weed species were used in a leaf bioassay to determine phytotoxicity of different toxins. Pyrenolide A was more toxic than other compounds. Lambsquarters, johnsongrass and sorghum were highly sensitive whereas bentgrass, ragweed and watercress were less sensitive. Ascochytine and pyrenolide A caused marked increase in electrolyte leakage from the lambquaters leaf tissue, inhibited most of the bacterial and fungal growth and also inhibited sorghum seed germination and seedling growth.

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TOXICITY OF THE TERPENOID PHYTOALEXIN DESOXYHEMIGOSSYPOL TO *VERTICILLIUM DAHLIAE* STRAINS: RELATION TO VIRULENCE. M. E. Mace, R. D. Stipanovic, J. Zhang and A. A. Bell. USDA, ARS, Cotton Pathology Research Unit, Route 5, Box 805, College Station, TX 77845

Desoxyhemigossypol (dHG) is a water soluble phytoalexin produced in the stem xylem tissue of cotton infected with *Verticillium dahliae*. Strains of *Verticillium dahliae* from Puhalla's v-c groups P1 (defoliating) and P2 (nondefoliating) were assayed for their sensitivity to dHG, the major phytoalexin in cotton. The quantitative assay is based on the determination of the absorption of fungal mass at 550 nm with a multiwell plate reader. No consistent differences in the sensitivities of the highly virulent P1 and weakly virulent P2 strains were detected. The data indicate that differential sensitivity to dHG is not a factor in the differences in virulence of the P1 and P2 v-c groups of *V. dahliae* strains.

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GLYCEOLLIN ELICITATION IN SOYBEAN HYPOCOTYLS BY HETEROLOGOUS AND HOMOLOGOUS SPP. OF *PHYTOPHTHORA* AND *PYTHIUM*. R. G. Bhat, A. F. Olah, and A. F. Schmitthenner, Dept of Plant Pathology, The Ohio State Univ., Wooster, OH 44691.

Phytophthora megasperma f. sp. *glycinea* (races 1, 3, 4, and avirulent), 21 *Phytophthora* spp., and eight *Pythium* spp. were hypocotyl-inoculated into the near isolines Williams or Williams 79. Glyceollin was determined at 48 hrs post-inoculation; symptomatology and plant kill were rated at 7 days. *Phytophthora* and *Pythium* spp. were grouped into homologous (HO) and heterologous (HE) pathogens of soybean based on plant kill. Cultivars did not differ significantly in glyceollin whereas the cultivar-pathogen interaction was significant. The avirulent *Phytophthora* isolate-cultivar interaction, all HE interactions and HO *Pythium* interactions were not different from compatible interactions. Induction by HE *Phytophthoras* was significantly different from HE *Pythia*. Glyceollin elicitation at 48 hrs appears to be the result of tissue damage and the resulting hypersensitive reaction.

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OUTCROSSING IN *PHYTOPHTHORA MEGASPERMA* F. SP. *GLYCINEA* AND THE INHERITANCE OF VIRULENCE GENES THROUGH THE SEXUAL CYCLE. R. G. Bhat[1], B. A. McBlain[2], and A. F. Schmitthenner[1], [1]Dept of Plant Pathology, [2]Dept of Agronomy, Ohio State Univ., Wooster, OH 44691

Metalaxyl (Mex) resistant and *p*-fluorophenylalanine (Fpa) resistant mutants of *Phytophthora megasperma* f. sp. *glycinea* (*Pmg*) races 1, 3, 4 and avirulent were crossed in nine different combinations using a mixed inoculum technique. Hybrids were selected on a double-inhibitor germination medium containing Mex and Fpa. Virulence of the hybrids and F_2 progeny was evaluated on soybean (*Glycinea max*) cultivars containing specific *Rps/rps* genes with a hypocotyl inoculation method. The genes governing inhibitor-resistance and virulence segregated independently. Race 1 was phenotypically dominant to races 3, 4 and avirulence; race 3 was dominant to avirulence; and race 4 was dominant to race 3. It is proposed that the soybean-*Pmg* system is different from the host-pathogen interactions as predicted by the gene-for-gene hypothesis since the observed virulence phenotypic classes of the progeny were not as expected.

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A *XANTHOMONAS CITRI* PATHOGENICITY GENE, *PTH*A, HAS PLEIOTROPIC AVR FUNCTIONS AND IS SIMILAR IN DNA SEQUENCE TO MEMBERS OF A MULTIGENE FAMILY, WIDELY DISTRIBUTED IN *XANTHOMONAS* SPP. S. Swarup, Y. Yang and D.W. Gabriel. Plant Pathology Department, University of Florida, Gainesville, FL 32611.

A virulence-enhancement approach was used to clone a pathogenicity locus, *pth*A, from the Asiatic citrus canker pathogen *Xanthomonas citri* 3213 by screening a library of its genomic DNA in the opportunistic citrus leaf spot pathogen *X. campestris* pv. *citrumelo* 3048 on grapefruit (*Citrus paradisi* cv. Duncan) leaves. Transconjugants were screened for production of Asiatic canker symptoms. Cloned *pth*A conferred canker-inducing ability to all strains of *X. campestris* tested that were compatible with citrus, including *X.c.* pvs. *alfalfae, citrumelo* and *cyamopsidis*. Marker exchange mutagenesis of *pth*A in *X. citri* 3213 led to a complete loss of both pathogenicity on citrus and the ability to induce a hypersensitive response on the heterologous host bean (*Phaseolus vulgaris* cv. Calif. Lt. Red). Both these functions were complemented by introduction of *pth*A in the *X. citri* mutant. Gene *pth*A could, therefore, be considered a *hrp* gene. Surprisingly, introduction of cloned *pth*A into strains of *X.phaseoli X.c.* pv. *cyamopsidis* rendered them avirulent on their respective hosts, bean and guar. *X.c.* pv. *malvacearum* N containing *pth*A gave gene-for-gene specific avirulence on cotton cv. Acala 44 congenic resistant lines. Gene *pth*A could, therefore, be considered an *avr* gene. Restriction mapping, Southern hybridizations and DNA sequencing to date reveal that *pth*A belongs to a family of pathogenicity/avirulence genes which consists of at least seven *avr* genes from *X.c.* pv. *malvacearum* and two from *X.c.* pv. *vesicatoria*. Gene *pth*A is 4.5 kb in size, has nearly identical 102 bp repeated units in the central region and has at least five homologous fragments in *X. citri* 3213.

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ISOZYME PROFILES OF NITROGEN METABOLISM IN PATHOGENICITY VARIANTS OF *VERTICILLIUM DAHLIAE* KLEB. J. S. Neck and A. A. Bell. Texas Agric. Experiment Station and USDA, ARS, Cotton Pathology Research Unit, College Station, TX 77845.

Tenfold increases in leaf tissue ammonia have been observed to occur when defoliating strains of *Verticillium dahliae* Kleb. infect *Gossypium* sp. Little or no increase in ammonia has been observed with non-defoliating strains of the fungus. Crude extracts of two defoliating and two non-defoliating isolates of *V. dahliae* from liquid culture were separated using horizontal electrophoresis. Screened enzymes included Glutamate-ammonia ligase [E.C. 6.3.1.2], aspartate aminotransferase [E.C. 2.6.1.1], alanine aminotransferase [E.C. 2.6.1.2], and glutamate dehydrogenase (GDH) [E.C. 1.4.1.4, 1.4.1.3, 1.4.1.2]. Different isoforms of the deaminating, NADP-specific GDH were consistently isolated from the defoliating and non-defoliating isolates of the fungus.

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REACTIONS OF CORN INBREDS TO *Bipolaris zeicola* RACES 1, 2 AND 3, AND THE NEW PATHOTYPE. E. J. Traut and H. L. Warren. Dept. of Plant Pathology, Physiology and Weed Science. Virginia Polytechnic Institute and State University, Blacksburg, VA 24061.

Three races and a recently described new pathotype (NP) of *Bipolaris zeicola* (= *Cochliobolus carbonum*) with differential pathogenicity on corn inbred lines have been described with the peculiarity of inducing different lesion shapes. Fourteen corn inbred lines were evaluated for reaction to races 1, 2 and 3 and the NP under greenhouse conditions and to races 1, 2 and 3 under field conditions. In the greenhouse, seedlings were inoculated at the V4-V5 growth stage, whereas in the field plants were inoculated at the V12-V14 stage. Lesion type and severity were assessed 7-9 days after inoculation in the greenhouse and after 10 days in the field. Disease severity was visually estimated on a 1-5 scale. Three inbreds showed resistance to all 3 races and the NP of *B. zeicola*. With limited test, races 1, 2 and 3, and the NP can be distinguished based on lesion types and shapes.

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VIRULENCE AND ORGANIC ACID PRODUCTION BY MUTANTS OF *SCLEROTINIA SCLEROTIORUM*. R. V. Miller, E. J. Ford, D. C. Sands, and C. A. Hertoghe. Department of Plant Pathology, Montana State University, Bozeman, MT 59717.

Stable mutants of *S. sclerotiorum* were generated by exposure to irradiation or chemical mutagens for the expressed purpose of obtaining strains useful for biological control of weeds. Auxotrophic and morphological mutants, including non-sclerotia forming ones, were obtained. These mutants were assayed for hydrolytic enzyme activity, virulence, sclerotia formation, and organic acid production. Preliminary observations failed to demonstrate a correlation between sclerotia formation, acid production, and/or virulence. The data suggest that assays of acid production are not sufficient for extrapolations to virulence.

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EVALUATION OF RESISTANCE TO WHITE RUST OF SPINACH IN FIELD AND GREENHOUSE INOCULATION TESTS. L.P. Brandenberger, J.C. Correll, and *T.E. Morelock. Dept. of Plant Pathology, and *Dept. of Horticulture and Forestry, University of Arkansas, Fayetteville, AR 72701.

Five spinach cultivars were evaluated for resistance to white rust (Albugo occidentalis) in field and greenhouse inoculation tests . Field resistance was evaluated in the spring of 1990 and 1991 by inoculating plants on two successive days and quantifying disease severity (% leaf area diseased) on whole plants over a 4-week period. Greenhouse inoculation tests were conducted on seedlings (2-6 true leaves) at different inoculum concentrations (10^3 to 10^4 spores per ml) and incubation temperatures (10-30 C). Seedlings were scored for disease severity over a 4-week period. Three cultivars, St. Helens, Grandstand, and Hybrid 424 were very susceptible to white rust in field tests, whereas Fall Green and Ozarka II had significantly (P=.001) lower disease severity ratings for all sample dates. These data indicate that both Fall Green and Ozarka II have a relatively high level of quantitative resistance to white rust. In greenhouse inoculation tests, disease severity varied considerably with inoculum concentration and incubation temperature. Measured differences in resistance between susceptible and resistant cultivars were less in the greenhouse then in the field tests.

417 Withdrawn

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EVALUATING FRONTANA WHEAT AS A SOURCE OF RESISTANCE TO LEAF RUST. Z. A. Pretorius, Department of Plant Pathology, University of the Orange Free State, Bloemfontein 9300, South Africa.

The origin of durable resistance to Puccinia recondita f. sp. tritici in several wheat (Triticum aestivum L.) cultivars is often attributed to Frontana. To reconstruct gene combinations conferring durable resistance, characterization of resistance expression and genetic analysis of Frontana should enhance breeding efforts. Primary leaf infection types showed that Frontana resistance could be identified at 10, 15, 20, 25 and 30 C in growth cabinets. Certain isolates, however, were virulent to Frontana seedlings at 15, 20 and 25 C, as well as to adult plants in a greenhouse. Of the Lr genes previously described in Frontana, only Lr13 could be confirmed. Susceptible F_2 seedlings, derived from crosses of Frontana with RL6058 and line 896, suggested that both Lr34 and LrT3 were absent. It appears that Frontana resistance should be thoroughly assessed before extensive breeding programs involving this cultivar are launched.

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QUANTITATIVE HYPERSENSITIVITY-RELATED RESISTANCE TO BACTERIAL SPOT IN TOMATO. J. F. Wang, J. B. Jones, J. W. Scott, and R. E. Stall. Dept. of Plant Pathology, Univ. of Florida, Gainesville, FL 32611.

Hawaii 7998 (H7998) is the only reliable source of resistance in tomato to Xanthomonas campestris pv. vesicatoria (Xcv). The resistance is associated with a hypersensitive (HR), which is temperature sensitive. The growth of strain 75-3 (race 1 of the tomato group) in leaves of H7998 changed from HR to compatible as the temperature was increased from 24 to 30 C. In subsequent testing at 24 C, plants of the F_1 generation of H7998 crossed with a susceptible line, 7060 displayed an intermediate phenotype based on the time to confluent necrosis and growth curves. The F_2 plants of the same cross were rated according to the percentage of necrosis every 8 hrs after infiltration. At each time period, no distinctive groups were observed.

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USE OF PCR TO DISTINGUISH BIOLOGICAL SPECIES WITHIN THE HETEROBASIDION ANNOSUM COMPLEX. T.E. Chase, W.J. Otrosina, P.T. Spieth, and F.W. Cobb, Jr. Dept. of Plant Science, South Dakota State University, Brookings, SD 57007; 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, CA 94720.

The polymerase chain reaction (PCR) technique was used to amplify DNA within the nuclear rDNA repeat of isolates in the Heterobasidion annosum biological species complex. ITS1 and ITS4 were utilized as primers. The study focused primarily on North American isolates of the S and P intersterility groups (ISGs) but also included representative Finnish isolates and Australian isolates of H. araucariae. All isolates yielded an amplification product of ca. 620 base pairs. Digestion of amplified DNA with restriction enzymes revealed several RFLPs diagnostic for North American S and P ISGs and Australian isolates of H. araucariae. These results are entirely consistent with our previous studies based on isozyme analysis. This study suggests that PCR will provide a rapid means to screen large field samples of H. annosum.

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DIFFERENTIATION OF THE WHEAT BUNT FUNGI BY RANDOM AMPLIFICATION OF POLYMORPHIC DNA. Hei Leung, Pat Loomis, and Yunling Shi. Dept. of Plant Pathology, Washington State University, Pullman, WA

The dwarf bunt (Tilletia controversa) and common bunt fungi (T. tritici and T. laevis) of wheat are closely related species with distinct disease cycles. To assess the genetic relationships among the three species, a randomly amplified polymorphic DNA (RAPD) assay (J. Williams et al. Nucl. Acids Res. 18:6531) was used to detect DNA polymorphisms specific to each species. Discrete DNA fragments (2 to 0.2 kb) were amplified from 0.1 ng of genomic DNA using 10-base random primers in a polymerase chain reaction. About 40% of the primers tested showed RAPD patterns that differentiate T. controversa from T. tritici and T. laevis. RAPD patterns of some isolates of T. controversa were identical to those of T. tritici and T. laevis suggesting potential gene flow among Tilletia species. The available markers offer an opportunity to study the population genetics of Tilletia and to develop diagnostic tools for T. controversa.

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DNA FINGERPRINTING OF HETEROBASIDION ANNOSUM CLONES WITH OLIGONUCLEOTIDE PROBES. R. A. DeScenzo and T. C. Harrington, Department of Plant Pathology, Iowa State University, Ames, Iowa, 50011.

Synthesized oligonucleotide probes of $(CAC)_5$ and $(CAT)_5$ were hybridized in gel with total, restricted DNA extracted from field isolates of the pine and fir type of Heterobasidion annosum from California, New York and New Hampshire. A Pst I digest of total genomic DNA with the probe $(CAT)_5$ gave the most useful RFLP pattern. Polymorphic markers segregated in a Mendelian pattern. Fingerprints were highly variable, even among isolates from a single pine stand in New Hampshire. Isolates of a given clone (based on mating-type alleles, isozymes and vegetative compatibility) had identical fingerprints based on banding patterns. Each clone had a unique fingerprint. Oligonucleotide probes for fingerprinting avoid DNA cloning, can be used for a wide range of higher fungi (including Leptographium wageneri and Ceratocystis coerulescens.), and can be used for in gel hybridizations, which avoids blotting.

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GAMETIC DISEQUILIBRIUM AMONG ANONYMOUS, NUCLEAR RFLP LOCI IN A *SEPTORIA TRITICI* POPULATION. B. A. McDonald. Department of Plant Pathology and Microbiology, Texas A&M University, College Station, Texas 77843-2132.

Gametic disequilibrium is the nonrandom association of alleles at different loci. Disequilibrium among 12 nuclear RFLP loci was measured in a sample of 93 isolates of *Septoria tritici* collected from a single field of wheat. Linkage relationships among the 12 loci were determined by DNA hybridization of RFLP probes to Southern blots of intact chromosomes separated by pulsed field gel electrophoresis. DNA fingerprinting and multilocus haplotype analysis showed that 22 different clones were present in the sample of 93 isolates. When all 93 isolates were utilized to measure disequilibrium, significant disequilibrium was found between 50 of the 66 possible pairwise combinations of RFLP loci. When a single representative of each clone was utilized to measure disequilibrium, significant disequilibrium was found between only 9 of the 66 combinations of loci. This result demonstrates that much of the disequilibrium detected in populations of fungi that reproduce asexually may be an artifact caused by repeated sampling of the same clone.

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GENETIC DIVERSITY AT RFLP LOCI IN AN OREGON *SEPTORIA TRITICI* POPULATION. J. M. Boeger and B. A. McDonald. Department of Plant Pathology and Microbiology, Texas A&M University, College Station, TX 77843-2132.

Restriction fragment length polymorphisms (RFLPs) were used to measure genetic variation in a population of *Septoria tritici* sampled from a field containing different wheat cultivars in Corvallis, Oregon. One hundred forty eight isolates were assayed at 10 single-copy RFLP loci. Nei's measure of gene diversity ranged from 0.11 to 0.82 for individual loci, and averaged 0.46 across all loci. DNA fingerprints showed that diversity was distributed on a fine scale. No variation was found among different single-spore isolates taken from the same pycnidium. In 3 out of 4 cases, different genotypes were collected from different pycnidia within the same lesion. In 4 out of 4 cases, different genotypes were collected from different lesions on the same leaf. Many of the alleles found in Corvallis, OR, were identical to those found previously in a California *S. tritici* population located approximately 500 miles to the south.

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PHYTOPHTHORA INFESTANS IN POLAND FROM 1987-1989. NUCLEAR DNA CONTENT, MATING TYPE AND RESPONSE TO METALAXYL. D.L. Bitch, University of Wisconsin-Green Bay, Green Bay, WI 54311-7001. C.D. Therrien, S.S. Daggett and J.H. Sim, Penn State University, University Park, PA 16802. L.S. Sujkowski, Institute for Potato Research, Mlochow, Rosalin, 05-832 Poland.

Isolates of Phytophthora infestans were collected from all potato growing regions of Poland during 1987-1989, and analyzed for ploidy (as determined by nuclear DNA content), mating type and response to metalaxyl. Only the A1 mating type was found among the 1987 isolates. The A2 mating type first appeared in 1988, comprising 4.7% of the population. By 1989 the frequency of the A2 mating type had increased to 47.6%. Coincident with this change in mating type frequency there has been a change in population ploidy. Whereas 3% of the 1988 isolates where diploid, 90% of the 1989 A2 isolates and 28.6% of the 1989 A1 isolates were diploid. The 1:1 ratio of the two mating types, and the predominance of diploidy suggests that the Polish population of P. infestans is becoming sexual. All 1987 isolates were sensitive to metalaxyl. In 1988 and 1989 45.5% and 55.3% of the isolates were sensitive to metalaxyl. The frequency of metalaxyl resistance increased from 25.6% in 1988 to 39.5% in 1989. The percentage of isolates which showed an intermediate response decreased during that period from 27.9% to 5.3%. Moreover, we have observed a significant association between the A1 mating type and metalaxyl resistance. This is probably indicative of the appearance and spread of the mutant allele within the A1 population prior to the introduction of the A2 mating type.

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GENETIC ANALYSIS OF SOYBEAN STRAIN SPECIFIC INCOMPATABILITY WITH RHIZOBIUM FREDII USDA 257. A. T. Trese, Botany Department, Ohio University, Athens, OH 45701.

A fast growing soybean symbiont, Rhizobium fredii, was found in China in 1982. However, many of the advanced U.S. soybean cultivars are ineffectively nodulated by these strains. R. fredii strain USDA 257 nodulates cultivar Peking effectively, but produces tumorous, empty nodules on cultivar Williams. An "avirulence" gene has been isolated from R. fredii 257 that conditions the

incompatabile reaction with cultivar Williams. In this study, segregation analysis of crosses between Williams and Peking, including F_2 and F_3 families, indicate that a single dominant gene is present in Williams that prevents nodulation with R. fredii 257. Thus, a gene for gene interaction exists in the Glycine max - Rhizobium fredii symbiosis.

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THE GENETIC STRUCTURE OF THE *PHYTOPHTHORA INFESTANS* POPULATION IN THE TOLUCA VALLEY AS DETERMINED BY MOLECULAR MARKERS J. M. Matuszak, S. B. Goodwin, W. E. Fry, *M. J. Villarreal-Gonzalez, and *J. Fernandez-Elguezabal. Department of Plant Pathology, 334 Plant Science, Cornell University, Ithaca, NY 14853 and * National Potato Program, INIFAP, Toluca, Mexico .

The Toluca region of Mexico is known to fall within the center of diversity of *Phytophthora infestans*. We have analyzed the genetic structure of the *Phytophthora infestans* population to determine if the population is randomly mating or if there is genetic substructuring. Information on over 250 isolates from 25 locations, in the summer of 1988, are presented. RFLP's revealed by single-copy nuclear DNA probes, a dispersed, moderately repetitive DNA "fingerprint" probe, allozyme markers and mating type are used to analyze the population. Contrary to expectations and a previous study a change in allele frequency and geographic substructuring are indicated.

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SDS PAGE AS A MEANS OF IDENTIFYING DIVERSITY IN COLLETOTRICHUM GLOEOSPORIOIDES. S. Digby and G.J. Weidemann. Department of Plant Pathology, University of Arkansas, Fayetteville, AR 72701.

Fungi assigned to the Colletotrichum gloeosporioides complex, including those with the teleomorphic state, Glomerella, are being examined using SDS polyacrylamide gel electrophoresis (PAGE) of total soluble proteins to assess genetic relationships at both the species and forma speciales level within this diverse species complex. SDS PAGE was used to examine single spore isolates from over 60 collections, obtained from a wide range of hosts and locations. Comparisons of Coomassie Blue stained gels of proteins from a number of single spore isolates obtained from single lesions show excellent protein banding similarity. Protein profiles of isolates obtained from the same host genus show good consistency. Comparisons are also being made between C. gloeosporioides and different species of Colletotrichum from related and identical hosts.

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RELATIONSHIP BETWEEN VEGETATIVE (HETEROKARYON) COMPATIBILITY GROUPS AND RACES OF COLLETOTRICHUM ORBICULARE. **L. Wasilwa, J. C. Correll, and T. E. Morelock. Dept of Horticulture and Forestry, and Dept. of Plant Pathology, University of Arkansas, Fayetteville, AR 72701.**

Over 60 isolates of the cucurbit anthracnose pathogen, Colletotrichum orbiculare, were examined for vegetative compatibility using nitrate nonutilizing (nit) mutants. Isolates were recovered or obtained from throughout the United States from cucumber, watermelon, honey dew, or cucuzzi gourd. At least one isolate from each of the seven described races was included. All isolates examined fell into 10 vegetative compatibility groups (VCGs). The majority of cucumber isolates from the U.S. belonged to a single VCG (VCG 1), whereas the majority of watermelon isolates from throughout the U.S. belonged to a second VCG (VCG 2). Several cucumber isolates belonged to a third VCG (VCG 3). Each of the other VCGs contained fewer than three isolates. A few isolates from VCG 1 and VCG 2 were vegetatively compatible with each other. Isolates representing the seven described races fell into a minimum of five VCGs. VCG 2 contains isolates described as race 2, 6, or 7, and VCG 3 contains three isolates described as race 1. Representative isolates of each of the described races, as well as selected isolates from each of the identified VCGs, are being compared for virulence on differential cucurbit hosts.

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GLOBAL MIGRATION OF *PHYTOPHTHORA INFESTANS*.

S. B. Goodwin and W. E. Fry. Department of Plant Pathology, 334 Plant Science, Cornell University, Ithaca, NY 14853.

Genetic diversity in *Phytophthora infestans* was analyzed in a worldwide culture collection of over 250 isolates. A single genotype predominated (frequency > 50%) in all samples from areas outside of Mexico (ten different countries on five continents) collected prior to about 1980. This genotype was characterized by a unique RFLP pattern when probed with a dispersed, moderately repetitive nuclear DNA probe that has the potential to hybridize to 28 different bands, and a single form (A) of mtDNA. Due to the low probability of the same genotype arising independently in many different environments, this pattern of variation is probably due to migration from a common source, and may represent the primary dispersal of the fungus out of Mexico in the 1840's. Overall genotypic diversity was higher in the United States than in Europe, and this is consistent with the hypothesis of an initial migration from Mexico first into the United States, and subsequently to Europe. These results confirm and expand an earlier report of migration based on allozyme and mating type data.

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REDUCED EFFECTIVENESS OF TRIADIMEFON FOR CONTROLLING CUCURBIT POWDERY MILDEW ASSOCIATED WITH FUNGICIDE RESISTANCE IN *SPHAEROTHECA FULIGINEA*. M. T. McGrath, Dept. of Plant Pathology, Long Island Horticultural Research Lab., Cornell Univ., Riverhead, N.Y. 11901

A field experiment was conducted in 1990 to test an action threshold identified in 1989 for initiating applications of triadimefon to manage cucurbit powdery mildew. In 1989, triadimefon, when applied in combination with chlorothalonil, greatly augmented the control which chlorothalonil provided on upper leaf surfaces by controlling *S. fuliginea* on lower leaf surfaces. In 1990, however, triadimefon provided only minor improvement in powdery mildew control. On 12 September, average powdery mildew severity (% symptomatic leaf area) on upper and lower leaf surfaces was 36% and 39% for nontreated pumpkin, 4% and 24% for pumpkin treated with chlorothalonil, and 2% and 17.5% for pumpkin treated with triadimefon plus chlorothalonil. An isolate collected on 25 September from pumpkin that had been treated with triadimefon plus chlorothalonil was insensitive to triadimefon (Bayleton 50 DF) at 50 ppm, while growth of an isolate collected from nontreated pumpkin was inhibited on triadimefon-treated foliage in growth-chamber trials.

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EFFECTS OF BICARBONATES AND COATING MATERIALS ON CUCURBIT FOLIAR DISEASES. O. Ziv and T. A. Zitter, Department of Field Crops, The Volcani Center, Bet Dagan, Israel, and Department of Plant Pathology, Cornell University, Ithaca, NY 14853.

Sodium, ammonium, and potassium bicarbonates inhibited the growth of four cucurbit pathogens (*Phoma cucurbitacearum*, *Ulocladium cucurbitae*, *Alternaria cucumerina*, and *Colletotrichum orbiculare*) when tested on potato dextrose agar. Fungal growth inhibition was positively correlated with bicarbonate concentrations. No fungal growth was observed for 9 days on media containing 2% (w/v) bicarbonate. Sodium and potassium but not ammonium bicarbonate controlled powdery mildew (*Sphaerotheca fuliginea*) for up to 14 days when applied once to cucumber and pumpkin plants before or after infection. Coating polymers (antitranspirants) also significantly ($P = 0.05$) reduced powdery mildew when applied before infection; better control was achieved when sodium or potassium bicarbonates (1%, w/v) were combined with Sun Spray Oil®(1%, v/v). Sun Oil performed consistently better than did the seven polymers tested. Pre-inoculation treatment of cucumber with each of the three bicarbonates combined with Sun Oil performed better than the bicarbonates or oil alone in reducing infection by *U.cucurbitae*.

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IDENTIY AND PATHOGENICITY OF TWO MARASMIUS SPECIES FROM THE STERILE WHITE BASIDIOMYCETE COMPLEX. R. E. Baird, J. P. Wilson and D. R. Sumner; Department of Plant Pathology, Coastal Plain Expt. Stn., University of Georgia, Tifton, GA 31793

Three fungal isolates with white mycelium, rhizomorphic strands, clamp connections and dolipore septa belonging to the plant pathogenic group known as the Sterile White Basidiomycetes (SWB) were cultured in flasks containing living sweet corn and snap-bean plants grown in vitro. Sporophores of Marasmius graminum and M. rotula were formed in flasks containing the SWB1 isolate. Reisolations from the pilei of both species were identical to the original SWB1, indicating that either this culture was contaminated with both species or the isolate has two teleomorphic states. Pathogenicity tests with the three original isolates and the two sporophore reisolations were conducted on six crops. Greenhouse results indicated that the five isolates were virulent to all six crops, but field results showed fewer significant differences compared to the control. SWB fungi were isolated from all six crops in the greenhouse and field studies.

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Verticillium dahliae: a Causal Agent of Internal Root Discoloration of Horseradish (*Amoracia rusticana*) in Illinois.

D. M. Eastburn, Department of Plant Pathology, University of Illinois, Urbana, IL 61801.

In a two year survey of horseradish fields in Illinois, isolations were made from 201 horseradish roots taken from 10 distinct field locations.

Verticillium dahliae was isolated from 74 percent of horseradish roots with symptoms of vascular discoloration, 37 percent of roots with areas of internal necrosis, and from 56 percent of roots with areas of internal rot. *V. dahliae* was never isolated from asymptomatic roots. Species of Fusarium, also reported as causing internal root discoloration of horseradish, were isolated from 6, 10, 19, and 9 percent of roots with vascular discoloration, internal necrosis, internal rot, and asymptotic roots respectively. Several other species of fungi and bacteria were also isolated, but none of them were consistently associated with specific symptoms.

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Phytophthora capsici strain characterization in southern New Mexico. C. L. Biles, C.M. Liddell, and G.F. Faubion. New Mexico State University, Department of Entomology, Plant Pathology and Weed Science. Las Cruces, NM 88003.

Isolates of Phytophthora capsici from southern New Mexico were tested for fungicide (Ridomil, Copper Sulfate) sensitivity, pathogenicity, growth rate, mating type, and electrophoretic protein profiles. There was a range of growth rates and responses to the fungicides. Mating types (A1 and A2) occurred within the same rows of the field. A range of virulence was also found among the isolates. Electrophoretic protein patterns revealed variation among the isolates. The intimate association between the A1 and A2 mating type provides an opportunity for sexual recombination, and the possibility of shifts in virulence and fungicide resistance. The potential for sexual recombination in the field should be considered when developing breeding programs and control methods for P. capsici in southern New Mexico.

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DISEASE DEVELOPMENT, YIELD LOSS, AND SOURCES OF RESISTANCE TO BLACK LEAF MOLD OF TOMATO. G. L. Hartman and T. C. Wang, Asian Vegetable Research and Development Center, P. O. Box 42, Shanhua, Tainan, Taiwan, 74199, ROC.

Field-grown tomato plants of a commercial cultivar (TN 2) and a breeding line (AVRDC CL 5915) were inoculated with Pseudocercospora fuligena (Roldan) Dieghton at several time different intervals or were fungicide-protected. The area under the disease progress curve (AUDPC) was significantly higher for CL 5915 than for TN 2, although the reduction in fruit weight was not significantly different between the two entries. Inoculated plants had 32% less fruit weight than plants grown in fungicide-protected plots. Inoculated CL 5915 plants had 11% less fruit and 20% less weight per fruit than plants in fungicide-protected plots; inoculated TN 2 plant had 28% less fruit and 7% less weight per fruit. A significant negative correlation occurred between yield parameters (t/ha, fruit/ha, and weight/fruit) and the AUDPC. A total of 541 accessions of Lycopersicon species were screened for resistance to black leaf mold under greenhouse and field conditions. Fourteen accessions representing L. glandifolia (1), L. peruvianum (4), L. hirsutum (5), and L. esculentum (4) had high levels of resistance.

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THE ROLE OF *MONOSPORASCUS CANNONBALLUS* IN A ROOT ROT-VINE DECLINE DISEASE OF MUSKMELON. J. C. Mertely, R. D. Martyn, and M. E. Miller, Department of Plant Pathology & Microbiology, Texas A&M University, College Station 77843 and Weslaco 78596, and B. D. Bruton, USDA,ARS,SCARL, Lane, OK 74555.

A disease of muskmelon (*Cucumis melo* L.) occurred in Texas in 1986 and has persisted through the 1990 crop season. Primary symptoms occur on roots and include extensive necrosis, vascular discoloration, and discrete, cortical lesions. Above ground, the disease is manisfested as a vine decline, characterized by dieback of older crown leaves which progresses rapidly to young, distal leaves near harvest. Four fungi (*Fusarium solani, Monosporascus cannonballus, Macrophomina phaseolina,* and *Stagonospora sp.*) were frequently isolated from diseased plants. In green house pathogenicity tests, *M. cannonballus* and *M. phaseolina* caused significant levels of root rot and reduced root weights of inoculated plants by 29 and 26%, respectively. *M. cannonballus* also caused significant reductions in vine length and formed perithecia on roots. Based on radial growth on PDA, optimum temperature for vegetative growth was 35 C; however, maximum number of fertile perithecia formed at 25-30 C. The pH optimum was 6-7, although substantial growth occurred at pH 9 but not at pH 4. This is the first report of *M. cannonballus* in Texas. The only other reports of this fungus are from Arizona, India, and Japan.

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STRUCTURE OF THE GLYCOPROTEIN AND SCRNA-4 GENES OF SONCHUS YELLOW NET VIRUS. K.-B. Goldberg, B. Modrell, L. A. Heaton, B. I. Hillman, B. G. Hunter, and A. O. Jackson. Department of Plant Pathology, University of California, Berkeley, 94720.

Sonchus yellow net virus (SYNV) is a plant rhabdovirus that undergoes replication in the nucleus and buds through the inner nuclear membrane into the perinuclear space. SYNV produces six poly(A)' mRNAs from a minus sense RNA genome. The scRNA-4 and G genes cistron are bordered by a consensus gene junction sequence that is common to all the SYNV genes, and both have a polyadenylation signal (AAUAAA) at the 3' mRNA terminus. The unusual sixth RNA, the scRNA-4, has the potential to encode a 36,400 dalton protein. This protein has not been detected in virion preparations and is presumed to be a nonstructural protein. Interesting features on this protein, as determined by sequencing data, are glycosylation signals (Asn-X-Thr/Ser) and an acid protease site (Val-Asp-Thr-Gly). Sequence analyses revealed that the open reading frame of the G gene codes for a 70,215 dalton protein; the 77,000 dalton native protein can be accounted for by glycosylation. In addition to glycosylation signals, the G protein contains a signal sequence, transmembrane anchor domain and a nuclear localization signal.

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GENOMIC ORGANIZATION OF THE IMPATIENS NECROTIC SPOT VIRUS M RNA. M.D. Law, J. Speck, and J.W. Moyer, NC State University, Raleigh, NC 27695-7616.

Impatiens Necrotic Spot Virus (INSV) is the type member of a distinct serogroup within the newly proposed Tospovirus genus of the Bunyaviridae. The nucleotide sequence of the M RNA was determined from cDNA clones which had been synthesized sequentially by primer extension from viral RNA. The clones were verified by hybridization with healthy and infected tissue blots and northern blots of INSV RNA. The INSV M RNA possessed the 8 nucleotide (nt) conserved terminal sequence found in both the INSV S RNA and in TSWV RNA's. The M RNA contained 2 open reading frames (ORFs). The large ORF located near the 3' end of the viral sense RNA was found in the viral complementary sense and encoded a 1110 amino acid protein with a predicted molecular weight of 125 KD. This ORF is capable of encoding the G1 and G2 proteins. The second ORF is capable of encoding a 303 amino acid protein with a predicted molecular weight of 34 KD. The two ORFs are separated by an A-T rich region of 450 nt which may have secondary structure. This is the first report of a second ORF in the M RNA of a Tospovirus.

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DIFFERENCES IN THE N PROTEIN NUCLEOTIDE SEQUENCE BETWEEN TOSPOVIRUS SEROGROUPS. M.D. Law, J. Speck and J.W. Moyer, NC State University, Department of Plant Pathology, Raleigh, NC.

Impatiens Necrotic Spot Virus (INSV), formerly tomato spotted wilt virus-I, is the type member of a distinct serogroup within the newly proposed Bunyaviridae genus, Tospovirus. The INSV N protein was found to be serologically distinct from the TSWV N protein which is the basis for the establishment of serogroups within genera (Law & Moyer, JGV 71, 933-938). The identity and orientation of the INSV N protein ORF cDNA clones were confirmed by in vitro transcription and translation. The INSV N protein, like that of TSWV, is encoded by a viral complementary sense, sub-genomic mRNA from the S RNA. The initiation codon for the INSV N protein is 150 bases from the 3' terminus while the termination codon is 935 bases from the terminus. This region encodes a 262 amino acid protein with a predicted molecular weight of 28.8 KD. Comparison of the INSV and TSWV N sequence showed 59% identity at the nucleotide level and 67% identity at the amino acid level. The 3' terminal region of the INSV S RNA is composed of an 8 nucleotide conserved sequence that is identical to the TSWV S RNA terminal sequence. An A-T rich intergenic region immediately follows the INSV N protein ORF.

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ESTABLISHING PRIMARY CELL CULTURES FROM THRIPS VECTORS OF TOMATO SPOTTED WILT VIRUS. H. T. Hsu, H. Hibino[1], T. Murai[2], and T. Omura[1], USDA-ARS, Beltsville, Maryland USA, [1]MAFF-NARC, Ibaraki, Japan and [2]Shimane Agr. Exp. Sta., Shimane, Japan.

Primary cell cultures were obtained from embryonic eggs of Thrips tabaci by culturing in a leafhopper medium (Proc. Am. Phytopathol. Soc. 3:234-235). One to two day old eggs were collected from T. tabaci that had fed on a mixture of tea and pine pollens and a 10% honey solution (Jap. J. Appl. Ent. Zool. 26:149-154). After additional 4 day incubation at 21C, eggs were surface sterilized with 70% ethanol, rinsed in Tyrode's solution, and crushed in Tyrode's solution containing 0.25% trypsin. After a 10 min trypsinization, culture media were added. Tissue fragments/cells were pelleted by centrifugation, resuspended in a small amount of fresh media and seeded on coverslips in a petri dish. Media were replenished every 4-5 days by replacing one half of spent media with an equal volume of fresh ones. Growth of fibroblasts and epithelial cells were observed around the peripheries of tissue fragments.

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RAPID DETECTION OF APPLE FRUIT MARKING VIROIDS BY DOT-BLOT HYBRIDIZATION USING A cRNA PROBE. L. J. Skrzeczkowski, G. I. Mink, and W. E. Howell. Washington State University, Irrigated Agriculture Research and Extension Center, Prosser, WA 99350.

A SP-6 generated [^{32}P]-cRNA probe was produced from PVAS 14-4 plasmid (provided by A. Hadidi, NPGQL, USDA, Beltsville, MD) which contained a 274 base cDNA copy of apple scar skin viroid (ASSV). The probe was used to detect viroids in two cultivars of greenhouse and growth chamber-grown maiden apple trees bud-inoculated with three sources that induced scar skin or dapple apple symptoms on fruit of field grown Red Delicious trees. Tissue samples were extracted in 0.2 M KH_2PO_4; 0.1% Triton X-100; 5mM dithiotreitol; 10mM 2-mercaptoethanol, denatured in 1.8 M NaCl, 0.18 M Na-citrate and 15% formaldehyde for 30 min at 60°C, blotted on nylon membrane and hybridized with [^{32}P]-ASSV cRNA probe. Extracts of leaf midribs and petioles produced stronger reactions than leaf lamella. Viroids were detected in petioles from all leaf positions on plants grown at constant 18°C but mostly from the expanded leaf positions of plants grown at 28°C.

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CLONING, PARTIAL NUCLEOTIDE SEQUENCE, AND COAT PROTEIN CONSTRUCTS FOR THE CARLAVIRUS OF SHEEP PEN HILL DISEASE OF BLUEBERRIES. B.I.Hillman, T.D.Cavileer, and B.T.Halpern. Dept. of Plant Pathology, Rutgers University, New Brunswick, NJ 08903.

Complementary DNA clones representing the 8.5 kb genome of the Carlavirus associated with Sheep Pen Hill disease (SPHDAV) of blueberries in New Jersey, similar to the Carlavirus that causes blueberry scorch disease in the Pacific Northwest, have been mapped. Approximately one half of the viral sequence, including both termini, has been analyzed. The SPHDAV sequence indicates that this virus is similar in genetic organization to potato virus M, and shares considerable similarity at both the 5' and the 3' termini. The viral coat protein gene has been successfully expressed from the bacterial expression vector pUR292 and has been subcloned into the plant expression vectors pMJD80, which contains the TMV leader sequence interjected as a translational amplifyer between the CaMV 35S promotor and the coat protein gene, and pBI121, which also contains the ß-glucuronidase (GUS) gene and T-DNA border sequences for stable introduction into plants.

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PROGRESS TOWARDS COAT PROTEIN MEDIATED RESISTANCE TO CASSAVA COMMON MOSAIC VIRUS IN CASSAVA. C. Fauquet[1], D. Bogusz[1], C. Franche[1], C. Schopke[1], P. Chavarriaga[1], L. Calvert[2] and R.N. Beachy[1]. 1: Washington University, St. Louis, Mo. 2: CIAT, Cali, Colombia.

Several strains of cassava common mosaic potexvirus (CCMV) have been isolated from different countries, and a cDNA containing the coding region of the coat protein (CP) from the Brazilian strain, has been sequenced. *Nicotiana benthamiana*, a host for CCMV, has been transformed with *Agrobacterium tumefaciens* containing different constructs made with the CCMV CP cDNA and many different lines have been regenerated. Many lines accumulated CP at a very high level, up to 1.6% of the total soluble protein, and are highly resistant to CCMV. The type and level of resistance of some of these lines has been evaluated and will be described. Other strains of CCMV as well as other potexviruses have been inoculated to one resistant line and results of these experiments will be reported. One construct has been succesfully expressed in transformed cassava calli, at a very high level. Regeneration of cassava is only possible through somatic embryogenesis and though it is difficult to transform embryos, chimeric cassava embryos expressing the GUS gene have been produced and expression of the CCMV CP in cassava embryos in underway.

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PORTAL OF ENTRY OF XANTHOMONAS CAMPESTRIS PV. HOLCICOLA INTO GRAIN SORGHUM PLANTS. P. Zvoutete, L. E. Claflin, and B. A. Ramundo, Department of Plant Pathology, Kansas State Univ., Manhattan, KS 66506-5502.

The mode of entry into grain sorghum (Sorghum bicolor) by Xanthomonas campestris pv. holcicola was determined by scanning electron microscopy (SEM). Wounded and healthy leaf tissue of 3-wk-old plants were mist-inoculated with a suspension of X. c. holcicola (strain KS 66) and examined by SEM 1, 2, 3, 5, 7 and 9 days after inoculation. Three days after inoculation, bacteria were observed predominately in and around stomates and wounds with evidence of bacterial lysis in "unprotected" sites. Small water-soaked lesions were evident 5 days after inoculation and vein-limited lesions became necrotic after 7 days. All plants wounded prior to inoculation developed symptoms whereas 20-30% of the healthy plants exhibited symptoms. X. c. holcicola enters sorghum plants via stomates and wounds; with preference for wound ingress.

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SYSTEMIC COLONIZATION OF GRAIN SORGHUM PLANTS BY XANTHOMONAS CAMPESTRIS PV. HOLCICOLA. J. Raghava Reddy, L. E. Claflin, and B. A. Ramundo, Department of Plant Pathology, Kansas State University, Manhattan, KS 66506-5502.

Two-week-old sorghum plants (Cv. 80B3039) were inoculated by injuring the roots with a knife and then pouring 10 ml of a streptomycin resistant suspension (1.3 X 10^9 colony forming units/ml) of Xanthomonas campestris pv. holcicola onto the soil surface. Tissue from inoculated and healthy plants was removed at monthly intervals and examined for X. c. holcicola by plating on NBY and MXP media amended with 100 µg/ml of streptomycin and by direct immunofluorescent staining (IFS). Cells of X. c. holcicola were observed by IFS and recovered from root, stem, and leaf tissue from inoculated plants although bacterial leaf streak symptoms were not observed. Bacteria were not recovered from non-inoculated plants. X. c. holcicola is systemic in sorghum plants and is probably translocated in the xylem tissue. Recovery of cells of X. c. holcicola was highest from stem and lowest from root tissue.

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INTERACTION OF *XANTHOMONAS ORYZAE* PV. *ORYZAE* RACES IN FIELD EXPERIMENTS. P.D. Roberts, T.W. Mew, and A.A. Alvarez. International Rice Research Institute, PI, and University of Hawaii, HI.

Xanthomonas oryzae pv. *oryzae* races 1 and 2, marked by antibiotic resistance, were inoculated alone and mixed onto the susceptible rice cultivar IR56 in field experiments. Incidence and spatial spread of the disease were recorded. The infecting strain from a symptomatic leaf from each infected hill was determined by recovery on antibiotic media and reactivity to monoclonal antibodies. No difference was found in disease progress of single and mixed treatments. Race 1 was always recovered more frequently than race 2 from mixed inoculated plots. In a separate study, race 1 exhibited a suppressive effect on the growth of race 2 in mixed inoculations on IR56. Race 1 appears to more fit than race 2 on rice cultivars carrying no resistance genes.

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CORRELATION BETWEEN SUSCEPTIBILITY OF POTATO CULTIVARS TO *STREPTOMYCES SCABIES* AND SENSITIVITY TO THAXTOMIN. L. M. Delserone, R. Loria and I. Arias, Dept. of Plant Pathology, Cornell University, Ithaca, NY 14853.

Phytotoxins, thaxtomin A and B (Phytopath. 80:606-608), may be determinants of pathogenicity in *Streptomyces scabies*. Our hypothesis is that, if thaxtomins are important in pathogenesis, a correlation should exist between the characterized susceptibility of potato cultivars to *S. scabies* and sensitivity to thaxtomin in vitro. Two-week-old tubers, grown on greenhouse-produced stem cuttings from cultivars with different levels of scab resistance, were exposed to thaxtomin A and assessed for degree of necrosis. Cultivars susceptible to *S. scabies* developed extensive necrosis, which in some cases extended into the vascular tissue of the tuber. Resistant cultivars were tolerant of the toxin, developing necrotic flecks around the lenticels, with no evidence of damage in the vascular ring. The results lend additional support to the role of thaxtomins in the pathogenicity of *S. scabies*.

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A LEAF SPOT OF PEARL MILLET CAUSED BY PSEUDOMONAS SYRINGAE. S. G. Jensen, P. Lambrecht, G. N. Odvody, and A. K. Vidaver, USDA-ARS, Texas A&M Univ. and University of Nebraska, Lincoln, NE 68583.

A bacterial pathogen was isolated from diseased leaves of pearl millet (Pennisetum americanum). In greenhouse studies the pathogen also caused severe leaf spotting symptoms on sorghum (Sorghum bicolor) and maize (Zea mays). The gram-negative bacterium produces fluorescing, opaque, colorless, smooth margined, domed colonies. Fatty acid methyl ester analysis, levan production, DNA genomic analysis, hypersensitive reaction on tobacco, and screening with the Biologs panel of tests all identify the pathogen as Pseudomonas syringae. The damaging disease, which appears following hard rains, is manifest by watersoaked spots that expand to form oval to elongate tan necrotic lesions with a thin dark brown margin.

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PLASMID-MEDIATED STREPTOMYCIN RESISTANCE IN ERWINIA AMYLOVORA IN MICHIGAN. C.-S. Chiou and A. L. JONES. Department of Botany and Plant Pathology and Pesticide Research Center, Michigan State University, East Lansing 48824.

Streptomycin-resistant Erwinia amylovora were isolated in 1990 from apple trees in Michigan. In colony blot hybridizations, a portion of the streptomycin-resistance gene (probe SMP3) from strain Psp36 of Pseudomonas syringae pv. papulans hybridized with all streptomycin-resistant strains of E. amylovora but not with streptomycin-sensitive strains. Probe SMP3 hybridized to 2.7-kb AvaI restriction fragments from total genomic DNA and from plasmid DNA of two resistant strains of E. amylovora and to a 1.5-kb fragment in DNAs from strain Psp36 of P. s. papulans. The probe did not hybridize with digested DNA from sensitive strains. Streptomycin resistance and a 33-kb plasmid were co-transferred in matings of two streptomycin-resistant donor strains with four streptomycin-sensitive recipient strains of E. amylovora. The gene for streptomycin resistance in E. amylovora is being characterized.

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EFFECT OF FREEZING ON DEVELOPMENT OF BACTERIAL CANKER OF SWEET CHERRY. P. Sobiczewski and A. L. Jones, Dept. of Botany and Plant Pathology and Pesticide Research Center; and J. A. Flore, Dept. of Horticulture; Michigan State Univ., East Lansing 48824.

Dormant 1-yr-old shoots of two sweet cherry cultivars were inoculated with Pseudomonas syringae pv. syringae (Pss) or P. s. morsprunorum (Psm), then sequentially incubated at 15, -10, and 15 C for 7, 1.5, and 10 days, respectively. Necrosis was significantly greater on these shoots than on shoots maintained at 15 C for 18.5 days. Inoculations with Pss gave significantly greater necrosis then inoculations with Psm. Multiplication and movement of bacteria in shoots prior to freezing was greater for Pss than for Psm. Susceptibility of shoots of Hedelfingen decreased, while those of Gold increased, as the degree of their dormancy increased. Susceptibility to Pss and freezing of 13 cultivars in deep dormancy was (in decreasing order): Napoleon, Emperor Francis, Gold, Nelson, Ulster, Sam, Vega, Windsor, Schmidt, Hedelfingen, Valera, Vic, and Viva. Ulster, Vega, and Napoleon were most susceptible to Psm.

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EVALUATION OF CALYX TISSUES OF SEVERAL APPLE CULTIVARS FOR THE PRESENCE OF ERWINIA AMYLOVORA. T. van der Zwet, E.W. Brown, and J.M. Wells. USDA, ARS, Appalachian Fruit Research Station, Kearneysville, WV 25430; Shepherd College, Shepherdstown, WV 25443; and USDA, ARS, ERRC, Philadelphia, PA 19118

Fruit of 6 apple cultivars (Empire, Granny Smith, Jonathan, Rome Beauty, Yellow Delicious, York) susceptible to fire blight were harvested in early October and stored at 1 C. From the beginning of storage to early February, pistils and stamen of each cultivar were dissected and plated on nutrient yeast dextrose agar (NYDA) plates at 5 different times. Three of these times, the surface of each fruit was also washed in sterile saline in a plastic bag and the wash plated on NYDA. In January, the calyx tissues without sepals were also removed with a cork borer, placed in a test tube with 2.5 ml phosphate buffer, sonicated for 30 sec, and 0.1 ml of sonicate was plated on NYDA. From all 5 plating dates, a total of 37 bacterial isolates resembling E. amylovora were recovered. Pathogenicity tests on pear seedlings and comparison with the fatty acid library proved that none of the isolates were E. amylovora.

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EFFECTS OF PRUNING ON YIELD AND DISEASE DEVELOPMENT IN TOMATO PLANTS SUPPORTING EPIPHYTIC POPULATIONS OF CLAVIBACTER MICHIGANENSIS SUBSP. MICHIGANENSIS. W. M. Carlton, M. L. Gleason, and E. J. Braun. Dept. of Plant Pathology, Iowa State University, Ames, Iowa, 50011.

In a field experiment, 8-wk-old caged tomato transplants (cv. 'Jet Star') were either spray-inoculated with a suspension of 10^4 cfu/ml of Clavibacter michiganensis subsp. michiganensis or left unsprayed. Two weeks after inoculation, these treatments were further split into plots in which suckers below the first flower cluster were either hand-pruned or left unpruned. Percentage defoliation and percentage stem tissue colonized by the pathogen were rated periodically. Mature fruits were harvested weekly. Pruning had no significant (P=0.05) effect on yield in either the inoculated or the uninoculated treatments. Disease symptoms developed more rapidly in the pruned-inoculated treatment than in the nonpruned-inoculated treatment. Yield was significantly less and disease incidence was significantly greater in inoculated treatments than in uninoculated controls.

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PATHOGENIC VARIABILITY IN ISOLATES OF *XANTHOMONAS CAMPESTRIS* PV. *GLYCINES*. I. Hwang, *S. M. Lim, and G. B. Lee. Department of Plant Pathology and *USDA-ARS, University of Illinois, Urbana, IL 61801.

Nine isolates of *Xanthomonas campestris* pv. *glycines* obtained from different geographical regions were evaluated in the field for their pathogenic variability on eleven soybean cultivars. The experiments were carried out over 3 years by inoculating the cultivars with individual isolates. Reactions of the cultivars to five of the nine isolates were identical but differed among the other four isolates. One of the four isolates was virulent on 'Williams' which developed typical bacterial pustules. This is a first report of an isolate of *X. campestris* pv. *glycines* virulent on the soybean cultivars carrying the gene *rxp* for bacterial pustule resistance. Based on different reactions of the cultivars, the isolates were classified and designated as races 1, 2, 3, 4, and 5. Five cultivars, 'Chippewa', 'Harosoy', 'Mukden', 'Pella', and 'Williams', were selected to differentiate these five races.

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SEEDBORNE ROLE AND LONGEVITY OF XANTHOMONAS CAMPESTRIS PV. HOLCICOLA IN GRAIN SORGHUM. P. Zvoutete, L. E. Claflin, and B. A. Ramundo, Department of Plant Pathology, Kansas State Univ., Manhattan, KS 66506-5502.

Xanthomonas campestris pv. holcicola, causal agent of bacterial streak of sorghum (Sorghum bicolor), was found on and inside the seed coat of grain sorghum. X. c. holcicola was isolated from surface sterilized and unsterilized seeds harvested from inoculated sorghum plants, as well as from leaf, lemma, and palea tissues. Recovery of cells of X. c. holcicola in the seed decreased gradually from 8.4×10^5 colony forming units (CFU) at harvest to 4.4×10^3 CFU after 24 mo in storage. Population of X. c. holcicola decreased from 8.8 and 4.1×10^6 CFU at harvest to 5.0 and 3.3×10^3 CFU for leaf and glume/lemma/palea tissue, respectively, after 24 mo storage in an unheated building.

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SPATIAL SCALE OF VARIATION OF THE BACTERIAL BLIGHT PATHOGEN OF RICE. R. Nelson, E.Y. Ardales, C.M. Vera Cruz and T.W. Mew. International Rice Research Institute, Los Banos, Philippines.

Previous studies aimed at assessing the genetic variability of *Xanthomonas oryzae* pv. *oryzae*, the bacterial blight pathogen of rice, have made use of strains collected from around the Philippines over a period of several years. In these studies, substantial variation was observed both within and between pathogenic races. To determine the spatial scale of pathogen variation, intensive sampling was conducted in farmers' fields at six sites. For each of four to five fields at each site, five symptomatic leaves (if present) were sampled from each of seven 1-m^2 areas in a W-walk. DNA types were determined using *Pst*I analysis for all sites, and by RFLP analysis using the probe pJEL101 at two sites. Pathogenic race was determined by testing on differential cultivars. For five of the six sites analyzed, a single race was present, while two races were present at one of the sites. For five of the six sites, a high degree of variability was observed at the DNA level, with multiple DNA types observed within a field. More than one DNA type was observed within many of the 1-m^2 areas sampled. These observations indicate that populations of *Xanthomonas oryzae* pv. *oryzae* can be very diverse in individual fields under natural conditions, and have implications for the design of sampling methods.

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DEVELOPMENT OF SEED ASSAYS FOR DETECTION OF PSEUDOMONAS SYRINGAE PV. GLYCINEA IN SOYBEAN SEED. Elizabeth Alvarez, D. C. McGee and E. J. Braun, Department of Plant Pathology, and Seed Science Center, Iowa State University, Ames, Iowa 50011.

Methods were developed to detect Pseudomonas syringae pv. glycinea (Psg), the causal agent of bacterial blight, in soybean seed. In one method seeds were washed in running tap water for 1 hr, then plated directly on King's B medium amended with cephalexin at 40 mg/l (KBC). After growth at 25 C

for 3 days in the dark, colonies with characteristic light-blue fluorescence of Psg were further tested by agglutination with antisera specific to Psg, and by lesion development on inoculated leaves of 20-day-old soybean seedlings, grown in the greenhouse. In the second method, unwashed seeds were ground in 0.85% saline solution and serially diluted on KBC. Presumptive colonies of Psg were tested as described in the first method. Both methods proved to be more rapid and sensitive than existing assays for this pathogen.

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CHARACTERIZATION OF PHENOLATE IRON-BINDING COMPOUND(S) PRODUCED BY GLOEOPHYLLUM TRABEUM. V. Chandhoke, J. Jellison, and F. Fekete. University of Maine, Orono, ME 04469.

Iron-binding compounds produced by brown-rot fungus G. trabeum were isolated and purified by reverse phase HPLC system using a methanol gradient. The analytical chemical characterization of one of the purified compound(s) was performed by GC/MS, NMR, and IR to confirm their phenolic nature. The electron impact mass spectroscopy studies of the purified fraction resulted in generation of ions 137 (base peak), 122, 94, 77, 51, and 210 (molecular ions). This fragmentation pattern shows the presence of aromatic ring with hydroxyl functionalities. The hydroxyl substitution pattern on the ring structure was determined by 1H NMR, and confirmed in subsequent IR studies. These studies show the presence of di-hydroxyl substituted phenolate moeity with high affinity for iron. Besides their role of transporting iron to the cell, these compounds may play a direct role in the process of lignocellulose degradation, and fungal pathology.

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EFFECTS OF CARBON SOURCE ON MACROCONIDIUM GERMINATION AND SPORE ATTACHMENT TO ROOT SURFACES BY *FUSARIUM SOLANI* F. SP. *PHASEOLI* IN HYDROPONIC NUTRIENT SOLUTION. A. C. Schuerger, D. T. Kaplan, and D. J. Mitchell. The Land, EPCOT Center, Lake Buena Vista, FL 32830, U. S. Department of Agriculture, ARS, Orlando, FL 32803, and Dept. of Plant Pathology, University of Florida, Gainesville, FL 32611.

Macroconidia of *Fusarium solani* f. sp. *phaseoli* attached immediately to roots of *Vigna radiata* when plants were inoculated in hydroponic nutrient solutions maintained at 25 C and pH 5.5. Asparagine, fucose, glucose, galactose, mannose, N-acetyl-D-glucosamine, N-acetyl-D-galactosamine, N-acetylneuraminic acid, and sucrose failed to inhibit spore attachment to roots at concentrations of 50 and 100 mM. Germination differed significantly between in situ processes on plant roots in nutrient solution and in vitro processes in 50 mM solutions of individual carbon sources. Macroconidia germinated primarily from terminal and foot cells when attached to roots, but primarily from lateral walls of intercalary cells during in vitro tests in individual carbon sources. Observations did not support the hypothesis that lectins were involved in spore attachment to roots.

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A MUTATION IN *XANTHOMONAS CAMPESTRIS* PV. *CITRUMELO* AFFECTS BOTH HOST-SPECIFIC-VIRULENCE AND EXOPOLYSACCHARIDE PRODUCTION. M.T. Kingsley and D.W. Gabriel. Dept. Plant Pathol., Univ. of Florida, Gainesville, FL 32611.

Xanthomonas campestris (*X.c.*) pv. *citrumelo* strain 3048, causal agent of citrus bacterial spot, has a broad host range that includes *Citrus* sp. and bean. A spontaneous, prototrophic Host Specific Virulence (Hsv) mutant of 3048 (M28) was isolated which was non-virulent on citrus but virulent on bean. Growth *in planta* showed M28 to die-out quickly in citrus leaves, while growth in bean was only slightly affected. In addition, M28 had other mutant phenotypes detectable in culture: decreased growth rate; reduction in exopolysaccharide (ca. 1/4 of 3048 levels); lack of capsules; and increased lipase activity. Co-inoculation into citrus of M28 with wild type pv. *citrumelo* strains prevented the die-off of M28. Cell-free preparations of 3048 exoployssaccharide also restored the pathogenic response of M28 when mixtures were inoculated into citrus. A single 2.3 Kb *SalI-SstI* fragment (pMK41.1) from 3048 restored M28 to pathogenicity on citrus, wild type levels of exopolysaccharide and lipase response. This region is at least partially conserved in other xanthomonads. A comparison of the DNA sequence is underway to determine if this region has homology with other known exopolysaccharide loci. The role of exopolysaccharide in virulence of citrus by 3048, as well as the role of this genetic locus in virulence of *X. phaseoli* in bean, and *X. citri* in citrus will be discussed.

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STRAIN DISTRIBUTION AND FURTHER CHARACTERIZATION OF THE *BRADYRHIZOBIUM JAPONICUM* MANNOGALACTURONAN EPS DEPOLYMERASE. M.F. Dunn and A.L. Karr; Department of Plant Pathology; 108 Waters Hall; University of Missouri; Columbia, MO 65211.

The production of extracellular polysaccharide (EPS)-degrading enzymes by bacteria has been documented in several systems, with most work being done on phage-associated or phage-induced depolymerases. Homologous, nonphage-induced or -associated depolymerases appear to occur more rarely and have been characterized in very few systems. Recently, we reported the isolation and preliminary characterization of an enzyme produced by *B. japonicum* strain 2143 which was capable of depolymerizing the EPS produced by that strain. We have now determined the distribution of EPS depolymerase (EPSD) among 36 strains of *B. japonicum* and related organisms. EPSD is present in most of the *B. japonicum* strains which produce an EPS of the same structural type as strain 2143 and in several others whose EPS composition has not been determined. The widespread occurrence of this enzyme suggests it may play some role in the ecology and/or life cycle of the strains possessing it. Also presented will be work aimed at further characterizing this enzyme, including progress on purification and preliminary studies on cellular localization.

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NITROGEN FIXATION LIMITATIONS: THE BIOCHEMISTRY OF HOST-DEFENSE RESPONSES IN *BRADYRHIZOBIUM*-SOYBEANS SYMBIOSIS. M. Mohammadi and A.L. Karr; Department of Plant Pathology; 108 Waters Hall; Univ. of Missouri; Columbia, MO 65211.

Both the decline in nitrogen fixation and bacteroid viability in nodules formed by strains (effective combination: 2143 & 2122 and ineffective combination: 3122) of *B. japonicum* on soybean cv. Williams 82 have been attributed to *in planta* accumulation of glyceollins following the peak in nitrogen fixation 27 days postinoculation (PI). In this work, membrane lipid peroxidation and catalase and peroxidase activities in nodules were found to be biphasic and much greater during early and late nodule development. Polyphenol oxidase (PPO) activity in effective combinations was high during early nodule development. Superoxide dismutase (SOD) activity in effective combinations gradually increased following the decline in nitrogen fixation and then leveled off. β-1, 3-glucanase activity was at a maximum between 17-21 d PI. Chitinase activity gradually increased during nodule development. Aryl-β-1, 3-glucanase activity was detectable in nodules of effective combinations at all times PI.

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AN ULTRASTRUCTURAL STUDY OF THE DEVELOPMENT OF SOYBEAN NODULES FORMED BY STRAINS/MUTANTS OF *BRADYRHIZOBIUM JAPONICUM* EXHIBITING A FIX$^-$ OR DELAYED NOD/FIX PHENOTYPES. M.C. Huber and A.L. Karr; Dept. of Plant Pathology; 108 Waters Hall; University of Missouri; Columbia, MO 65211.

Bradyrhizobium japonicum strain 2143 and 2122 exhibit a Nod$^+$Fix$^+$ phenotype. These strains initiate a normal process of nodule development. This includes infection thread formation, bacteroid release and initiation of nitrogen-fixation which reaches a maximum at 28 d and, thereafter, declines. After 28 d, infected cells begin forming vacuoles and degradation of bacteroids is obvious. Strain 3122 (a mutant of strain 122 which grows abnormally on C_4 acids) exhibits a Nod$^+$Fix$^-$ phenotype. Development of nodules formed by strain 3122 appears normal up to the point of release when bacteria fail to multiply and apparently disintegrate. Typical of DNA homology group II strains of *B. japonicum*, strain 117 displays a delay of at least one week in nodulation and the onset of nitrogen fixation. Strain 117 nodules contain bacteroids surrounded by a large electron-translucent area. Mutant II-14 (a Tn5 mutant of strain 2143) exhibits the same delay in nodulation and nitrogen fixation as strain 117. Unlike its parent strain (2143), strain II-14 continues optimal nitrogen fixation for at least 43 d.

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THE GALACTURONIC ACID UPTAKE SYSTEM OF ERWINIA CHRYSANTHEMI.
C. Anderson and M. J. D. San Francisco, Department of Biological Sciences, Texas Tech University, Lubbock, TX 79409.

Members of the phytopathogenic genus Erwinia produce enzymes necessary to degrade the pectinaceous component of the plant cell wall. Pectin degradation yields monomers, saturated and unsaturated dimers and oligomers of galacturonic acid (GA). These compounds are internally catabolized by the phytopathogens, generating molecules involved in regulating the production of pectin-degrading enzymes. Intracellular catabolism necessitates uptake of the molecules being catabolized. The uptake of the products of pectin degradation must, therefore, be an integral part of the pathogenic process. Using [^{14}C]-GA we have been investigating the uptake systems for GA in two strains of E. chrysanthemi EC 16 and 0873. Uptake of GA is induced in cells grown on GA or pectin, the latter being a more powerful inducer. The rate of uptake was greatly reduced however, in cells grown on glucose as a sole source of carbon. The uptake of GA is energy-dependent; exhibiting sensitivity to the metabolic inhibitors 2,4 dinitrophenol and potassium cyanide. A number of different membrane proteins are observed to be induced in cells grown on GA as compared to glucose. These membrane proteins are potential candidates for the GA uptake permease(s).

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IN VITRO ACTIVITY OF SELECTED PROTEINS AND PEPTIDES AGAINST PLANT PATHOGENIC FUNGI. T. A. Rood, J. P. Duvick and A. G. Rao, Pioneer Hi-Bred International, Inc., 7250 NW 62nd Ave., Johnston, IA 50131

An *in vitro* test was used to screen commercially available proteins and peptides for activity against six filamentous fungi. The test consists of a spore germination/hyphal elongation assay done in microtiter plates. Proteins are generally tested at 20-200 ug/ml final concentration. The fungi tested are pathogens of crop plants, and include *Aspergillus flavus, Alternaria alternata, Fusarium graminearum, Fusarium moniliforme, Sclerotinia sclerotiorum* and *Sclerotinia trifoliorum.* Antifungal activity was found among members of several classes of proteins, including polycations, small basic peptides, peptide hormones, enzymes, and proteinases.

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CHARACTERIZATION AND BIOLOGICAL SIGNIFICANCE OF FUSARIUM-INDUCED CELERY HYDROLASES. S. L. Krebs and R. Grumet, Department of Horticulture, Michigan State University, East Lansing, MI 48824.

The hydrolytic enzymes, B-1,3-glucanase (B13G) and endochitinase (CHIT), increase in celery roots upon infection by compatible (race 2) and incompatible (race 1) pathovars of the soil borne fungus, *Fusarium oxysporum* f. sp. *apii*; preinduction of these enzymes by chitosan treatment is correlated with a delay in symptoms. IEF activity gels indicate 1 acidic B13G, 3 acidic CHITs and 1 basic CHIT; both fungal and chitosan treatment induce the same isoforms of the enzymes. Preliminary data suggest that fungal growth in the presence of induced root extract is inhibited relative to growth on medium with control root extract. Race 1 is a better CHIT substrate than race 2; race 2 is a better B13G substrate than race 1. Comparisons of +/- chitosan root preps show that the difference in CHIT activity on fungal substrate corresponds to the difference in B13G activity on pure substrate (ca. 2 fold) rather than the difference in CHIT activity on pure substrate (ca. 15-20 fold). We are testing the possibility of B13G limitation of CHIT activity on *F. o.* f. sp. *apii* substrate.

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CUTICLE- AND WALL-DEGRADING ENZYMES OF FUSARIUM SOLANI AND PHYTOPHTHORA PARASITICA FROM CITRUS TISSUES AND SOILS. S. Nemec and W. Osswald, USDA, ARS, 2120 Camden Rd., Orlando, FL 32803.

Enzymes of F. solani (F.s.) isolated from root-rotted fibrous and scaffold roots and soil, and P. parasitica (P.p.) from infected bark and soil in Florida citrus groves were detected in 10 to 12 isolates from each source. Fifty to 100% of all F.s. isolates produced cellulase, P.p. produced none. Sixty to 100% of all P.p. and 70-100% of all F.s. produced cutinase. Lipase was produced by 42-50% of all P.p., but not by F.s. F.s. from roots produced pectin methyl esterase (PME), polygalacturonase (PG), and pectin methyl galacturonase (PMG), soil isolates produced only PMG. About 100% of P.p. from soil and bark produced all three pectinases while 0-72% of all F.s. produced them. Neither F.s. nor P.p. produced xylanase. Sixty-seven percent of F.s. from fibrous roots produced low levels of chitinase. Results reveal soil and root isolates of F.s. have high enzymatic potentials to breach cell walls and compared to P.p., mechanisms to this differ in regard to cellulase and pectinase production.

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A Comparison of the Effects of an Ethylene Biosynthesis-Inducing Xylanase on Tobacco Cell Cultures and Leaf Disks, B. A. Bailey, R. F. Korcak and J. D. Anderson. USDA/ARS, BARC, Beltsville, MD 20705.

We have previously demonstrated that an ethylene biosynthesis-inducing xylanase (EIX) purified from *Trichoderma viride* liquid cultures elicits extensive necrosis in tobacco (*Nicotiana tabacum* cv. Xanthi) leaves. The necrosis is accompanied by extensive leakage of electrolytes which has been further shown to include potassium. Cell cultures of Xanthi respond to EIX within 15 min by rapid efflux of potassium, uptake of calcium and alkalinization of the media. Cell cultures of Xanthi, in contrast to leaf tissue, respond to EIX by reduced production of ethylene. All the effects of EIX studied so far in leaf tissue and cell cultures are inhibited by treatment with lanthanum, a calcium channel blocker. EIX appears to be a host-specific substance that elicits strongest effects in certain strains of Xanthi. The observed responses further characterize the EIX-tobacco interaction as a hypersensitive response similar to many plant-pathogen interactions.

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TOPOISOMERASE INTERACTIONS WITH PEA DEFENSE *GENE 49*. David Gerhold and Lee A. Hadwiger, Department of Plant Pathology, Washington State University, Pullman, WA 99164-6430.

Topoisomerases are enzymes which can relieve transcriptional DNA supercoiling (topoisomerases I and II) and mediate DNA attachment to chromatin "scaffolds" (topoisomerase II) *in vivo*. Our interest in topoisomerases stems from the ability of several topoisomerase inhibitor drugs to stimulate transcription of pea defense response *gene 49*. This gene is also flanked by putative consensus recognition sites for the *Drosophila* topoisomerase II enzyme. Clusters of such sites are indicative of "Scaffold Attachment Regions" (SAR's) which are believed to interact with chromatin proteins. DNA is thus reportedly organized into discrete loops which impact gene regulation. We are characterizing topoisomerases from tobacco callus with respect to drug sensitivities, and consensus DNA binding/cleavage sites. We hope to understand the sites of topoisomerase action in and around *gene 49* and how this gene is induced by topoisomerase inhibitors as well as by fungal attack.

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ACTIVE OXYGEN INDUCTION IN TOBACCO CELL SUSPENSIONS TREATED WITH PSEUDOMONAS FLUORESCENS CONTAINING THE COSMID pHIR11 AND WITH STRAINS CONTAINING TNPHOA MUTATIONS IN THE HRP CLUSTER. J. A. Glazener[1], H.-C. Huang[2] and C. J. Baker[1]. [1]U.S.D.A., A.R.S., Microbiol. & Plant Path. Lab., Beltsville, MD 20705. [2]Dept. of Plant Path., Cornell Univ., Ithaca, NY 14853.

In tobacco suspension cells active oxygen production is increased after 2-3 hr when treated with Pseudomonas syringae pv syringae (Pss) which causes the hypersensitive response (HR) in tobacco leaves. Cosmid pHIR11 contains a cluster of hrp genes from Pss that enables P. fluorescens (Pf) to elicit the HR. Pf with the cosmid pHIR11 elicited an increase in active oxygen production after 2-3 hr. Strains with TnphoA mutations within this hrp cluster were tested to determine which complementation groups were responsible for active oxygen production. These results indicate that the full complement of hrp genes may be necessary for active oxygen prodution and HR in tobacco cells.

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GROWTH OF TOMATO FROM SEEDS EXPOSED TO SPACE RADIATION BY NASA. E.M. Sutker, J.D. Hamilton, J.M. Speer, Dept. of Botany, Eastern Illinois University, Charleston, IL 61920.

Tomato seeds exposed to radiation in space as part of "Space Exposed Experiment Developed for Students" were supplied by the National Aeronautics and Space Administration. Doses were not revealed, but four exposures and Earth Based (control) seeds were provided. Emergence ranged from 78 to 90% in the greenhouse but differences were not significant according to t-tests. Seedlings (18/treatment) were transplanted to a field plot in three randomized blocks. Measurements of plant height over time indicated no significant differences among treatments when B_1 coefficients from linear regression analysis were compared. At the end of the season, plant height, fruit number, and fruit weight were not significantly different among treatments (ANOVA, $P<0.05$). Early and late blight incidence was low and all plants appeared equally susceptible. Cytological examination revealed no chromosome abnormalities in pollen spore mother cells. Therefore, radiation in space at doses used in this experiment was not detrimental to seed viability and subsequent plant growth.

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IDENTIFICATION OF PATHOGENICITY GENES IN *COCHLIOBOLUS HETEROSTROPHUS*. P. R. Thorson and C. R. Bronson, Department of Plant Pathology, Iowa State University, Ames, Iowa 50011-1020.

Effort is currently underway to obtain pathogenicity mutants of *Cochliobolus heterostrophus*, the incitant of Southern corn leaf blight. Survivors of ultraviolet light and diepoxybutane mutagenesis are being screened on maize seedlings in the greenhouse for either failure to infect or failure to form spreading lesions, while growing normally on agar media. Several putative mutants have been identified. These are being crossed to determine whether the phenotype is genetically controlled, backcrossed to remove second site mutations, and intercrossed to determine the number of independent loci. To characterize infection phenotypes, confirmed mutants will be analyzed cytologically. This work will form the basis for future efforts to clone these pathogenicity genes and to determine their function.

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SPECIFIC ASSOCIATION OF BARLEY YELLOW DWARF VIRUS WITH OAT ROOT ENDOPLASMIC RETICULUM. I. S. Lamboy[1] and D. P. Briskin[2], [1]Plant Pathology Department and [2]Agronomy Department, University of Illinois, Urbana, IL 61801

A protocol was established to isolate enriched endoplasmic reticulum (ER) and plasma membrane (PM) fractions from healthy and barley yellow dwarf virus (BYDV strain PAV-IL) infected oat roots. Monoclonal antibodies to BYDV-PAV-IL detected virus particles in the PAV-infected oat root ER but not PM fractions. ELISA analysis of floatation gradients (purified virus, membrane suspension, and 60% sucrose mixed to form the bottom of a sucrose step gradient centrifuged 18 h at 80,000 g) demonstrated that the very dense, free virus particles were in the pellet, while virus associated with ER was collected at the 15%/27% sucrose interface. This phenomenon was observed with healthy oat root microsomal membrane suspensions and enriched oat root ER, but not with enriched oat root PM or red beet microsomal membranes. Anti-BYDV-PAV-IL polyclonal antibodies added to the virus suspension prevented membrane association, perhaps by competing for binding sites. Pre-immune rabbit serum did not block the association. These results suggest the existence of a host membrane component acting as a putative virus docking site.

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ELECTROPHYSIOLOGICAL STUDY OF HYPERSENSITIVE REACTION TO *XANTHOMONAS MALVACEARUM* IN RESISTANT COTTON (*GOSSYPIUM HIRSUTUM* CV. IM216). S.M. Pike, H. Urbanek, and A. Novacky; Department of Plant Pathology; 108 Waters Hall; University of Missouri, Columbia, MO 65211.

The membrane potentials of cotton cotyledons of *Gossypium hirsutum* cv. Im216, a cultivar resistant to *Xanthomonas malvacearum* (Xm), were measured after inoculation with 10^8 cfu ml^{-1} Xm, race 1. Segments cut 2.5 h after inoculation and measured 4 to 12 h after inoculation behaved similarly to water-infiltrated controls. Segments cut 5 h after inoculation and measured 6 to 12 h after inoculation often had very low potentials, but if aerated overnight under fluorescent light, increased in resting potential. However, aging overnight greatly altered light/dark responses in bacteria-inoculated segments cut at both times. These alterations are similar to those seen in viral HR (cowpea inoculated with tobacco ringspot virus) and suggest a change in cytosolic pH.

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THE EFFECT OF KINETIN CONCENTRATIONS ON K^+ LEAKAGE DURING BACTERIA-INDUCED HR IN SUSPENSION CULTURED CELLS. P.L. Popham, S.M. Pike, D. Zemlova, and A. Novacky; Department of Plant Pathology; 108 Waters Hall; University of Missouri; Columbia, MO 65211.

During the bacterial hypersensitive reaction (HR), plant cells die and the tissue becomes necrotic. Active oxygen production, lipid peroxidation, electrolyte leakage, and changes in pH are among several physiological alterations that accompany the HR. The visible symptoms observed during HR of cotton (*Gossypium hirsutum* cv Im216) cotyledons infiltrated with a *Pseudomonas syringae* (Ps) pathovar can be delayed or prevented with a pretreatment of kinetin. However, Im216 suspension cultured cells grown in 0.5 mg/L of kinetin and treated with Ps pathovars release significantly more K^+ into the reaction media by 24 h after exposure as do similar cells grown in 0.1 mg/L of kinetin when treated with the same bacteria. This apparent discrepancy between the HR suppressive effect of kinetin and the increased K^+ leakage during HR in suspension cultured cells grown under high kinetin conditions will be discussed.

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ANALYSIS OF INTERCELLULAR WASH FLUIDS FOR THE PRESENCE OF ELICITOR-ACTIVE OLIGOGALACTURONIDES IN COTTON COTYLEDONS. Edgar T. Miranda, Margaret Lee Pierce, and Andrew J. Mort, Department of Biochemisty, Oklahoma State University, Oklahoma Agricultural Experiment Station, Stillwater, OK 74078-0454.

Pectic fragments of cell walls may be intermediates in the defense response of plants against pathogens because oligomers of galacturonic acid of the size 11-13 residues are active in plants, including cotton cotyledons, as elicitors of phytoalexins. We are attempting to quantitate galacturonic acid oligomers in intercellular wash fluids (IWF) from cotton cotyledons to test the hypothesis that elevated levels of elicitor-active oligomers are formed during a resistant response to the bacterial blight pathogen. Two size-exclusion HPLC steps followed by PA-1 anion exchange HPLC were developed to fractionate IWF spiked with standards. As little as 10 pmol of oligomer labelled with 2-aminopyridine could easily be detected by its fluorescence. When healthy cotyledons were infiltrated with a 0.5 mg/ml solution of undecamer and the IWF was prepared using 50 mM CDTA as extractant, recovery of the oligogalacturonide from the cotyledons was ~70%. Current focus is directed towards analyzing materials rinsed from bacterially inoculated cotyledons.

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DIFFERENT MODES OF ACTION OF AM-TOXIN PRODUCED BY THE APPLE PATHOTYPE OF ALTERNARIA ALTERNATA AMONG APPLE CULTIVARS. N. Shimomura, K. Kohmoto, H. Otani and M. Kodama, Plant Pathology Lab., Fac. of Agric., Tottori University, Tottori 680, Japan.

Apple cultivars are classified into three groups with respect to reaction against AM-toxin: highly sensitive (HS), moderately resistant (MR) and resistant (R) cvs. HS cvs. had a striking tissue-specificity in their response to toxin. Toxin seriously injured HS leaves, but not at all petals. MR leaves and petals were comparably affected by toxin. No damages were detected in R leaves and petals. Green calli derived from HS leaves were sensitive to toxin, but white calli from leaves and petals were insensitive. Green and white calli from MR cvs. were affected by toxin. Pretreatments with SH-reagents before toxin exposure protected HS, but not MR, leaves from toxin action. Protein synthesis inhibitors reduced veinal necrosis on HS leaves induced by toxin. In contrast, the inhibitors stimulated necrosis on MR leaves. The mode of action process of AM-toxin in HS cultivars appears to differ from that in MR cultivars.

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AN ELICITOR THAT INDUCES CULTIVAR-SPECIFIC RESPONSES IN SOYBEAN. D.M. Lawrence and M.M. Stayton, Department of Molecular Biology, University of Wyoming, Laramie, WY 82071.

Avirulence geneD was isolated from Pseudomonas syringae pv. tomato by virtue of its ability to confer avirulence on strains of Pseudomonas syringae pv. glycinea. Over-expression of this gene in both Pseudomonas syringae and E. coli leads to the production of a cultivar-specific elicitor (SE) of the soybean defence response. Following treatment of soybean tissue with SE, a typical phytoalexin defence response is induced only in cultivars that express the disease resistance gene, Rpg4. Protein phosphorylation has been implicated in a variety of signal transduction systems. Therefore, the effects of the SE on the phosphorylation of proteins in plasma membranes, microsomal membranes and soluble fraction isolated from soybean cultivars, Rpg4+ and Rpg4-, were investigated.

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HISTOLOGY OF INFECTION OF HYDRILLA VERTICILLATA BY MACROPHOMINA PHASEOLINA. G.F. Joye and R. Paul. Landis Int'l, Inc. 3025 Madison Highway, Valdosta, GA 31603 and USDA/ARS/SWSL Stoneville, MS 38776

The infection of Hydrilla verticillata by Macrophomina phaseolina was investigated using transmission electron microscopy. Sprigs of plants in petri plates were inoculated with hyphal suspensions. Samples of inoculated and non-inoculated plants were taken over time. Within 16 hr after inoculation, fungal cells attached to the wall of lower epidermal cells, but not to upper epidermal cell walls of leaves. Within 40 hr, penetration through the cell wall was completed and colonization of host cells was observed. Penetration of the upper epidermis occurred through the cell wall adjacent to a lower epidermal cell. Inhibition of penetration through the out cell wall of the upper epidermis may be attributable to an osmiophilic layer below the cell wall. The disruption of the host cell walls and subsequent host cell death was preceeded by massive colonization of the host.

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Nichole R. O'Neill. Defense responses and the role of medicarpin in resistance in alfalfa to Colletotrichum trifolii. USDA, ARS, Beltsville, Maryland 20705.

The role of phytoalexins in resistance to anthracnose in alfalfa was examined by comparing the effectiveness of medicarpin in inhibiting growth stages of races 1 and 2 of Colletotrichum trifolii, and by quantifying the accumulation of medicarpin and phenylalanine ammonia lyase in resistant and susceptible seedlings and cotyledons. Medicarpin was the major phytoalexin among 7 fungitoxic compounds extracted and was inhibitory to spore germination and growth of pre-germinated spores but not to hyphal growth of race 1 and 2 isolates. The activity of defense enzymes PAL and chalcone synthase related to the accumulation of alfalfa phytoalexins in cultivars with specific resistance genes. RNA extracted from incompatible cotyledons hybridized with heterologous soybean DNA probes for PAL and CHS. A cDNA expression library was constructed from mRNA extracted from resistant and susceptible alfalfa tissues will be used to identify defense related genes.

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EFFECT OF CLAYS AND LACTOSE ON SURVIVAL OF RHIZOBACTERIA IN POWDER FORMULATIONS. L. M. Dandurand[1], M. J. Morra[2], and C. S. Orser[1] [1]Dept. of Bacteriology & Biochemistry, and [2]Dept. of Plant, Soil, & Entomological Sciences, University of Idaho, Moscow 83843.

Two strains of *Pseudomonas fluorescens* (W4F393, 2-79RN$_{10}$) were suspended in phosphate buffer or 10% lactose and mixed into Ca^{2+} saturated kaolin, montmorillonite, vermiculite, zeolite, talc, or pyrophillite (ca. 10^9 cfu/g). Formulations were air dried and held at 20 C and 15% RH. Populations of 2-79RN$_{10}$ decreased in all formulations over 6 weeks. Population decline ([log$_{10}$ at 0 wk] - [log$_{10}$ at 6 wk]) was least in montmorillonite with lactose (1.06) or without lactose (1.21), in zeolite with (1.62) or without lactose (1.76), in vermiculite with (2.00) or without lactose (2.07), in pyrophillite with lactose (2.03), and in talc with lactose (2.13), and intermediate in kaolin with lactose (2.57). Decline of 2-79RN$_{10}$ was greatest in talc (3.74), in kaolin (4.32), and in pyrophillite (4.83), without lactose. Populations of W4F393 declined to nearly undetectable levels over 6 weeks. Specific clay minerals may enhance the survival of biocontrol bacteria, but protection is strain-dependent.

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COLONIZATION OF PEAR STIGMAS BY BENEFICIAL BACTERIA FOR CONTROL OF FIRE BLIGHT. V. O. Stockwell[1], J. E. Loper[1], and K. B. Johnson[2]. [1]USDA-ARS, Horticultural Crops Research Lab. and [2]Dept. of Botany & Plant Pathology, Oregon State University, Corvallis, OR 97330.

The stigmatic surface of pear blossoms is the site of primary colonization and bacterial growth leading to infection of floral tissues by Erwinia amylovora, the causal agent of fire blight. Timely application of beneficial bacteria may preempt colonization of stigmas by the pathogen and prevent subsequent infection of floral tissues. Colonization of pear blossoms by two bacterial biological control agents of fire blight, Pseudomonas fluorescens A506 and Erwinia herbicola C9-1, was evaluated. In April 1990, pear trees (c.v. Bartlett) in three locations in Oregon were sprayed with bacterial suspensions (10^8 cfu/ml) during pre-pink and 10-30% bloom. Population sizes of these bacteria, which were estimated from stigmas of individual flowers, averaged 10^5 to 10^6 cfu/blossom. Although these bacteria were applied when only 10-30% of flowers were open, E. herbicola C9-1 was detected on stigmas of 50-60% of open flowers throughout the bloom period. Similarly, P. fluorescens A506 was detected on 60-80% of pear blossoms. These two biocontrol agents of fire blight developed large populations on pear blossoms in three diverse regions of Oregon.

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PLANT CELL WALL-DEGRADING ENZYMES FROM THE POTENTIAL MYCOHERBICIDE COLLETOTRICHUM COCCODES. Leathers, T. D. and N. J. Alexander. National Center for Agricultural Utilization Research, Agricultural Research Service, U.S. Department of Agriculture, 1815 N. University St., Peoria, Illinois 61604.

The fungus Colletotrichum coccodes is a pathogen of the weed pest Velvetleaf (Abutilon theophrasti). We examined 14 naturally occurring strains of C. coccodes isolated from diverse sources and geographical locations. Strains were cultured in vitro on host-specific (Velvetleaf) and non-host-specific (V8) media. Nearly all strains secreted detectable levels of both xylanase and cellulase, but no polygalacturonase, on these media. Enzyme production was generally slightly higher on non-host-specific medium. Quantitative virulence assays are in progress to test correlations with enzyme production.

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BIOLOGICAL CONTROL OF RHIZOCTONIA SOLANI ON SOYBEAN WITH BINUCLEATE RHIZOCTONIA. F. U. Khan, B. Nelson and T. Helms*. Depts. of Plant Pathology and *Crop and Weed Science, North Dakota State University, Fargo, ND 58105.

Nine cultures of binucleate Rhizoctonia (BNR) were tested for biological control of R. solani on Ozzie soybean in the greenhouse. The soil was infested with R. solani (AG4), and then the BNR mycelia were placed in the soil in direct contact with the soybean seed. Germination and disease severity was recorded after three wk. Cultures of binucleate Rhizoctonia differed in their effects on disease and seed germination. Four BNR significantly decreased germination in the absence of AG4 and five significantly increased germination in the presence of AG4. Three BNR significantly increased germination in the presence of AG4, but did not reduce germination in the absence of AG4. Four BNR reduced disease severity on surviving plants.

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SELECTION OF ISOLATES OF *Phytophthora parasitica* var. *nicotianae* FOR BIOCONTROL OF PRE-EMERGENCE DAMPING OFF OF *Catharanthus roseus* CAUSED BY *Phytophthora parasitica*. K. A. Holmes and D. M. Benson, Dept. of Plant Pathology, North Carolina State University, Raleigh 27695-7616.

Isolates of *P. p. nicotianae* were evaluated as biocontrol agents for pre-emergence damping off of *C. roseus* caused by *P. parasitica*. Parameters for identifying antagonistic isolates were established. Inoculum of *P. p. nicotianae* cultured on rice grains for 14 or 21 days caused more disease than 7-day-old inoculum. Inoculum concentrations of 0.33 or 0.58 g of colonized rice/m^2 caused 90% damping off compared to 70% at 0.19 g/m^2. When antagonists were incubated in a peat:vermiculite mix at 60% soil moisture for 7 days prior to seeding plug trays, pre-emergence damping off was 46, 33, and 30% with the best isolates of *P. p. nicotianae* compared to 61% in the control. In experiments without *P. parasitica* present, these same isolates caused a mild stunting of plant growth and were isolated from 24, 23, and 14% of the roots sampled respectively. Some isolates of *P. p. nicotianae* may protect *C. roseus* from pre-emergence damping off, but the mechanism of biocontrol has not been determined.

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SCREENING IN THE GREENHOUSE OF SOME TREATMENTS TO CONTROL TAKE-ALL PATCH ON TURFGRASS. P. Lucas and A. Sarniguet, Station de Pathologie végétale, INRA, BP 29, 35650 Le Rheu, France.

Three fungicides, three fluorescent pseudomonads, and two sources of nitrogen fertilizer were compared for controlling take-all patch on two cultivars of Agrostis palustris (Penneagle and Penncross). Plants were grown in pots of sand inoculated with Gaeumannomyces graminis var. avenae. Two weeks after treatment (T+2), significant reduction in disease index (DI) occured for the three bacteria tested (27-45%) but not with the fungicides. At T+5, the disease index was reduced by triadimenol (61%), nuarimol (52%) and cyproconazole (48%). At this stage, only one bacterium gave still a good control (22%). No effect was observed with either nitrate or ammonium forms of nitrogen. Penncross appeared to be much more susceptible to take-all than Penneagle (DI=2.4 and 1.3, respectively). Results with bacteria and nitrogen will be discussed with regard to the low nutrient concentration and microbial population in the sand substrate.

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DISPERSAL OF COLLETOTRICHUM GLOEOSPORIOIDES UNDER NATURAL AND CONTROLLED CONDITIONS. X.B. Yang and D.O. TeBeest, Dept. of Plant Pathology, Univ. of Arkansas, Fayetteville, Ar 72701.

The dispersal of Colletotrichum gloeosporioides, a pathogen of northern jointvetch, was quantified after point inoculating source plants in flooded circular plots. Disease was detected on plants more than 1-m from source plants, about 5 days after sporulation was observed on the source plant (one disease cycle). Early in the experiment, lesions appeared to occur on the parts of plants at or near the level of the free standing water, indicating that horizontal spread of inoculum may be related to the surface water. Later in the season, lesions were also found on upper parts of the plant, indicating vertical spread. More lesions were observed on down-wind plants than up-wind plants. Spread of disease was also quantified under controlled conditions where inoculum spread was much greater when surface water was present. Both distance and slope of dispersal were significantly reduced when weeds were in rice. Results indicate that rice limits spore dispersal and is a key constraint to disease spread.

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SUCCESSFUL INOCULATION OF CANADA THISTLE (CIRSIUM ARVENSE) WITH TELIOSPORES OF PUCCINIA PUNCTIFORMIS IN FIELD STUDIES. R. C. French, USDA, ARS, Ft. Detrick, Frederick, MD 21702.

Systemic infection of Canada thistle can be obtained by placing teliospores of P. punctiformis on dormant buds of root cuttings (French & Lightfield, Phytopath. 80:872-877, 1990). This method was compared to inoculation of one bud on each of 100 root balls of potted thistle, and to spraying teliospores onto chopped thistle roots (1.0 kg) (5/25/89). Inoculated root cuttings did not survive well in the field. Inoculated root balls survived; 10% produced systemically infected shoots in 1989. In spring ('90) the 100 root balls produced over 280 systemically infected shoots. The infected chopped roots produced 164 infected shoots in '89 and 215 in '90. Of the techniques tested, inoculated chopped roots appeared the most efficient. Although many inoculated root cuttings did not survive in the field, and few became systemically infected, appreciable systemic infection was later observed in plots left undisturbed for over a year.

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BIOLOGICAL CONTROL OF COLLETOTRICHUM GRAMINICOLA, CAUSAL AGENT OF THE SORGHUM LEAF ANTHRACNOSIS, BY USING BACTERIA. S.J. Michereff and R.L.R. Mariano, Depto. de Agronomia, Universidade Federal Rural de Pernambuco, Recife, PE, Brazil.

The antagonism of Pseudomonas fluorescens (isolates P2, BJ22 and SDR2), P. marginalis (C21) and Bacillus subtilis (B16) against Colletotrichum graminicola was evaluated through the following parameters (1) micelial growth inhibition, by using paired and cellophane tests, on PDA and King's B medium (KMB) and, (2) diseasy severity reduction in plants under greenhouse conditions, evaluated by a note scale. "In vitro", B16 and P2 showed best eficiency in the cellophane test, reaching the first one, inhibitions of 94.34% on PDA and, the second one, 56.44% on KMB. Under greenhouse conditions, P2, C21 and B16 were applied in 5 different periods of time. The best results were achieved by applying simultaneously with pathogen inoculation, where C21 reached disease reduction percent of 21.94, however without statistical significant difference from the other bacteria.

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BIOLOGICAL CONTROL OF COLLETOTRICHUM GRAMINICOLA, CAUSAL AGENT OF THE SORGHUM LEAF ANTHRACNOSIS, BY USING TRICHODERMA SPECIES. S.J. Michereff and M. Menezes, Depto. de Agronomia, Universida de Federal Rural de Pernambuco, Recife, PE, Brazil.

The antagonism of Trichoderma viride (isolate TR2), T. koningii (T15), T. aureoviride (T10), T. harzianum (T25) and T. pseudo - koningii (T26) against Colletotrichum graminicola was evaluated through the following parameters (1) size of the inhibition zone, by using paired test, (2) micelial growth inhibition, by using cellophane test, both in PDA medium and, (3) disease severity reduction in plants under greenhouse conditions, evaluated by a note scale. "In vitro", T25 and TR2 showed best eficiency, with inhibitions zones of 2.50 and 2.44 mm, as well 100 and 99% for micelial growth inhibition, respectively. Under greenhouse conditions, TR2, T15 and T25 were applied in 5 different periods of time. The best disease severity reduction was obtained by aplying antagonists 48 hours before pathogen inoculation where T15 reached disease reduction percent of 17.89, however without statistical significant difference from the other isolates.

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MODIFIED RECURRENT SELECTION FOR TOLERANCE TO BARLEY YELLOW DWARF VIRUS IN WINTER WHEAT. E. M. Bauske, Dept. of Plant Pathology, F. L. Kolb, Dept. of Agronomy, and A. D. Hewings, USDA ARS, University of Illinois, Urbana, IL 61801.

Progeny from three cycles of a modified recurrent selection scheme were field tested to determine the effectiveness of this strategy for improving tolerance to barley yellow dwarf virus (BYDV) in a winter wheat population. Seventeen parents were crossed and F_1 seed was planted in rows. Female rows were sprayed with a chemical hybridizing agent and inoculated with BYDV-PAV-IL. Untreated rows provided pollen. Seed was harvested from female rows. Tolerant plants should contribute more seed to the subsequent generation than sensitive plants. This cycle was repeated 4 times. F_3 seed from cycles 2, 3, and 4 (C_2, C_3, and C_4) was planted in a RCB design with 6 replications. The 17 parents and the cultivars, Abe and Elmo, were included as controls. Inoculated hills were evaluated for BYDV tolerance using a visual rating scale and percentage yield of the control. At an early rating date, ratings were slightly lower in the C_2 population compared to the mean of the parents; no improvement was detected in the C_3 or C_4 populations. At heading, no differences were detected among the populations. The percentage yield of the control was unaffected by the selection scheme. Although genetic variability was detected in the three cycles and the parents using the rating scale, it was not large.

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EARLY-GROWTH STAGE DETECTION OF RESISTANCE CONFERRED BY Lr37 TO LEAF RUST OF WHEAT. F. J. Kloppers and Z. A. Pretorius, Department of Plant Pathology, University of the Orange Free State, Bloemfontein 9300, South Africa.

In addition to the potential of Lr37 as a new source of adult-plant resistance to leaf rust (Puccinia recondita f. sp. tritici) of wheat (Triticum aestivum L.), the gene has also been associated with increased protein in kernels. To facilitate early-growth stage detection of Lr37 in backcross breeding programs, the effects of pathotype and temperature on the resistance of 7-, 14-, 21- and 28-day-old RL6081 plants were investigated. Infection types indicated that interactions among temperature, pathotype and growth stage occurred. Resistance in primary leaves (infection types ;1 to 2-) was most clearly detected at 15 C. At 20 and 25 C, the highest degree of resistance was expressed by 28-day-old RL6081 plants. Certain pathotypes, however, were virulent to Lr37 in 28-day-old plants at 25 C.

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ADULT PLANT RUST RESISTANCE ASSOCIATED WITH LEAF PUBESCENCE IN COMMON BEANS. M. T. Mmbaga and J. R. Steadman, Dept. of Plant Pathology, University of Nebraska, Lincoln, NE 68583-0722.

Rust disease reactions of pubescent and glabrous genotypes of Phaseolus vulgaris from diverse genetic origins were compared at seedling and adult plant stages under glasshouse and field environments. All pubescent genotypes expressed moderate susceptibility (MS) or moderate resistance (MR) to individual or mixed single uredinia cultures of Uromyces appendiculatus on the primary leaves. Reduced uredinia size and density were observed on the upper trifoliolate leaves of these genotypes to give MR or R reactions. The use of 24 rust cultures produced similar results and indicated a race-nonspecific response. These results indicated reduced susceptibility of the upper leaves which has been termed adult plant resistance.

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VARIATION IN SUGAR BEET SUSCEPTIBILITY TO ISOLATES OF FUSARIUM OXYSPORUM F. SP. BETAE FROM TEXAS AND OREGON. C. M. Rush and R. D. Martyn. Texas Agricultural Experiment Station, P.O. Drawer 10, Bushland, Texas 79012.

Isolates of Fusarium oxysporum f. sp. betae from sugar beets in Texas are morphologically and genetically distinct from those from Oregon. It was unknown whether these differences related to pathogenicity, so a study was conducted to determine if sugar beet germplasm reacted differently to Fusarium isolates from Texas and Oregon. Seed from 90 entries were planted and later seedlings were inoculated with each pathogen using the tray dip method. Poor seed quality resulted in erratic seedling emergence and only 60 entries were evaluated for disease severity, and top and root weight. When data was sorted by isolate, high variability resulted in little difference in disease rating among entries. However, 16 entries differed significantly in their susceptibility to the two isolates with nine more resistant to the Texas isolate and seven more resistant to the Oregon isolate. Fourteen entries had a disease rating value ≤1.2 on a 0-3 scale. Root and top weight were not highly correlated to disease rating.

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SCREENING OF COCOYAMS FOR RESISTANCE/TOLERANCE TO COCOYAM ROOT ROT DISEASE IN CAMEROON. R. P. Pacumbaba, J. G. Wutoh, and Muyali Mary B. Meboka. Department of Plant and Soil Science, Alabama A&M University, Normal, AL., U.S.A., and ROTREP, USAID/GOC, Ekona Res. Centre, Buea, Cameroon.

Cocoyam root rot disease (CRRD) is caused by *Pythium myriotylum* (Pm). Pm inoculum was grown for 3-5 days at 31 C on petri dishes containing lima bean sucrose agar. The screening box measuring 100 x 72 x 14 cm was filled with autoclaved soil and inoculated with 2.4 x 10^4 mycelial strands/ml of Pm. Screening period was for 21 days after planting the cocoyams in a screening box. Cocoyams that remained green for 21 days in the screening box were transplanted to a CRRD-affected field. The ratings for determining the levels of resistance/tolerance to CRRD were from 1-3 where; 1=Resistant (plants remained green for 21 days in screening boxes), 2=Tolerant (slight root rotting and yellowing only of older leaves after 5 days and succeeding leaves remained green), and 3=Susceptible (roots are rotted and general chlorosis in 5 days followed by wilting and death of the plants). There are presently 72 and 212 resistant and tolerant cocoyams, respectively, out of 626 cocoyams screened for resistance/tolerance against CRRD and planted in a CRRD-affected field in Mamu, Ekona. These cocoyams will be further screened every month for 3 months under field conditions before final selection of plants resistant/tolerant to CRRD can be made.

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SOURCES OF COMPOUNDS WHICH INDUCE SYSTEMIC RESISTANCE IN CUCUMBER TO COLLETOTRICHUM LAGENARIUM, THE CAUSAL ORGANISM OF ANTHRACNOSE. L. FOUGHT AND J. KUC, DEPARTMENT OF PLANT PATHOLOGY, UNIVERSITY OF KENTUCKY, LEXINGTON 40546.

Canola, lettuce, green bean, dwarf pea, tobacco, tomato, poplar, corn, wheat, and cucumber leaves were extracted in boiling 95% ethanol to obtain water-soluble fractions containing compounds with a molecular weight <1,000 D. Leaves 1 & 2 of cucumber plants were sprayed with each plant fraction (induction) and 7 days later leaf 3 was inoculated with a spore suspension of C. lagenarium (challenge). All fractions induced systemic resistance in leaf 3 to C. lagenarium. Commercially available compounds were screened to determine structural similarities among the compounds which can induce systemic resistance. Leaves sprayed with the compounds were damaged but leaf damage was not correlated with induced systemic resistance in leaf 3. Galacturonic acid, glucuronic acid, salicylic acid, m-hydroxybenzoic acid, p-hydroxybenzoic acid, γ-resorcylic acid, protocatechuic acid, gallic acid, hemimelletic acid, trimelletic acid, trimesic acid, and phloroglucinol induced systemic resistance. Structural similarities are not evident among either compounds which induce systemic resistance or those that do not.

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GENETIC VARIABILITY FOR MELOIDOGYNE INCOGNITA RESISTANCE IN WHITE CLOVER (TRIFOLIUM REPENS L.). G. A. Pederson and G. L. Windham, USDA, ARS, P. O. Box 5367, Miss. State, MS 39762

Meloidogyne incognita resistance has recently been identified in white clover. Three M. incognita resistant (ND 1, 2, & 3) and three susceptible (ND 4, 7, & 10) clones of white clover were evaluated in a 6-parent diallel to determine the inheritance of this resistance. General (GCA) and specific (SCA) combining ability mean squares were significant (P=0.01) for both percent of root system galled (PRSG) and egg index (EI). GCA effects were more important than SCA effects. There were no maternal or reciprocal effects for PRSG or EI. Crosses with ND 1 and ND 3 had the least amount of root galling and crosses with ND 3 had the least nematode reproduction. SCA effects (P=0.05) were observed for ND 1 x ND 3 and ND 4 x ND 7 with more root galling than expected and for ND 4 x ND 7 with more nematode reproduction than expected. Selection methods that utilize additive genetic variance should be effective in breeding for M. incognita resistance in white clover.

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FUNGAL FLORA OF PEARL MILLET GRAIN AS AFFECTED BY DATE OF PLANTING. J. P. Wilson and W. W. Hanna. USDA-ARS Forage and Turf Unit, Coastal Plain Experiment Station, Tifton, GA 31793.

Pearl millet hybrid Tift 90DAE x Tift 8677 was planted April 20, May 10, May 30, June 21, and July 10, 1990. Grain was harvested 30 days after anthesis. Three hundred seeds from each planting date were surface sterilized and plated on either V8 agar to examine total fungi or 10% malt-salt agar to detect Aspergillus flavus. The most frequently isolated fungi were Alternaria spp. (37%), Fusarium semitectum (22%), Curvularia spp. (8.7%), and Exserohilum rostrata (5%). Total Fusarium isolations averaged 29.5%. Infection by the four most frequently isolated fungi increased an average of 323% from the first to the last planting date. Percentages of seed infected by any fungus were 32, 60, 83, 89 and 100% for the different planting dates. No A. flavus was isolated on either medium.

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TOXIGENIC FUNGI FROM CORN AND PEARL MILLET IN GEORGIA. J.P. Wilson, D.M. Wilson, R.W. Beaver, W.W. Hanna, N.W. Widstrom and W.W. McMillian. USDA-ARS and Dept. of Plant Path., UGA, Coastal Plain Expt. Stn., Tifton, GA 31793.

Infection by mycotoxin-producing fungi on corn and pearl millet was compared in 1990, a severe year for infection by Aspergillus flavus and aflatoxin contamination in corn from Georgia's Coastal Plain. Infection by A. flavus in eight corn samples from Tift Co. ranged from 0 to 20% and averaged 6.4%. Aflatoxin contamination ranged from 0 to 318 ppb and averaged 54.4 ppb. Infection by Fusarium moniliforme averaged 50.1%. A. flavus infection in six samples of pearl millet ranged from 0 to 0.1% and averaged 0.02%. Aflatoxin contamination ranged from 0 to 1.8 ppb and averaged 0.4 ppb. Five of the samples were examined for Fusarium species. F. semitectum was most frequently isolated, and infection averaged 21.5%. Although pearl millet appears to be less prone to infection by A. flavus, potential problems from infection by toxigenic Fusarium species exist.

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UTILIZATION OF IPM TECHNIQUES FOR CONTROL OF APPLE SCAB AND CODLING MOTH IN AN IOWA APPLE ORCHARD. M. K. Ali, M. L. Gleason, P. A. Domoto, D. R. Lewis, and M. D. Duffy. Horticulture Department, Iowa State University, Ames, Iowa 50011.

Five spray strategies for control of apple scab and codling moth were compared in a Red Delicious apple orchard at the Horticulture Research Station, Ames, Iowa, during 1989 and 1990. Three IPM-based treatments, incorporating monitoring of weather and pest populations, were compared to a traditional spray schedule and a control. A partial budget technique was used to compare economic data from all treatments. IPM-based treatments saved an average of six pesticide sprays per year compared to the traditional treatment, with equivalent pest control efficacy. Yield of IPM-based treatments was comparable to or greater than yield of the traditional treatment. The IPM-based treatments were comparable in cost to the traditional treatment. However, IPM-based treatments had an increasing cost advantage as orchard size increased.

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MINIMUM USE OF FUNGICIDES FOR CONTROL OF FOLIAR FUNGAL PATHOGENS OF TOMATO. F.J. Louws, M.K. Hausbeck and C.T. Stephens. Dept. of Botany and Plant Pathology, Michigan State University, East Lansing, MI 48824-1615.

A minimum pesticide use production system for processing tomatoes (Lycopersicum esculentum 'Ohio 7870') was initiated in 1990 using cultural practices and Tom-Cast, a program that calculates a daily disease severity value

(DSV) for early blight (EB), caused by Alternaria solani, based on the average temperature during hours when foliage is wet. A standard weekly spray program from Jul 5 to Sep 5 required nine chlorothalonil applications compared to either four or three sprays applied at every 20 or 25 cumulative DSVs. Final amount of defoliation resulting from EB was 30% in untreated controls compared to 9 to 15% for sprayed plots. Total mold on fruit caused by Colletotricum coccodes, Rhizoctonia solani or Alternaria solani was 17% in untreated controls, 7.7% for DSV 20, 7.4% for DSV 25 and 3.4% for the weekly spray treatment. Zone tillage limited defoliation caused by EB to 11% compared to 18% for conventional tillage plots.

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THE EFFECTS OF ALUMINUM TOXICITY ON THE WATER UPTAKE PATTERNS OF LOBLOLLY PINE (*PINUS TAEDA*) SEEDLINGS AS STUDIED BY MAGNETIC RESONANCE MICROSCOPY (MRI). L.M. Werrell, J. S. MacFall, G. A. Johnson, Duke University, Durham, NC 27706.

The toxicity of soil-solution aluminum to seedlings was examined using morphological, chemical and water extraction indices. Over the three month period examined, only alterations in water extraction proved significant. Analysis of proton MR images acquired indicate significant shift in water extraction patterns from lateral extraction by the controls (untreated) to taproot extraction only by the aluminum treated plants. This indicates that prior to obvious gross morphologic changes or nutrient content alterations, the water relations of aluminum treated plants are severely impacted and could then act as a more sensitive indicator of aluminum toxicity.

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SOYBEAN MATURITY AS A COMPONENT OF RESISTANCE TO SCLEROTINIA WHITE MOLD. P. L. Gross and J. R. Venette. North Dakota State University, Plant Pathology Department, Fargo, ND 58105-5012.

White mold (Sclerotinia sclerotiorum) severity and incidence were evaluated on fifteen soybean (Glycine max) cultivars which differed in maturity. Soybeans were grown in a furrow-irrigated plot that was naturally infested with the pathogen. All cultivars were harvested 115 days after planting and separated into one of seven maturity classes. Disease severity (average length of longest stem lesion on each plant) and incidence (percentage of infected plants) were compared to maturity classes. On immature plants, disease severity and incidence were inversely related to maturity. Mature plants (dark-brown to dried pods) had <10% infection and severity of ca. 1 cm. The least mature lines had green pods and stems and had 53% infection with severity of 13 cm.

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W. Khoury. Components of resistance to Ascochyta rabiei in chickpea. International Center for Agricultural Research in the Dry Areas (ICARDA), Aleppo, P.O.Box 5466, Syria.

Ascochyta rabiei (Pass.) Lab is a major pathogen on winter-sown chickpeas in the Mediterranean region. Large variability in the reaction of the chickpea genotypes to the pathogen has been observed and several races of the A. rabiei have been identified. Under controlled environment, the interaction between 19 chickpea genotypes of various resistance levels and 6 races of the pathogen was studied in detail. Large differences were observed in the various genotype-race combinations for components of resistance such as the incubation and latent periods, lesion growth rate, pycnidial number and size and sporulation capacity.

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DISEASES WITH STRIP INTERCROPPING. K. M. Tubajika and C. A. Martinson, Plant Pathology, Iowa State Univ. Ames, IA 50011.

Diseases were assessed in corn, soybean, and an oat-alfalfa mixture planted contiguously in narrow (3-4 meter wide) strips with yearly crop rotation; this practice has increased yields of corn and oats. Plants on one edge of a strip are contiguous with the land planted to the same crop the prior season and with debris of the prior crop. Minimum tillage is required for strip intercropping. Five field experiments and two commercial fields were studied in 1990; disease incidence and severity were measured in both outside rows (or edges) of each strip. Inoculum in crop debris was sparse because of drought in 1989. With oats, soybean and alfalfa, foliar diseases developed first and more severely on the edge contiguous to crop debris than on the opposite side of the strip. The diseases included a complex of alfalfa leaf diseases, Septoria blights of oats and soybean, and bacterial blight of soybean. Eyespot, gray leaf spot, and Northern leaf blight of corn developed heavily and uniformly late in the season. Rotations with three crops appeared inadequate for foliar disease control. Stalk rot of corn was less prevalent in exterior strip rows than inner rows.

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RECOVERY OF RACE NON-CLASSIFIABLE PHYTOPHTHORA MEGASPERMA F. SP. GLYCINEA FROM SOYBEAN ROOTS IN INDIANA IN 1990. P.W. REESER, D.H. Scott, and G.E. Ruhl. Purdue University, West Lafayette, IN 47907.

Following cool, wet weather in July 1990, numerous soybean fields in Indiana exhibited root rot symptoms. Plants either wilted suddenly or were chlorotic and stunted and had internally discolored taproots. The degree of root deterioration varied. Phytophthora was detected serologically in 139 of 196 taproot samples collected from 137 sites. P. megasperma f. sp. glycinea was isolated from taproots in 31 samples from 25 sites. In hypocotyl-inoculation tests on 8 differential soybean cvs. routinely used for race identification 10 of 31 isolates were classified as races 1, 3, 4, 7, 8, or 13, all previously found in Indiana. Twenty isolates, including 13 from varieties with the Rps1-k gene for resistance, could not be classified by the existing race designation scheme, but were virulent on 2 or more differentials. Race non-classifiable isolates were often less aggressive than race classifiable isolates. One isolate was not pathogenic on the cvs. tested.

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EFFECT OF SILICON FERTILIZER GRADES ON BROWN SPOT DEVELOPMENT AND YIELD OF RICE. L. E. Datnoff, G. H. Snyder, and C. W. Deren, University of Florida-EREC, P. O. Box 8003, Belle Glade.

Brown spot of rice, caused by Bipolaris oryzae, can be severe on rice grown on organic soils (Histosols) of Florida because of their low silicon (Si) content. Amending these soils with Si has reduced the severity of brown spot and increased rice yields, but relatively high rates of Si fertilizers are required. A study was conducted to determine the efficacy of lower rates of a finely-divided Si material, with or without pelleting to facilitate application. Si was applied at 0, 2.5, and 5.0 Mg/ha as a standard grade material (90% < 2.36 mm), a fine grade (100% < 0.15mm) or as pellets (100 % > 1mm, < 3.35mm) made from the fine-grade material. Si applied at all rates and grades significantly ($P \leq 0.05$) decreased the severity of brown spot, increased silicon content in the plant and increased rice yields over the control. Si applied at 5.0 Mg/ha as a fine grade material was the best treatment.

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DEVELOPMENT OF GREENHOUSE INOCULATION TECHNIQUES FOR THE PRODUCTION OF FOLIAR SYMPTOMS OF SUDDEN DEATH SYNDROME OF SOYBEAN. P.A. Donald and J.A. Wrather; Department of Plant Pathology; 108 Waters Hall; University of Missouri; Columbia, MO 65211 and Delta Research Center; P.O. Box 60; Portageville, MO 63873.

Sudden death syndrome of soybean, caused by a blue isolate of *Fusarium solani*, is a root-rotting disease occurring in high yield environments. In the field, this disease is diagnosed by the appearance of interveinal chlorosis of the foliage which sometimes is followed by necrosis of the tissue. An inoculation technique is urgently needed so that soybean germplasm resistance may be determined by the reliable, reproducible development of symptoms in the greenhouse instead of by unpredictable field screening methods. Preliminary results indicate that a fungal slurry root-dip inoculation technique is most reliable.

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Effects and Control of Northern Corn Leaf Spot Disease in Wisconsin. G. L. Worf and K. A. Delahaut, Department of Plant Pathology, University of Wisconsin-Madison, Madison, WI 53706

Foliage damage by a recently identified pathotype of Bipolaris zeicola (Stout) Shoemaker (Helminthosporium carbonum Ullstrup) reduced yields of inbreds having a B73 background in some producers' fields by 25-40% in 1989 and 1990. Seed size, but not germination, was also affected. However, a post-emergence crown rot resulted in losses from 15-50% due to seedling mortality in greenhouse trials. Similar crown symptoms, e.g., a dry, brown to black internal and external discoloration, had been noted in the field. Roots appeared unaffected. Isolations from both crown and leaf tissue confirmed that the crown rot and foliage symptoms were caused by the same pathogen. Inoculation of greenhouse-grown corn seedlings with agar plugs containing the pathogen placed in close proximity to the crown resulted in crown rot 21-28 days later. Likewise, foliar inoculations made with an atomizer contained mycelia and spore suspensions isolated from both crown and leaf tissue produced leaf lesions. Propiconazole (Tilt), chlorothalonil (Bravo 720) and mancozeb (Dithane M45) controlled foliage symptoms in the greenhouse, but two "early symptom" post-tasseling applications at 13 day intervals in the field were ineffective. Seed transmission evaluation is implicated because of symptom development in greenhouse studies, although we have been unable to isolate the organism from seeds to date. Seed treatments with either captan or mancozeb, but not benomyl (Benlate) effectively controlled the crown rot phase of the disease.

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CHARACTERIZATION AND PATHOGENICITY OF RHIZOCTONIA FROM SOYBEAN. B. Nelson, I. Kural, T. Christianson, and T. Helms*. Depts. of Plant Pathology and *Crop and Weed Science, North Dakota State University, Fargo, ND 58105.

Rhizoctonia was isolated from stems and roots of soybeans collected from the Red River Valley of North Dakota, characterized to anastomosis groups (AG) and tested for pathogenicity in the greenhouse. Two binucleate Rhizoctonia and 41 isolates of R. solani consisting of 28 AG4, 10 AG5, 2 AG2-2, and 1 AG3 were obtained. AG4 and AG2-2 were highly pathogenic on soybean, AG5 was less pathogenic, AG3 was not pathogenic, and the binucleates caused minute lesions on roots. AG4 and AG2-2 were also pathogenic on dry bean, alfalfa, flax, sugarbeet, mustard and rape and AG2-2 caused root rot of corn. AG5 was pathogenic on dry bean but weakly pathogenic on the other crops and not pathogenic on corn.

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STUDIES ON THE SOUTHERN CALIFORNIA STRAIN OF VERTICILLIUM WILT OF ALFALFA. A.B. Howell and D.C. Erwin, Dept. of Plant Pathology, University of California, Riverside, CA 92521.

In 1987, Verticillium albo-atrum was found on alfalfa in desert areas of southern California where air temperatures often exceed 40 C. Studies were undertaken to compare isolates of this strain with those from northern temperate regions. Isolates from northern latitudes grew more rapidly at 27 C than those from southern California. None of the isolates grew at 33 C but regrowth of the southern isolates occurred after subsequent incubation at 22 C. Differences in colony morphologies were observed between the northern and southern isolates. In the field, greatest numbers of the southern strains were isolated from stem sections near the soil line where air temperatures were 5-10 C cooler than at the top of the canopy. The fungus survived for over 18 mo in baled hay and in stem pieces buried in field soil.

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EFFECTS OF PEANUT STUNT VIRUS AND MELOIDOGYNE INCOGNITA ON GROWTH OF WHITE CLOVER. M. R. McLaughlin†, G. L. Windham† and A. S. Heagle††, †USDA-ARS, Crop Science Research Laboratory, Forage Research Unit, Mississippi State, MS 39762 and ††USDA-ARS, Air Quality Research Program, Raleigh, NC 27606.

Diseases caused by peanut stunt virus (PSV) and Meloidogyne incognita (MI) and injury from ozone are major factors affecting white clover (Trifolium repens) in the southeastern U.S. Greenhouse experiments were conducted to measure separate and combined effects of PSV and MI on stolon growth of white clover clones, NC-R and NC-S, which are resistant and susceptible, respectively, to ozone injury. Stolon tip cuttings from PSV-infected and healthy plants of both clones were transplanted to soil in clay pots. Ten days later, 6000 MI eggs were added per pot. Plant growth and MI reproduction were measured after 8 wk. Root, stolon and top growth were reduced by PSV, which affected NC-R more than NS-S. Root, stolon and top growth were also reduced by MI. Nematode reproduction (eggs per gram of root) was greater on NC-S than NC-R, but not affected by virus treatment.

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PHYTOPHTHORA ROT OF GRAFTED CACTI. R. D. Raabe. Department of Plant Pathology, University of California, Berkeley, CA 94720.

Moon or star cacti consist of a number of different genera (many forms of which appeared originally as chimeras and because they contain little or no chlorophyll, if not left on the parent plant, must be maintained as grafted scions) grafted on rootstocks, a common one of which is *Hylocereus trigonus*. Recently, chlorophyll-less forms of *Chamaerocereus sylvestris*, and more commonly *Gymnocalcium nickolovii*, grown under hanging foliage plants in a greenhouse were found to have a rot which in early stages was dry but in advanced stages became soft. *Phytophthora parasitica* was isolated from the infected tissues. Zoospores were produced by flooding oatmeal agar cultures of the fungus and warming them after chilling for 2 hours. Plants inoculated with these were placed in a moist chamber for 24 hours, then moved back into the greenhouse. Symptoms appeared in 7 days. Control was attained by moving the plants from under the overhanging foliage plants.

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EVOLUTION OF BACTERIAL POPULATIONS RELATED TO DECLINE OF TAKE-ALL PATCH ON TURFGRASS. A. Sarniguet and P. Lucas, Station de Pathologie végétale, INRA, BP 29, 35650 Le Rheu, France.

Symptoms of take-all on turfgrass (Gaeumannomyces graminis var. avenae = Gga) occurs as patches of destroyed plants. On a golf course, at Benodet (Brittany), we observed a recolonization of the patch centers by the same grass species (Festuca sp.) previously killed by the fungus. The population of total bacteria was nearly the same in all zones across the patches. In contrast, the ratio of "fluorescent Pseudomonas / total bacteria" was respectively 1/26, 1/24.5, 1/3.5 and 1/3 in the disease free area, the front margin of the patch, in the destroyed part of the patch, and in the recolonized central part. Furthermore, in this last zone, 44 to 82% of the fluorescent Pseudomonas were antagonistic in vitro to Gga, vs only 12 to 34% from the disease free area. The development of take-all on turf induced quantitative and qualitative changes in fluorescent pseudomonads populations, illustrating on a space scale what probably occurs overtime during establishment of take-all decline on small grains.

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A NEW EXTENSION EDUCATION PROGRAM IN MARYLAND - THE HOME AND GARDEN INFORMATION CENTER. David L. Clement, Mary K. Malinoski, Raymond V. Bosmans and Denise D. Sharp, Regional Specialists, Cooperative Extension Service, University of Maryland System, 12005 Homewood Rd., Ellicott City, MD 21043.

The Home and Garden Information Center was started in 1989 in response to steadily increasing numbers of phone inquiries by homeowners in Maryland for gardening advice. The Center staff is made up of regional specialists, horticulture consultants and master gardener volunteers. The Center has provided a toll free phone answering facility for homeowner questions on horticulture and pest control. The Center also features an automated phone tape system on various gardening topics which is accessible 24 hours a day. The Center staff is involved with development of fact sheets, phone scripts, TV, radio and video tapes, master gardener classes, inservice training, and applied research. In its first year of operation the Center received a total of 35,079 phone calls. The distribution of the 19,688 calls answered by the staff were as follows: 36% on ornamentals, 25% on pest control, 12% on fruit and vegetables, 11% on turf, and 16% were on miscellaneous topics. Plant diseases made up 9% of the assisted calls and 24% of the plant samples. In the first year of operation the Center reduced the phone calls of the participating counties by approximately 30%.

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BICARBONATE SALTS OF POTASSIUM AND SODIUM CONTROL POWDERY MILDEW OF ROSES. R. K. Horst, S. O. Kawamoto, and L. L. Porter, Department of Plant Pathology, Cornell University, Ithaca, NY 14853.

Frequent fungicide applications are the only means of control of powdery mildew on rose; environmental safety is a logical concern. Powdery mildew on *Rosa* spp. caused by *Sphaerotheca pannosa* var. *rosae* was significantly controlled by weekly sprays of 0.05% (w/v) aqueous solution of either potassium or sodium bicarbonate plus 0.5% or 1.0% (v/v) Sunspray Ultra-fine Spray Oil, respectively. Control was evaluated on seven cultivars which were arranged into four significantly different ($P \leq 0.0002$) disease susceptibility groups ranging from highly susceptible to nonsusceptible as follows: (i) Samantha; (ii) Sonia, Bridal Pink and Royalty; (iii) Prive and Lavande; and (iv) Gold Rush. Control was by pathogen eradication by bicarbonates; analysis showed that combinations of sodium bicarbonate plus 1.0% oil was more effective ($P = 0.0002$) than either alone. Moreover, control of powdery mildew was also exhibited by potassium bicarbonate plus 0.6 ml Triton B 1956/3.8 l water, potassium bicarbonate plus 0.5% oil, or 0.5% oil. Therefore, potassium and sodium bicarbonates and oil appear to be effective biocompatible fungicides.

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A HISTOLOGICAL STUDY OF DOGWOOD ANTHRACNOSE PATHOGENESIS. E. T. Graham, M. T. Windham, K.R. Malueg, and D. A. Brown. P.O. Box 1071, Knoxville, TN 37901.

Leaf disks from greenhouse-grown Cornus florida were inoculated with Discula sp. and incubated on wet filter paper at 23 C. Disks were recovered at 24 hr intervals, cleared with chloral hydrate or embedded in paraffin, mounted in resin, and examined microscopically. Discula germ tubes were variable in length before they penetrated the cuticle and epidermis. Penetration was direct and evidence of enzymatic digestion of the surrounding entry site was not observed. Appressoria were not observed nor was penetration associated with

any particular anatomical structure of the leaf surface. Hyphae were not observed to penetrate through stomata. Rather conidia, deposited on guard cells, germinated and developing germ tubes grew away from stomatal pores.

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ROOT AND STEM ROT OF BRACHYCHITON POPULNEUS CAUSED BY LASIODIPLODIA THEOBROMAE. C. M. Sandlin and D. M. Ferrin, Dept. of Plant Pathology, Univ. of California, Riverside 92521.

Lasiodiplodia theobromae (Pat.) Griffon & Maubl. was found to be the causal agent involved in the death of several hundred Australian bottle trees (Brachychiton populneus Schott & Endl.) at a nursery in Oxnard, California. The taproots of the 18-mo-old trees, shipped bare-root from Israel, had been cut and treated with rooting hormone (without fungicides) before repotting. Infection, which appeared to have occurred at the cut surfaces of the taproots, resulted in deeply sunken lesions or complete collapse of the root tissue. Inoculation of healthy plants reproduced field symptoms. Internally, infected tissue of inoculated roots is soft and discolored, becoming cotton-like and green-grey to grey in color as the disease progresses. Spread of the fungus within root tissue was rapid; the fungus was reisolated 5 cm above the point of inoculation after only 5 days. Colonization of root tissue is followed by spread of the fungus into the stem, and death of the plant.

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Blights of Nematanthus and Radermachera caused by Phytophthora nicotianae in Hawaii. M. Aragaki and J. Y. Uchida, Department of Plant Pathology, University of Hawaii, Honolulu, 96822.

Foliar necrosis, defoliation, stem dieback, and stem and crown rot were symptoms of a severe blight problem in Nematanthus gregarius D. L. Denh. (guppy plant) confirmed to be caused by Phytophthora nicotianae B. de Haan. Flower blight and drop of open flowers and buds were early symptoms, followed by scalded leaves, brown-black rots of leaf lamina and petioles, shrivelling, and leaf drop. No disease symptoms were seen in plants up to four weeks following root inoculation, although approximately a third of the root tips were infected. Dull, grayish-brown irregular lesions on foliage of Radermachera sinica (Hance) Hemsl. (China doll), were also caused by P. nicotianae. Water-soaked, dark gray, irregularly-shaped expanding lesions on oldest leaves (unifoliolate or trifoliolate), and restricted tan spots on younger, bipinnately compound leaves were early symptoms of this disease. Infection progressed into stems, eventually killing most plants.

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Leaf blight of Leea coccinea caused by Phytophthora meadii in Hawaii. J. Y. Uchida and M. Aragaki, Department of Plant Pathology, University of Hawaii, Honolulu, 96822.

Leaf blight on red leea and green leea (Leea coccinea Planch.) caused by Phytophthora meadii Peries was observed in 1987. On red leea, spots were dark greenish-purple and irregularly shaped with water-soaked borders; these expanded rapidly, appeared scalded, became purplish-olive-green to brown and followed by wilting and considerable defoliation. Foliar necrosis was difficult to discern, because of leaf coloration. On green leea, expanding spots and blights were grayish-green to brownish-green, and readily visible; older lesions turned dark brown, then black. The disease spread into petioles and stems of both red and green leea, frequently resulting in plant death. The causal agent was confirmed as P. meadii. Sporangia of this fungus are deciduous with moderately long pedicels (means 12-15 um). Mean sporangial dimensions for eight isolates were 43-46 x 26-27 um, with L:D ratios 1.6-1.8.

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A BRIGHT-FIELD AND SCANNING ELECTRON MICROSCOPE STUDY OF TALL FESCUE INFECTED WITH PUCCINIA GRAMINIS SSP. GRAMINICOLA. M. M. Kulik and P. D. Dery. USDA, ARS, SARL, Beltsville Agricultural Research Center, Beltsville, MD 20705.

In 1989, a rust incited by Puccinia graminis ssp. graminicola caused serious losses of tall fescue in Oregon. The occurrence of this rust at Beltsville, MD and at New Brunswick, NJ provided additional material for a study of pathogen development within host tissues. Development was characterized by the formation of a dense layer of hyphae just below the cuticle. Concurrently, less dense hyphae ramified throughout the mesophyll layer, producing haustoria. Mycelium almost completely replaced host tissue by the later stages of infection. Uredinia and urediniospores arose from the dense layer of subcuticular hyphae. By late summer, teliospores began to form in uredinia, but eventually developed only in telia.

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IDENTIFICATION OF OPHIOSPHAERELLA HERPOTRICHA WITH A CLONED DNA PROBE. K. Sauer, N. Tisserat, and S. Hulbert. Kansas State University, Manhattan, 66506-5502.

Differentiation of Ophiosphaerella herpotricha from other ectotrophic fungi associated with spring dead spot of bermudagrass is difficult because of similarities in colony morphology and the inability to induce ascocarp formation in some isolates. Therefore, a DNA hybridization technique was developed to detect O. herpotricha in infected plant tissue. DNA of O. herpotricha was digested with XbaI and cloned. A 1.5 Kb insert (pOh29) strongly hybridized to total DNA of 29 isolates of O. herpotricha from four states, but not to DNA of 29 other fungal species, including Leptosphaeria korrae and Gaeumannomyces graminis var. graminis. pOh29 also hybridized to DNA of O. herpotricha isolated from 1 μg lyophilized mycelium and from 200 mg (wet weight) of infected bermudagrass roots, but not to DNA of healthy root tissue.

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DETECTION OF *Rhizoctonia* spp. CAUSING STEM ROT IN POINSETTIA CUTTINGS WITH ELISA. D. M. Benson, Department of Plant Pathology, North Carolina State University., Raleigh, NC 27695-7616.

A multiwell ELISA kit available from Agri-Diagnostics Assoc., Cinnaminson, NJ, was evaluated for detection of binucleate and multinucleate isolates of *Rhizoctonia* spp. on poinsettia cuttings. Rice grain inoculum of each test isolate was positioned 2 cm from the cutting. Poinsettia stem samples, 4 cm in length, were split in half for ELISA or cultured on acidified PDA. *Rhizoctonia* spp. were detected by ELISA and by culture within 2 days of inoculum placement for multinucleate isolates and 3 days for binucleate isolates. Binucleate isolates of *Rhizoctonia* spp. that did not cause stem rot were not detected with ELISA. Generally, multinucleate isolates which caused severe stem rot in 5 to 7 days gave absorbance readings between 1.0 and 2.0. A multinucleate isolate was detected in as little as a 1-mm long stem lesion (8.6 sq. mm) with the multiwell kit. In a survey of poinsettia cuttings with symptoms of stem rot from commercial greenhouses, the multiwell kit was reliable in detection of *Rhizoctonia* spp. compared to visual assessment and culture results. The multiwell kit may be useful for plant clinics.

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DETECTION OF RHIZOCTONIA SOLANI IN ASYMPTOMATIC CREEPING BENTGRASS BY ISOLATION AND ELISA. G. Y. Yuen and K. N. Kim. Department of Plant Pathology, University of Nebraska, Lincoln, NE 68583-0722.

The brown patch fungus, Rhizoctonia solani, was found to spread through asymptomatic foliage from active disease foci in a creeping bentgrass (Agrostis palustris) green. Cores of turf collected at various distances from the margins of symptomatic brown patch areas were dissected into foliage, thatch, and root fractions. Each fraction was tested separately for the presence of the pathogen by isolation on water agar and by Rhizoctonia-specific ELISA (Agri-Diagnostics). The fungus was detected consistently in foliage 30 cm from the margin of disease patches by isolation and ELISA, with good agreement (83%) between the 2 methods. The pathogen was isolated from thatch material most often at the margin, but was not isolated from any sample of root tissue. There was little agreement between isolation and ELISA when thatch or root tissues were tested, and isolation appeared to be the more reliable method.

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PRELIMINARY SURVEY OF ORCHID VIRUSES IN HAWAII. J. S. Hu, M. Wang, and S. Ferreira. Department of Plant Pathology, University of Hawaii, Honolulu, HI 96822.

Approximately 1000 orchid plants in three orchid collections and four commercial farms in Hawaii were surveyed for cymbidium mosaic virus

(CyMV), odontoglossum ringspot virus (ORSV), tomato spotted wilt virus (TSWV), and potyviruses using ELISA. CyMV and ORSV were detected in 63% and 34% of 30 genera surveyed, respectively. Double infection was found in 30% of the genera. Most commercial *Dendrobium* orchids grown in Hawaii are seed-propagated hybrids produced by the HorticultureDepartment, the University of Hawaii (UH). Commercially grown UH hybrids less than three years old were all virus free, indicating that CyMV is not transmitted through the seeds. However, UH hybrids older than five years were virus free only in one farm where tool and handling sanitation was enforced. This example suggests that the use of virus-free materials and proper sanitary practices in orchid production is an effective control of CyMV. Although thrips and aphids are common insect pests in commercial orchid farms in Hawaii, TSWV and potyviruses were not detected in any of the samples.

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PERSISTENCE AND ENDEMICITY OF PATHOGENS IN PLANT POPULATIONS OVER TIME AND SPACE. D. W. Onstad, Illinois Natural History Survey, 607 E. Peabody Dr., Champaign, Illinois, 61820.

A simple model was simulated on a Connection Machine supercomputer to study pathogen persistence and the temporal and spatial dynamics of a hypothetical pathosystem consisting of one foliar pathogen and one host species in a region of 8,192 host sites. The pathogen persisted longer when the potential reproduction per lesion iR, host density, and spatial heterogeneity of iR were increased. Host growth also enhanced persistence. Theorems of Van der Plank and Jeger were challenged by the results. Spatial scales and appropriate predictor variables must be included in theorems so that they can be properly tested.

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BACTERIAL RING ROT SYMPTOM EXPRESSION IN POTATO IS INFLUENCED BY GEOGRAPHIC LOCATION. S. A. Slack and Alan Westra. Plant Pathology Department, Cornell University, Ithaca, New York 14853.

Three potato cultivars, Russet Burbank, Norchip, and Norland (late, medium, and early maturity respectively), inoculated with 10^2, 10^6, and 10^9 CFU of *Clavibacter michiganensis* subsp. *sepedonicus* (CMS), were planted as a randomized complete block design in diverse potato producing regions of the United States (CO, ME, NY, ND, OR, WI, and WA) during 1986-1990. Plant growth (emergence, plant height, senescence, and yield) and disease incidence and severity were monitored over the course of the growing season. Plant growth was influenced by geographic location, but generally was not affected by inoculation with CMS. Maximum disease incidence and severity ranged from 1.6% (CO, 1986) to 90.0% (ME, 1988), and 1.8% (ND, 1988) to 49.2% (WI, 1988), respectively. Regions could be grouped, based on disease incidence, into three categories: Low (CO), Medium (ND, WI) and High (ME, OR, WA), indicating that environmental conditions influence symptom expression. Relative foliar and tuber symptom expression in the three cultivars generally remained constant (Russet Burbank > Norchip > Norland) irrespective of geographic location.

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EFFECT OF PLANT DENSITY ON THE SPREAD OF GRAY LEAF SPOT OF CORN. N. R. X. de Nazareno, L. V. Madden and P.E Lipps, Dept. of Plant Pathology, The Ohio State University and Ohio Agricultural Research and Development Center, Wooster, OH 44691.

Spread of gray leaf spot, caused by Cercospora zeae-maydis, was studied under different plant densities. Plots consisted of sixteen rows (12.2 m long, 0.76 m apart) of the susceptible hybrid Pioneer Brand 3569. Inoculum in the form of infected corn residue was spread over 2.3 m^2 (85% soil coverage) in the center of each plot. Disease severity was estimated weekly by counting lesions on three leaves of plant pairs within and across rows up to 6 m from the inoculum center. Disease proportions were obtained by dividing total estimated lesion area by estimated leaf area. Disease gradients were described by the exponential model; temporal progress was described by the logistic model. Gradient slope was significantly affected by plant density x direction interaction; average disease severity was affected by plant density x time and direction x time interactions (P=0.05). At 91 days after planting, disease severity was 0.9%, 1.3%, and 1.7% for densities of 72600, 46200, and 19800 plants/ha, respectively.

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LONGEVITY OF TELIOSPORES OF USTILAGO SCITAMINEA IN SOIL. J. W. Hoy and Zheng Jiaxie. Dept. of Plant Path. and Crop Phys., La. Agr. Experiment Station, Louisiana State Univ. Agricultural Center, Baton Rouge, LA 70803.

Teliospores of Ustilago scitaminea, the causal agent of sugarcane smut, were affected by fungistasis when placed in field soils. Spore germination after 8 hr ranged from 1-14%. Few spores (1%) remained viable after 4 wk in saturated soil, and none were viable after 6 wk. Spore viability was lost after 6-9 wk in five soils at three moisture levels. Spores also lost viability in sterilized soils. Variation in spore longevity was observed for different spore collections in air-dried soils. Spores in air-dried soils remained viable when kept under desiccation, and spores from five locations maintained free of soil at ambient relative humidity lost viability within 6 mo. Results indicate that spores produced during one season will not persist to the next season. The absence of inoculum during the Spring tillering season represents an additional factor limiting the increase of sugarcane smut in Louisiana.

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ESTABLISHMENT AND SPREAD OF MOSAIC IN SUGARCANE. M. P. Grisham, USDA, ARS, Sugarcane Research Unit, Houma, LA 70361.

Establishment and spread of mosaic, caused by sugarcane mosaic virus (SCMV) strain H, was monitored in three sugarcane field of a susceptible cultivar, CP65-357. Two fields were planted in the fall of 1988 with sugarcane stalks of which <5% were systemically infected with SCMV. A third field was established in the spring of 1989 with plantlets from apical meristem cultures free of SCMV. Mosaic infection was monitored in randomly distributed 3 m plots in the fall-planted fields, and among all plants in the spring-planted field. Mosaic incidence was recorded monthly (April to November) for two years. Initial establishment of mosaic, other than that introduced by planting infected stalks, was random, except in one fall-planted field where mosaic incidence was higher in plots adjacent to the bordering drainage ditches (20%) than in plots in the interior of the field (6%). The plants and plots infected with mosaic in the first year did not appear to be foci for infections the second year.

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APPLICATION OF A STOCHASTIC MARKOV CHAIN TO MODEL SPATIAL AND TEMPORAL PROGRESS OF ANTHRACNOSE. S. Chakraborty[1], G.K. Smyth[2], A.N. Pettitt[3] & R.G. Clark[2]. [1]CSIRO, 306 Carmody Road, Australia 4067, [2]University of Queensland, Australia, [3]Queensland University of Technology, Australia.

Spatial and temporal progress of anthracnose (Colletotrichum gloeosporioides) in quantitatively resistant accessions of the tropical pasture legume Stylosanthes scabra was modelled using a Markov chain. Inoculated susceptible plants, raised in the center of each plot, initiated an epidemic and plants raised at radial distances of 0.35, 1.06, 1.77, 2.47 and 3.18m from the center were regularly rated for severity. Proximity of plants to the inoculum source and the resistance level of accessions determined the rate of disease spread. The probability of individual plants becoming infected or increasing in severity was analysed with an ordinal regression model using severity of first and second order neighbors and time between successive ratings as covariates. The probability of a disease-free plant with disease-free neighbors becoming infected within a one week period was estimated to be 52% for a susceptible, 2.8% for a resistant, and between 6.5 & 23% for quantitatively resistant accessions. Accession ranking based on an accession effect parameter was highly correlated with the ranking based on the area under the disease progress curve.

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INTERSPECIFIC ASSOCIATIONS AMONG FIVE FOLIAR PATHOGENS OF WHITE CLOVER IN A CLOVER/TALL FESCUE PASTURE. Scot C. Nelson and C. Lee Campbell. Department of Plant Pathology, North Carolina State University, Raleigh 27695-7616.

Incidence of five foliar pathogens (Cercospora zebrina, Colletotrichum sp., Pseudomonas andropogonis, Rhizoctonia sp., and Stagonospora meliloti) of white clover was monitored from July to September, 1990 in eight 64-plant plots of virus-resistant (Southern Regional Virus Resistant germplasm) and virus-susceptible (cv. Regal) white clover in a 10 ha clover/tall fescue pasture. Indices of interspecific association revealed a continually changing pattern of associations among pathogens. In mid-July, an overall net negative association among species was found in most plots; by late September, species were positively associated. Pathogens were more positively associated after the mid-season harvest. Associations among specific pairs of pathogens on virus-free plants were found to be reversed (e.g., from positive to negative) on virus-infected plants in some plots.

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SPLASH DISPERSAL OF TWO STRAWBERRY FUNGAL PATHOGENS BY SINGLE DROP IMPACTION. X. Yang, L. V. Madden, M. A. Ellis, and L. L. Wilson. Dept of Plant Pathology, OARDC/OSU, Wooster, OH 44691.

Dispersal of *Colletotrichum acutatum* and *Phytophthora cactorum* from strawberry fruit by single drop impaction was studied using a drop-generating system. Uniform water drops, 0.5-4 mm in diameter, were released from heights of 25-100 cm above infected target fruits with spores labeled with fluorescent tracer. Splash droplets were collected on water sensitive paper up to 50 cm from the fruit. Size and distance of each droplet trace were determined using an image analysis system, and number of spores contained in each trace were counted by means of fluorescent microscopy. Size and release height of impacting drops were shown to have a significant effect on mean travel distance and total numbers of droplets and dispersed spores, but not on mean number of spores per droplet. Number of spores per droplet were fairly well described by the log-normal distribution for *C. acutatum* and the negative-binomial distribution for *P. cactorum*.

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Influence of Temperature and Wetting Duration on Infection of Apple Leaves by *Alternaria mali*. N. Filajdić and T. B. Sutton, Department of Plant Pathology, North Carolina State University, Raleigh 27695-7616.

The effects of combinations of nine different temperatures and eight wetness durations on the infection of Delicious apple seedlings by *Alternaria mali* were examined. Disease severity increased with greater wetness duration for all temperatures and was greatest from 12-28 C. The relationship of temperature and wetness duration to infection of apple seedlings by *A. mali* was adequately described by the model: $Y_{ij}=2.2927 + 0.4221T_i + 0.0422W_j - 0.0104T^2$, where Y=(% leaf area with lesions + 0.5)$^{0.5}$, T=temperature (C), W=wetness duration (hr), i=temperature treatment, and j=wetness duration treatment. The predicted optimum T for infection was 20.3 C. At this temperature, 5.34 hr of W were required for light infection (2% leaf area covered with lesions). The model was tested in the field using Delicious apple seedlings. Conidia of *A. mali* were trapped during 16 wetting periods; infection occurred only when predicted, although no infection occurred in six periods when it was predicted.

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NATURE OF SILVERLEAF SYNDROME IN SQUASH. N. Bharathan, K.R. Narayanan, and L.J. Ramos, University of Florida, IFAS, TREC, 18905 SW 280 Street, Homestead, FL 33031.

There is a high correlation between sweetpotato whitefly (*Bemisia tabaci* Genn.)-associated silverleaf syndrome and the presence of two double-stranded RNA's of sizes 4.2 and 4.6-kb in squash (Plant Pathology 39: 530-538, 1990). The severity of silverleaf symptoms was correlated with the density of adult *B. tabaci* and level of dsRNA. Crude extracts from symptomatic leaf tissue had a 100-fold increase in RNA-dependent RNA polymerase activity when compared to extracts from healthy tissue. The dsRNA appears to be translocatable in the plant with limited synthesis in the host plant in the absence of *B. tabaci*. The putative causal agent of silverleaf syndrome was graft transmissible as evidenced by the occurrence of the symptoms in grafted plants not exposed to *B. tabaci*. All *Cucurbita pepo* cultivars tested were susceptible to silverleaf syndrome and also contained the dsRNA's. In contrast, *Citrullus lanatus*, *Cucumis melo*, *Cucumis sativus*, *Cucurbita maxima* and *Lagenaria siceraria* did not exhibit silverleaf symptoms and did not contain the dsRNA.

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DISEASES OF *BRASSICA CAMPESTRIS*, *CRAMBE ABYSSINICA*, AND OTHER ALTERNATIVE CROPS IN MISSOURI. J. D. Mihail, S. J. Taylor, E. R. Champaco, Dept. of Plant Pathology, and R. L. Myers, Dept. of Agronomy, University of Missouri, Columbia, MO 65211.

Recent interest in diversification of Missouri agriculture has resulted in the examination of a number of plant species as alternatives to traditional crops. In 1990, experimental plantings of alternative crops were monitored in three locations in central Missouri for the occurrence of pathogens which might limit future commercial production. Of the 20 plant species evaluated, those with the most potentially damaging pathogens were: *Amaranthus cruentus* (grain amaranth), affected by charcoal rot (*Macrophomina phaseolina*); *Brassica campestris* (spring rapeseed), affected by blackleg (*Phoma lingam*) and powdery mildew (*Erysiphe* sp.); *Crambe abyssinica*, affected by bacterial stem rot (*Xanthomonas campestris*); and *Carthamus tinctorius* (safflower), affected by charcoal rot (*M. phaseolina*). Details of the etiology and impact of these diseases will be presented.

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EFFECT OF TEMPERATURE ON AGGRESSIVNESS OF *RHIZOCTONIA SOLANI* KUHN ON SOYBEAN LEAVES AND SEEDLINGS. C.S. Kousik and J.P. Snow. Dept. of Pl. Path. & Crop Phys., La. Ag. Expt. Sta., La. St. Univ. Ag. Center, Baton Rouge, LA 70803

Forty-five isolates of *Rhizoctonia solani* Kühn from various geographic locations and host plants, representing 11 of the 12 known anastomosis groups (AG's) were tested for pathogenicity and aggressivness on soybean leaves (V5 stage) at 15, 20, 25, 30 and 35 C, and on soybean seedlings (Ve stage) at 20, 25, and 30 C. Numbers of infection cushions formed on soybean leaves by the isolates at these five temperatures were recorded using light microscopy. Isolates of AG-1 IA and IB and AG-5 were significantly more aggressive on soybean leaves at 20, 25 and 30 C (optimum 25-30 C) than any other isolates. AG-1 IC and AG-4 were aggressive at 25 C but, significantly less aggressive than AG-1 IA, IB and AG-5. Maximum numbers of infection cushions were formed on soybean leaves by AG-1 (IA, IB and IC), AG-4 and AG-5 at 25 and 30 C. The other AG's tested did not form infection cushions on soybean leaves although some caused minimal disease severity. Isolates of AG-1 IA formed significantly more infection cushions and caused greater disease severity than AG-1 IB and other isolates at 35 C. Maximum seedling infection, based on mean disease severity, disease rating, and lesion length per seedling occurred at 25 C for AG-1 (IA, IB and IC). AG-4 and AG-5, caused greater seedling infection at 20 C than at 25 and 30 C. Of the other AG's tested, AG-2 and AG-9 caused minimal damage to the seedlings and the rest were not pathogenic. Isolates of AG-4 and AG-5 are not known to cause Rhizoctonia foliar blights of soybean in Louisiana, but our data indicates their potential to be very destructive pathogens. Infection cushion formation by *R.solani* appears to be a better measure of pathogenicity than disease severity alone.

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PHENOTYPIC AND GENETIC VARIATIONS IN *DIAPORTHE PHASEOLORUM* VAR. *CAULIVORA*, THE SOYBEAN STEM CANKER PATHOGEN. Y.H. Lee and J.P. Snow. Department of Plant Pathology and Crop Physiology, Louisiana Agricultural Experiment Station, Louisiana State University Agricultural Center, Baton Rouge, LA 70803.

Fourteen isolates of *Diaporthe phaseolorum* var. *caulivora* (Dpc) representing six southern states (AK, FL, GA, LA, MS, and TN) and two northern states (IO and OH) were compared for colony morphology, response to two different temperatures (22 C and 30 C), style of perithecia formation, phenol oxidase activity, and virulence to the soybean cultivar 'Bedford'. All northern isolates produced white colonies with dense tufts of mycelia, whereas southern isolates exhibited uniform white, dark brown, or light brown mycelia. Mycelial growth of all isolates tested was significantly inhibited at 30 C. Observation of perithecia with light and scanning electron microscopes showed singly-borne and caespitose perithecia formed by southern (except FL40) and northern isolates, respectively. Northern isolates showed strong phenol oxidase activity compared to southern isolates. Northen isolates were highly virulent to 'Bedford', but southern isolates exhibited diverse virulence. To compare genetic relationship between the two populations, phenol-soluble polypeptides from selected isolates were compared by two-dimensional electrophoresis. Greater genetic diversity was observed among southern isolates than among northern isolates. This genetic diversity might reflect phenotypic diversity in southern isolates. The strong relationship between the two populations in polypeptide analysis suggested that two populations of Dpc in northern and southern areas of the United States are the same fungus.

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RESTRICTION FRAGMENT LENGTH POLYMORPHISMS OF MITOCHONDRIAL AND NUCLEAR DNA AMONG GEOGRAPHIC ISOLATES OF PHYTOPHTHORA CAPSICI. B.K. Hwang. Department of Agricultural Biology, Korea University, Seoul 136, Korea.

Seventeen isolates of Phytophthora capsici from various pepper-growing countries of the world were examined for restriction fragment length polymorphisms of mitochondrial and nuclear DNA. Several homogeneous RFLP groups could be distinguished in mitochondrial and nuclear DNA of the geographic isolates. Isolates of the same group showed identical RFLPs, with only one exception. The size of mtDNA ranged from 35.4 to 36.5 kb, due to length mutation within a short sequence of the genome. RFLPs were correlated to some extent with geographic origin of the isolates. Mit DNA variation was not similar to that observed with the nuclear DNA. In particular, the great diversity in mt and nuclear DNA was detected in the European isolates.

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HAZARD RATING MODEL DEVELOPMENT FOR DECLINE IN BOTTOMLAND OAKS IN THE SOUTHEAST. Vernon Ammon, Evan Nebeker, Francis McCracken, and Jim Solomon, Plant Pathology and Weed Science, P.O. Drawer PG, Mississippi State University, Mississippi State, MS 39762, and USFS Southern Hardwoods Laboratory, Stoneville, MS 38776.

Oak decline is a disease of unknown etiology. Interactions among the abiotic and biotic agents implicated are poorly

understood. Our hypothesis is that oak decline in southern hardwood bottomlands is associated with specific site and stand characteristics and that these characteristics can be measured and utilized in analytical techniques to separate decline from non-decline (healthy) sites. Biological and edaphic data were collected from over 3,000 trees growing in 272 field sites at 22 locations in seven states. Canonical discriminant analysis was used to determine the subset of these variables that best differentiated between decline and non-decline sites. A set of seven variables for the Tennessee-Tombigbee River basin explained approximately 85% of the differences between decline and non-decline sites. Similar analyses of data collected form the Mississippi River basin identified a set of 10 variables that accounted for approximately 82% of the variation between decline and non-decline sites.

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IMPORTANCE OF HEARTROT FUNGI IN ASPEN IN CREATING POTENTIAL NESTING SITES FOR PICIDS. John H. Hart and D.L. Hart, Department of Botany and Plant Pathology, Michigan State University, East Lansing, MI 48824

To determine the number of cavity-containing stems in aspen (Populus tremuloides) >80 years-old, we counted the number of aspen which contained cavities in 132 0.02-ha plots in Wyoming. There were 8.7 stems with cavities/ha. At least 84% of the stems were alive when the initial cavity was constructed. Phellinus tremulae basidiocarps were present on 70% of all cavity-bearing stems but on only 9.6% of stems >15 cm dbh. Cavities were present in 7.7% and 0.2% of living stems with and without basidiocarps, respectively. The frequency of P. tremulae fruiting bodies increased from 6% on stems <15 cm dbh to 13.5% for stems >30 cm dbh. Average dbh of cavity-bearing stems was 27.4 cm. P. tremulae caused heartrot in aspen while the sapwood remained intact, protecting the nest cavity. These data indicate that primary cavity-nesting birds preferentially selected living aspen stems with heartrot as nest sites.

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WHITE-SPORED PERIDERMIUM HARKNESSII IN NORTH DAKOTA. J.A. Walla, G.A. Tuskan & J.E. Lundquist, North Dakota State Univ., Fargo, ND, 58105, Oak Ridge Natl. Lab, Oak Ridge, TN, 37831, and U.S. Forest Service, Rapid City, SD, 57701, respectively.

Nearly 2% of the normally orange-spored *P. harknessii* galls in a native *Pinus ponderosa* stand in North Dakota had white spores. Habitat, host reaction, occurrence on the same gall, spore size and germination, axenic culture morphology, and isozyme phenotypes were all similar between white- and orange-spored isolates. Several papers report differences, including habitat, host reaction and spore germination, between white- and orange-spored isolates of *P. harknessii* from the southwestern U.S.A. Such differences may indicate that white spore color in the southwestern isolates is characteristic of genetically distinct populations. In contrast, white spore color in North Dakota is notable in that it occurs in isolates that are phenotypically similar to orange-spored isolates. This trait may be valuable as a marker to distinguish between similar isolates in pathogenic and genetic studies.

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CONTROL OF DECAY IN DOUGLAS-FIR AND SOUTHERN PINE BY TRICHODERMA. T. L. Highley and L. Ferge, U.S. Dept. of Agric., Forest Service, Forest Products Laboratory, One Gifford Pinchot Drive, Madison, WI 53705-2398.

Field trials were conducted to determine the ability of a biological control product (Binab T pellets and wettable powder, Trichoderma spp.) to colonize and prevent decay in Douglas-fir and Southern pine. The ends of freshly-cut Douglas-fir piling were brush-treated with Trichoderma wettable powder. Squared Douglas-fir and Southern pine timbers exposed above ground were inoculated with Trichoderma pellets. Material was exposed in three different climates. Douglas-fir was poorly colonized by Trichoderma. Trichoderma extensively colonized Southern pine timbers, but did not prevent decay by the brown-rot fungus, Gloeophyllum trabeum. Wood blocks removed from areas of Southern pine colonized by Trichoderma were not resistant to attack by decay fungi in laboratory tests.

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PROPICONAZOLE AS A TREATMENT FOR OAK WILT IN *QUERCUS RUBRA* AND *Q. ELLIPSOIDALIS*. N.K. Osterbauer and D.W. French, Department of Plant Pathology, University of Minnesota, St. Paul, MN 55108.

In Minnesota, mature *Quercus ellipsoidalis* and *Q. rubra* at high risk to natural infection by *Ceratocystis fagacearum* were injected with propiconazole as a possible control for oak wilt, caused by *C. fagacearum*. Trees were treated preventively in 1989 and 1990 at a rate of 0.21 g/L A.I. and 0.8 L/cm dbh. Five of the 49 trees injected in 1989 wilted. A total of 3 out of 50 control (untreated) trees wilted. In 1990, 88 trees were injected and 4 of these wilted the same summer. Of 80 control trees, 36 wilted. Results from 1990 suggest propiconazole is effective as a preventive treatment for oak wilt in *Q. ellipsoidalis* and *Q. rubra*. Injections of two *Q. ellipsoidalis* indicated propiconazole may not be effective as a therapeutic treatment.

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ROLE OF PECTATE LYASE IN THE PATHOGENICITY OF FUSARIUM ROOT ROT OF RED PINE SEEDLINGS. Finan, E.I., Bruhn, J.N., and Podila, G.K. Department of Biological Sciences and School of Forestry and Wood Products, Michigan Technological University, Houghton, MI 49931

Fusarium oxysporum f.sp. pini causes heavy losses of conifer seedlings in nurseries and greenhouses. Typical symptoms on young seedlings include maceration of root tissue just below the soil level. While all tested isolates of this fungus were found to produce cell wall degrading enzymes, only virulent isolates were found to produce high levels of pectate lyase (PL). Thus the PL gene seem to play a critical role in the pathogenicity of F. oxysporum f.sp. pini. Both pectin, and cell wall components of pine seedling roots induced in vitro production of pectate lyase by F. oxysporum f.sp. pini. Using heterologous probe, we have determined that there are two copies of PL gene in our most virulent isolates of F. oxysporum f.sp. pini. Our ultimate objective is to develop an effective biocontrol for F. oxysporum f.sp. pini by selectively "knocking out " the PL gene through cloning and gene replacement. Progress made in the characterization and the cloning of PL gene will be discussed.

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ISOLATION OF A PHYTOTOXIN FROM MYCOSPHAERELLA DEARNESSII, THE CAUSAL AGENT OF BROWN SPOT NEEDLE BLIGHT OF PINE. Z. H. Huang[1], E. B. Smalley[1] and C. D. Li[2], [1]University of Wisconsin-Madison, Madison, WI, and [2]Nanjing Forestry University, Nanjing, China.

Toxins associated with the production of symptoms by the brown spot fungus, Mycosphaerella dearnessii, are recognized, but have not been identified or characterized. Slash pine needles placed in cell-free aqueous extracts from M. dearnessii rice or wheat cultures became yellow and wilted in 4 days. Response time of immersed needle fragments, or pine calli, were more rapid (e.g. 24 hrs and 3 hrs, respectively). Toxic extracts were not host specific since elm cuttings in the 2-4 leaf stage without roots were immersed in extracts and also wilted within 24 hrs. Toxic concentrates were heat resistant (e.g. 10 min at 100°C), non-proteinaceous, and the molecular weight was <1 Kd as determined by dialysis procedures. Purification and chemical structure determinations are continuing.

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VEGETATIVE COMPATIBILITY AND HYPOVIRULENCE CONVERSION OF CRYPHONECTRIA PARASITICA ON AMERICAN CHESTNUT TREES IN MASSACHUSETTS. Jong-Kyu Lee, T. A. Tattar, P.M. Berman, and M. S. Mount. Dept. of Plant Pathology, University of Massachusetts, Amherst, MA 01003.

From Dec. 1989 to May 1990, 102 virulent (V) strains of Cryphonectria parasitica were isolated from the cankers of American chestnut (Castanea dentata) trees in western MA. The diversity of vegetative compatibility groups (VCGs) of C. parasitica was investigated. The 102 strains represented 54 VCGs; 38 VCGs had only 1 strain each, 6 VCGs had 2 strains each; and the 10 most common VCGs had 52 strains. We attribute the great diversity in VCGs to the increasing numbers of VCGs over time (pathogen has been in MA for 80 yrs) (Anagnostakis, S.L., Kranz, J. 1987. Phytopathology. 77:751-754). Ten vegetative compatibility (v-c) strains were selected from the 10 most common VCGs and converted to hypovirulent (H) strains through pairing with H strains (4 strains with French dsRNA, 17 strains with Italian dsRNA) from S.L. Anagnostakis, Conn. Agric. Exp. Sta.

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COMPARISON OF CRYPHONECTRIA PARASITICA STRAINS FROM MASSACHUSETTS IN CULTURAL CHARACTERISTICS, PATHOGENICITY, AND PHENOL OXIDASE ACTIVITY. Jong-Kyu Lee, T. A. Tattar, P.M. Berman, and M. S. Mount. Dept. of Plant Pathology, University of Massachusetts, Amherst, MA 01003.

Ten vegetative compatibility (v-c) strains from MA, 21 hypovirulent (H) strains, and 29 converted hypovirulent (CH) strains were compared in cultural characteristics, pathogenicity, and phenol oxidase(PO) activity. The growth of strains was higher on PDA with ground bark tissue than on PDA with ground wood tissue. Pathogenicity was tested in 3 methods: tree inoculation, stem sections (2-3 cm dia.), and bark-wood tissue sections. The latter test was best and consisted of 2.5 cm long stem sections split longitudinally. The inner bark (phloem) tissue was separated from wood (xylem) tissue. Both tissues were inoculated with 5 mm dia. mycelial plugs, and kept on moist filter paper in Petri dishes at 25°C. Percent colonization and color reaction were measured (at 5 days). PO activity on media amended w/different phenolic compounds was tested. Tannic acid media was the best source of color reactions for PO activity. MA strains appear physiologically similar to those from other U.S. locations.

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RELATIONSHIP OF TISSUE WATER POTENTIAL AND VIABILITY TO GROWTH OF HYPOXYLON ATROPUNCTATUM IN OAK STEMS. J.D. Mason, L.C. Chaudoir and P. Fenn. Department of Plant Pathology, University of Arkansas, Fayetteville, AR 72701.

Drought stress is presumed to initiate the rapid colonization of infected oaks by Hypoxylon atropunctatum. The relationships among tissue water potential (WP), host tissue viability and fungal growth were investigated in inoculated stem segments incubated at ca. 100% or ca. 50% relative humidity (RH) at 25° C. At 100% RH, WP remained constant averaging -0.90 MPa for at least eight days and the fungus showed little or no growth from the inoculation wound. At ca. 50% RH, rapid growth of the fungus began when the WP dropped to an average of -1.66 MPa after 3-4 days incubation. That decreased WP, as such, was not the stimulus for fungal growth was suggested by experiments with stem segments killed by mild heat (57° C, 6 hr). In killed stems the time and rate of fungal growth were the same regardless of differences in water content. This suggests that changes in host tissue viability (determined by vital staining with triphenyltetrazolium) may be responsible for changes in susceptibility to fungal colonization in water-stressed stems. Time course studies in drying stems indicated that fungal growth began when tissues were still viable, but that the tissues soon lost their viability. The relationship of changes in host tissue viability to fungal growth was observed in both actively growing and dormant stems.

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GENES REQUIRED FOR CELLULOSE SYNTHESIS BY AGROBACTERIUM TUMEFACIENS. A. G. Matthysse, K. S. Sears, and T. M. Rosche. Department of Biology, CB# 3280, University of North Carolina, Chapel Hill, NC 27599-3280.

A. tumefaciens was found to synthesize cellulose fibrils when the bacteria were grown in contact with plant cells or plant extracts. Transposon Tn5 mutants which fail to synthesize cellulose as well as Tn5 mutants which overproduce cellulose were isolated. All of these mutations were located on the bacterial chromosome. A clone was identified from a library of A. tumefaciens strain NT1 DNA which complemented all of the cellulose minus mutants. Insertions of Tn3 HoHo1 were used to map the region of the cloned DNA required for cellulose synthesis. This appeared to be a region of about 15 kb. Subclones of this region were examined and sequenced. In addition cell free extracts of A. tumefaciens were able to incorporate UDP-^{14}C-glucose into cellulose. Extracts from cellulose minus mutants were unable to carry out this reaction. The ability of mixtures of extracts from various mutants to synthesize cellulose was examined in an attempt to determine which mutations affected different functional steps in cellulose synthesis.

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GENE ACTION OF ADULT-PLANT RESISTANCE TO STRIPE RUST IN WHEAT CULTIVARS DRUCHAMP AND STEPHENS. Xianming Chen and Roland F. Line. USDA/ARS, Department of Plant Pathology, Washington State University, Pullman, WA 99164-6430.

Gene action of adult-plant resistance in Druchamp (DRU) and Stephens (STE) to *Puccinia striiformis* was studied based on means and variances of areas under the disease progress curve of parents and F1, F2, and BC progeny from diallel, reciprocal crosses of DRU with STE and of DRU and STE with Paha and Michigan Amber (MA). Adult-plant resistance in DRU and STE was partially dominant or recessive. Additive gene action was the major component for the adult-plant resistance. In crosses with susceptible MA, additive, dominance, and non-allelic interactions were significant for DRU but only the additive component was significant for STE. In the other crosses, additive and dominance components were usually significant and non-allelic interactions were sometimes significant. Cytoplasmic effects were evident only in reciprocal crosses of resistant STE with susceptible Paha.

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GENE NUMBER AND HERITABILITY OF ADULT-PLANT RESISTANCE TO STRIPE RUST IN WHEAT CULTIVARS DRUCHAMP AND STEPHENS. Xianming Chen and Roland F. Line. USDA/ARS, Department of Plant Pathology, Washington State University, Pullman, WA 99164-6430.

Wheat cultivars Druchamp (DRU) and Stephens (STE) have durable, adult-plant resistance to *Puccinia striiformis*, as well as race-specific resistance expressed in seedlings. Parents and F1, F2, BC, F3, and F5 progeny from diallel, reciprocal crosses of DRU with STE and of DRU and STE with Paha and Michigan Amber (MA) were tested at Pullman, WA in two plots, one inoculated with a race virulent on DRU, STE, and MA and one with a race virulent on Paha, and at Mt. Vernon, WA exposed to races virulent on DRU and STE. Gene number and heritability were estimated based on infection type and means and variances of areas under the disease progress curve. Results were similar at all three sites. DRU and STE each have two to three genes for adult-plant resistance. The genes in DRU and STE were different from each other and from genes detected in seedlings. Both broad-sense and narrow-sense heritabilities were high (75.6% - 99.8%).

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DIVERSITY OF VIRULENCE IN PUCCINIA CORONATA ON OATS IN THE USA. K. J. Leonard, USDA, ARS Cereal Rust Lab, University of Minnesota, St. Paul, MN 55108.

Single-pustule isolates obtained from 55 collections of Puccinia coronata (one/collection) from 12 states of the USA were tested for virulence on 30 oat lines with known single genes for crown rust resistance and on 46 backcross lines with unidentified resistance genes from Avena sterilis. Frequency of virulence to these lines was bimodal; 40% of the lines were resistant to less than 1/3 and 48% were resistant to more than 2/3 of the isolates. Three lines were resistant to all and 8 were susceptible to all isolates. On average, individual isolates were virulent on about half the lines (mean 50.2%, median 48.6%, range 32-70%). Frequencies of virulence to 11 of the lines differed significantly between isolates from north central (SD, MN, and WI) and south central (TX, KS, LA, and AR) states.

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THE CONTRIBUTION OF DEGRADATIVE ENZYMES TO THE PATHOGENICITY OF *COCHLIOBOLUS HETEROSTROPHUS*. L. K. Lyngholm and C. R. Bronson, Iowa State University, Ames, Iowa 50011.

The goal of this research is to determine the significance of degradative enzymes in the pathogenicity of *C. heterostrophus*. Survivors of UV mutagenesis are being screened for more, less or no secretion of five extracellular enzymes. Mutants are being backcrossed and intercrossed to determine the genetic control of the phenotypes and to remove unwanted mutations. Genetic control has been demonstrated for 8 low and 4 high producers of β-xylosidase, 1 low and 4 high producers of polygalacturonase, 6 low and 5 non-producers of protease, and 1 low producer of xylanase. Genetic control is being determined for an additional 120 putative mutants. Mutants are being quantitatively assessed for their ability to cause disease on maize by measurements of infection efficiency and lesion size.

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ISOZYME VARIATION IN USTILAGO HORDEI. R. Hellmann and B.J. Christ. Dept. of Plant Pathology, Pennsylvania State Univ., Univ. Park, PA.

Haploid isolates of the smut fungus, Ustilago hordei, were examined for enzyme variation by starch gel electrophoresis. Fifty-five isolates from North Dakota varying in virulence and eight isolates from Ethiopia, of unknown genotype, were tested. Twenty-one enzymes were screened, activity was detected for nine. A single allele common to all isolates was detected in aconitase (ACO), adenylate kinase, glucose-6-phosphate dehydrogenase (G6P), phosphoglucose isomerase, 6-phosphogluconate dehydrogenase, and peptidase. Two alleles were detected for isocitrate dehydrogenase (IDH) and malate dehydrogenase (MDH). Three alleles, including a null, were detected for phosphoglucomutase (PGM). Analysis of F_1 progeny revealed that PGM is coded by different alleles at a single locus. For IDH, MDH, and PGM, greater than 70% of the isolates had a common allele. Using diversity and cluster analysis, five electrophoretic types and 2 clusters were identified. Clusters based on these analyses did not coincide with those based on virulence data. Mean diversity was 0.189 and 0.401 based on isozyme and virulence data, respectively. In 1990, ten additional sporidial isolates were obtained from teliospores collected in Pennsylvania. Preliminary results of these isolates revealed additional alleles for MDH, G6P, and ACO.

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Isolation of mating-defective mutants of *Ustilago hordei* . Alfredo D. Martinez-Espinoza and John E. Sherwood. Dept. of Plant Pathology, Montana State University. Bozeman 59717.

Mating in *Ustilago hordei* (covered smut of barley), which is controlled by a single locus with two alleles (*MATA* and *MATa*), results in the conversion of haploid, non-pathogenic yeast-like sporidia to dikaryotic pathogenic mycelium. To date, more than twenty mutants of *U. hordei* that fail to form dikaryotic mycelium (*Fuz-*) have been isolated from auxotrophic strains of race 8 *MATA* and race 14 *MATa* using UV irradiation. *Fuz-* mutants were selected by replica plating colonies arising from irradiated sporidia onto Holliday's complete agar media supplemented with 1 % activated charcoal and over-spraying with sporidia of the opposite mating type. The mating reaction was observed after incubating at 21°C for 2 days. After a primary morphological characterization as *Fuz-*, the mutants were classified into different subclasses: those that continue to grow in the presence of cell of the opposite mating-type, those that fail to form conjugation bridges (*Cnj-*), those that form conjugation bridges but fail to maintain further growth, those that form greatly reduced amount of mycelium, and those that produce a halo inhibiting sporidia of the opposite mating type. The isolation of *Fuz-* mutants is the first step towards the cloning and characterization of genes involved in mating of *U. hordei*.

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ISOZYME VARIATION IN ALTERNARIA SOLANI AND A. ALTERNATA. D.M. Petrunak and B.J. Christ. Department of Plant Pathology, The Pennsylvania State University, University Park, PA 16802.

Using starch gel electrophoresis, 56 isolates of Alternaria solani, the causal agent of early blight of potato, and 94 isolates of A. alternata from various hosts and geographic locations were examined for isozyme variability. Thirteen loci were identified for 10 enzymes (MDH, PEP, IDH, ACO, PGM, G6P, MPI, MPD, G3P, PGI). Thirty-five electrophoretic types (ETs) were detected. The most common ET had 47 isolates whereas 18 ETs had one isolate each. The genetic diversity for a given enzyme ranged from 0.000 to 0.763 with an average genetic diversity of 0.500. The number of alleles detected per locus ranged from 1 to 4 with an average of 2.9. Cluster analysis revealed that the isolates could be divided into groups which almost completely separated the isolates according to species. Two A. solani isolates and one A. alternata isolate did not fit this pattern. Three enzymes (MDH, IDH, ACO) differentiated the isolates according to species for a majority of the isolates. No significant correlation was found between isozyme phenotype and either host or geographic origin of the isolates.

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MODELS OF GENETIC POLYMORPHISM IN PLANTS AND THEIR PATHOGENS. S. A. Frank, Department of Ecology and Evolutionary Biology, University of California, Irvine, CA 92717.

Theory is developed to determine, in wild populations, which factors control genetic polymorphism for resistance in hosts and virulence in pathogens. These models assume that disease is determined by a gene-for-gene genetic system and that epidemiology and population dynamics are determined by the standard Lotka-Volterra equations of ecology. These assumptions are the simplest possible while maintaining a degree of realism about genetic complexity and the fluctuating abundance of pathogens and disease. Several surprising results are obtained. For example, the factors that most strongly influence the number of resistance alleles carried by each host are: (1) the cost to a pathogen of carrying a virulence allele, (2) the intrinsic rate of population growth of the pathogens, or, equivalently, the potential for drastic epidemics, and (3) the rate at which new resistance and virulence alleles immigrate into the local population or arise by mutation. The cost of resistance has little influence on the number of resistance alleles carried by hosts. A second set of models examines the factors that influence spatial variation in the distribution of resistance and virulence alleles, and considers how this spatial variation can be exploited to infer the temporal dynamics of coevolutionary genetics.

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IDENTIFICATION OF GENES FOR RESISTANCE TO BACTERIAL WILT IN TOMATO USING RFLPS. S. R. Aarons and N. D. Young, Department of Plant Pathology, University of Minnesota, St. Paul, MN 55108.

Bacterial wilt, caused by *Pseudomonas solanacearum*, can result in crop losses up to 100%. However, previous forms of genetic resistance in tomato have been short-lived. A highly resistant tomato line (L285) has very small fruit size linked to disease resistance as determined by crosses with a susceptible large fruited variety (CLN286). In greenhouse comparisons, L285 was consistently resistant, irrespective of whether stem or root inoculation methods were used. Cytological examination of stem sections revealed cell necrosis restricted to one vascular bundle in L285 and not extending into multiple vascular bundles, as in CLN286. Initial restriction fragment length polymorphism (RFLP) surveys indicate that 40% of tomato genomic clones exhibit DNA sequence differences between L285 and CLN286. These RFLPs will be used to map genes for resistance, identify correlations between specific disease response characters and resistance genes, and break the apparent linkage between resistance and small fruit size.

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GENETICS OF RESISTANCE IN MUNG BEANS (*VIGNA RADIATA*) TO POWDERY MILDEW (*ERYSIPHE POLYGONI*). D. Danesh and N. D. Young, Dept. of Plant Pathology, University of Minnesota, St. Paul, 55108.

Most genotypes of mung bean (MB), an important legume in developing countries, are susceptible to powdery mildew (PM). While examining various MB genotypes for their reaction to a local isolate of PM, we identified one (PM-2) that is highly resistant. Scanning electron microscopy of leaf tissue showed that stomate density is significantly lower in PM-2 than in two highly susceptible lines (TC1966 and VC1628) and preliminary results suggest this may be related to PM resistance. F1 progeny from crosses between PM-2 and the susceptible lines were intermediate in reaction to PM and number of stomata. However, ten days after inoculation, F1 plants developed a severe hypersensitive reaction. Restriction fragment length polymorphism (RFLP) analysis will be carried out in segregating progeny populations to map genes controlling PM resistance and identify correlations between histological characters and PM resistance.

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RFLP ANALYSIS OF COMPLEX INTERACTIONS BETWEEN MUNG BEAN (*VIGNA RADIATA*) AND POWDERY MILDEW (*ERYSIPHE POLYGONI*). N. D. Young and D. Danesh, Department of Plant Pathology, University of Minnesota, St. Paul, MN 55108.

Mung bean (MB), a legume crop with a small genome ($4\text{-}5 \times 10^8$ base pairs), little repetitive DNA, and a restriction fragment length polymorphism (RFLP) linkage map, is well suited for studies of plant-pathogen interactions. In order to characterize MB genes involved in resistance to powdery mildew, 58 F2 progeny from a cross between a moderately resistant (VC3890) and a highly susceptible (TC1966) MB genotype were analyzed using 115 RFLP markers. RFLPs in four regions of the MB genome were associated with disease reaction. Two regions contained resistance genes acting in a partially recessive/partially additive manner. Two other genomic regions contained genes where heterozygotes were significantly more resistant than either homozygote, indicating a heterotic type of disease response. These results demonstrate that genes with subtle effects on disease resistance can be mapped and analyzed using RFLPs.

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DEVELOPMENT OF A RESTRICTION FRAGMENT LENGTH POLYMORPHISM (RFLP) MAP FOR *UROMYCES APPENDICULATUS*. J. P. Martinez, J. V. Groth, and N. D. Young, University of Minnesota, Department of Plant Pathology, St. Paul, MN 55108.

An F2 population of the bean rust fungus was obtained by selfing a single F1 isolate of a cross between two dissimilar parents. To develop the map, we are using both genomic and cDNA clones from *U. appendiculatus* as putative RFLP markers, and 27 randomly selected F2s as a mapping population. Twelve genomic clones have been surveyed for polymorphisms between the parents using five restriction enzymes. Of these clones, eight showed distinct polymorphisms, two as condominant markers. Initial segregation analysis indicated that linkage mapping will be feasible, although two of the RFLPs showed significant segregation distortion, and four clones did not segregate in the F2. An RFLP map for *U. appendiculatus* will provide a better understanding of genome organization and chromosome number, as well as a basis for map-based cloning.

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GENETIC CHARACTERIZATION OF MULTIPLE POTYVIRUS RESISTANCE IN THE CUCUMBER LINE TMG-1. T. Wai and R. Grumet, Department of Horticulture, Michigan State University, East Lansing, MI 48824.

The inbred Chinese cucumber line TMG-1 is resistant to three potyviruses: ZYMV, WMV-2, and PRV-W. Resistance to ZYMV is due to a single recessive gene (Provvidenti, 1987); resistance to the other two viruses has not yet been characterized. We sought to determine: (1) the genetics of resistance to WMV-2 and PRV-W, and (2) the relationship of the three resistances to each other. TMG-1, WI-2757 (a susceptible inbred line), and their F_1 and F_2 progeny, were screened for resistance to all three viruses by monitoring symptom expression and virus level using ELISA. Resistance to WMV-2 appears to be due to two recessive genes, and resistance to PRV-W to a dominant gene. ELISA data indicate that the mechanisms of resistance to WMV-2 and ZYMV may differ. While WMV-2 levels are virtually non-detectable in TMG-1, ZYMV levels are ca. 50% in TMG-1 relative to 2757. The F_1 levels were intermediate to the parental levels for both viruses. The relationship among the resistance genes is being studied using vegetatively propagated clonal sets of F_2 individuals.

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SEQUENCE ANALYSIS OF PCR-AMPLIFIED DNA FOR PHYLOGENETIC STUDIES OF RUSTS IN PUCCINIA AND RELATED GENERA. P. J. Zambino and L. J. Szabo, USDA-ARS Cereal Rust Laboratory, Dept. of Plant Pathology, Univ. of Minnesota, St. Paul, MN, 55108.

To test the usefulness of DNA sequence data for phylogenetic studies of the rusts, DNA sequences from six isolates of Puccinia graminis tritici (PGT) of diverse geographic origin and two isolates of P. coronata avenae (PCA) were compared using PCR-amplified DNA from the 5' end of the large rDNA subunit (LRS) and the internal transcribed spacer (ITS) region of the ribosomal repeat unit. PGT strains differed from PCA strains at 6/106 bases of the LRS region and 59/435 bases of the ITS region, but no differences were detected among strains of either forma specialis. To determine if estimates of evolutionary divergence based on sequence data from rDNA are strongly biased, other regions, such as introns of the highly-conserved gene glyceraldehyde-3-phosphate dehydrogenase, are also being explored.

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mtDNA RFLP ANALYSIS OF THE CUCURBIT ANTHRACNOSE PATHOGEN, COLLETOTRICHUM ORBICULARE. J. C. Correll, D. D. Rhoads[2], and J. C. Guerber. Department of Plant Pathology, and [2]Department of Biological Sciences, University of Arkansas, Fayetteville, AR. 72701.

A collection of anthracnose isolates from cucumber, watermelon, and cucuzzi gourd from throughout the United States were examined for mitochondrial DNA restriction fragment length polymorphisms. All isolates had previously been characterized into ten vegetative compatibility groups (VCGs). Multiple isolates within a VCG, as well as isolates representing the ten VCGs, were examined. RFLP analysis and cloning of mtDNA restriction fragments indicated that a reference strain (isolated from cucumber in Arkansas in 1990) had a mitochondrial genome size of approximately 36 kb. Restriction patterns of mtDNAs with several restriction enzymes were similar or identical among all cucumber and watermelon isolates tested which represent the three predominant VCGs. Restriction patterns of mtDNAs among isolates from the other VCGs differed from those of the isolates from the three predominate VCGs. mtDNA RFLPs appear to be minor or nonexistent in the cucumber and watermelon anthracnose populations examined.

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MOLECULAR ANALYSIS OF GENETIC RELATEDNESS OF TILLETIA CONTROVERSA AND T. CARIES. B. W. Russell*, and D. Mills*+. Genetics Program*, Dept of Botany and Plant Pathology+. Oregon State University, Corvallis, OR, 97331-2902.

Electrophoretic karyotypes of Tilletia controversa, T. caries, and interspecific progeny obtained by crossing compatible strains of these fungi, reveal close genetic relatedness. Radiolabelled probes from either highly conserved fungal genes or DNA fragments from either organism, identified linkage groups common to both pathogens and their progeny. The number of chromosome-size DNA bands in four strains of T. controversa varied from 13 to 18 that ranged in size from 680 kilobases (kb) to ca. 5,000 kb, whereas in the T. caries strains, 13 to 15 bands were detected that ranged in size from 370 to 4,490 kb. Genome sizes ranged from ca. 30 to 40 megabases (mb); 30 mb is predicted to be the haploid genome size and strains with genomes nearing 40 mb are assumed to be aneuploid. Among five F1 progeny, 16 to 18 bands of 830 to 3,700 kb were detected. Evidence for the occurrence of recombinant chromosomes among F1 progeny will be presented.

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GENETIC VARIATIONS AMONG XYLELLA FASTIDIOSA STRAINS. J. Chen[1], C. J. Chang[1], R. Jarret[2], and N. Gawel[3]. [1]Dept. Plant Pathology and [3]Dept. of Horticulture, University of Georgia, and [2]USDA, ARS, Dept. Plant Introduction, Griffin 30223.

A genomic library was constructed from Xylella fastidiosa [Pierce's disease (PD) strain R112V2]. Restriction fragment length polymorphism analysis was performed (12 probes-HindIII and BamH1 digestions) among 15 PD strains, 9 ATCC strains [35868 (mulberry, MB68), 35869 (mulberry, MB69), 35870 (almond, AM), 35871 (hybrid plum, HP), 35873 (American elm, AE), 35876 (ragweed, RW), 35877 (grape), 35878 (periwinkle, PW), and 35879 (grape)] and one strain of Xanthomonas campestris pv. campestris (Xcc). Similarities among PD strains ranged from 0.90 to 1. Similarities of PD strain R112V2 to MB68, MB69, AM, HP, AE, RW, PW and Xcc were 0.77, 0.83, 0.93, 0.67, 0.67, 0.94, 0.68, and 0.07, respectively. A plasmid (about 1 kb) was observed in PD82-21, MB68, and PW.

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Amplification, cloning and sequence analysis of the 16S ribosomal RNA gene of the bacterialike organism (BLO) associated with citrus greening disease. K. H. Chen, M. Metzler and T. A. Chen, Department of Plant Pathology, Rutgers University, New Brunswick, NJ 08903

A phloem limited bacteria-like organism (BLO) associated with citrus greening disease was partially purified. The 16S ribosomal RNA gene (rDNA) from BLO was selectively amplified via PCR using a set of synthetic oligodeoxynucleotides that are broadly homologous to conserved eubacterial 16S rDNA. Purified healthy plant organelle DNA was subjected to the same treatment as controls. The 1kbp PCR products from both the BLO and the healthy DNA were cloned in pGEM-3Zf(+). Restriction pattern analysis using four-cutter enzymes revealed that three different groups from PCR products of BLO DNA. Clones of one group have the same restriction patterns as that from the healthy plant preparations. Clones of the other two groups showed uniquely different patterns. The two latter groups were chosen for sequencing analysis. The analysis of the sequencing data will be presented.

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COMPARISON OF PROTEINS OF SPIROPLASMA CITRI M200H AND TWO VIRUS RESISTANT MUTANTS. Y. -H. Sha, J. Fletcher, and R. E. Davis. Department of Plant Pathology, Oklahoma State University, Stillwater, OK 74078-9947 and USDA-ARS, Beltsville, MD 20705.

Spiroplasma citri M200H is a triply cloned isolate derived from S. citri Maroc. Plaques are formed when M200H is infected by spiroplasma virus SVTS2, which was isolated from S. melliferum TS2. Two spontaneous mutants, MR2 and MR3, were derived from colonies growing in the center of cleared plaques on lawns of M200H inoculated at a high MOI with SVTS2. The mutants did not produce plaques when inoculated with whole virus particles of SVTS2. The protein profiles of the mutants were compared with each other and with the parent strain using 1- and 2-D gel electrophoresis. There was no significant difference in the 1-D protein profiles of the mutants and the parent strain. In 2-D gels, three proteins seen in the parent strain were lacking or significantly reduced in both mutants. The roles of these proteins in virus infection of S. citri are under investigation.

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ARABIDOPSIS THALIANA AS AN EXPERIMENTAL HOST PLANT OF SPIROPLASMA CITRI. J. Fletcher and C. E. Eastman. Department of Plant Pathology, Oklahoma State University, Stillwater OK and Illinois Natural History Survey, Champaign, IL.

Beet leafhoppers, Circulifer tenellus, previously confined on Spiroplasma citri BR3-infected turnip plants, were caged with seedlings of Arabidopsis thaliana cv. Lansburg ER or cv. Columbia for 4-7 days. Symptoms, beginning after 14 days, included stunting of the basal rosette, curled and deformed cauline leaves, floral stunting and necrosis, reduced silique size and seed set, and reduced internode length on the floral stalk with terminal bunching of flowers and siliques. Inoculation of test plants occurred with 1,2,4 or 8 leafhoppers. Spiroplasmas were cultured from 23 of 26 exposed plants, but not from control plants. PAGE protein patterns of reisolated spiroplasmas were indistinguishable from those of cultured S. citri BR3, but different from S. kunkelii or other spiroplasmas. These findings extend the utility of Arabidopsis as a host plant for genetic studies of host-pathogen interactions.

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TRANSLOCATION OF THE CORN STUNT SPIROPLASMA IN CORN. J. S. Gussie and J. Fletcher. Department of Plant Pathology, Oklahoma State University, Stillwater, OK 74078-9947.

Corn leafhoppers, Dalbulus maidis, previously fed on Spiroplasma kunkelii (CSS) - infected corn (Zea mays, cv. Early Golden Bantam), were caged (day 0) on the youngest leaf of corn at two different stages of plant maturity (V-1 and V-4). At intervals from day five to day 42, samples were collected for ELISA testing from the roots, youngest leaf, inoculated leaf, oldest leaf, and tassel. S. kunkelii was detected as early as day 14 in youngest leaves of corn plants in both maturity groups, at least two weeks before symptom appearance. The pathogen was detected most frequently in roots of V-1 inoculated plants and in tassels of V-4 inoculated plants. In

general, tissues of the V-1 inoculated plants were positive by ELISA earlier than those of V-4 inoculated plants. Spiroplasmas were detected in all assayed regions of the corn plant by day 25. These findings extend our understanding of translocation patterns of S. kunkelii in corn.

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XYLEM-LIMITED BACTERIA IN CITRUS FROM ARGENTINA WITH SYMPTOMS OF CITRUS VARIEGATED CHLOROSIS. R. H. Brlansky, C. L. Davis, L. W. Timmer, D. S. Howd, and Jesus Contreras*. University of Florida, CREC, Lake Alfred 33850, and *INTA, Monte Carlo, Misiones, Argentina.

Sweet oranges on various rootstocks exhibiting symptoms similar to citrus variegated chlorosis (CVC) were examined for xylem-limited bacteria. Diseased and healthy petioles were prepared for scanning (SEM) and transmission (TEM) electron microscopy and for membrane entrapment immunofluorescence (MEI) assay. Using SEM, bacteria were found in the xylem vessels of petioles from all samples with CVC symptoms and in one sample from an asymptomatic tree. Bacteria in ultrathin sections were similar in structure to those from other xylem-limited bacterial diseases caused by Xylella fastidiosa. Fluorescing bacteria were observed when MEI was used with antisera to two strains of X. fastidiosa and not with antisera to other bacteria. Culturing of the bacterium and fulfillment of Koch's postulates are needed to confirm this bacterium as the causal agent of CVC.

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CAUSAL AGENT OF TOMATO BIG BUD DISEASE IN CALIFORNIA IS THE BEET LEAFHOPPER TRANSMITTED VIRESCENCE AGENT. M. E. Shaw, B. C. Kirkpatrick and D. A. Golino*. Department of Plant Pathology and *USDA/ARS, University of California, Davis, CA 95616.

Mycoplasma-like organisms (MLOs) cause big bud disease of tomatoes in many areas of the world. However, the MLOs associated with the disease have been characterized in only a few cases. Biological and genetic data established that the causal agent of California tomato big bud disease is the beet leafhopper transmitted virescence agent-MLO (BLTVA-MLO). Healthy Circulifera tenellus (Baker) leafhoppers acquired the BLTVA-MLO from field-collected symptomatic tomato big bud plants, and transmitted it to healthy tomato plants which developed typical big bud symptoms. The California tomato big bud isolate caused symptoms typical of BLTA, including premature induction of flowering, on a standard plant host range. Southern blot analysis of DNA from plant samples showed that all samples possessed plasmid homologous to a cloned BLTVA-MLO plasmid.

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DAPI FLUORESCENCE VERSUS DNA PROBES FOR DETECTING MYCOPLASMALIKE ORGANISMS IN WOODY PLANTS AND INSECTS. H. M. Griffiths, W. A. Sinclair, I.-M. Lee[1], and R. E. Davis[1], Dept. Plant Pathology, Cornell Univ., Ithaca, NY 14853; and [1]USDA-ARS, Beltsville, MD 20705

DNA was extracted from root or stem phloem of seven ash (*Fraxinus* spp.) and three lilac (*Syringa* spp.) plants and hybridized with a cloned biotin-labeled probe (pBB115) and with ^{32}P-labeled pBB115. This probe detects ash and lilac mycoplasmalike organisms (MLOs). MLO DNA was detected by the biotinylated probe in three plants and by the ^{32}P-labeled probe in four, whereas MLO DNA had been visualized with DAPI (4',6-diamidino-2-phenylindole•2HCl) by fluorescence microscopy in the same specimens from all 10 plants. Healthy ash and lilac plants tested negative with DAPI and both probes. DAPI and biotin-labeled, digoxygenin-labeled (Genius™), and ^{32}P-labeled pBB115 were tested for detection of MLO DNA in insects. DAPI was not suitable, and the nonradioactive probes often gave hybridization signals with extracts from healthy insects. The ^{32}P-labeled pBB115 hybridized only with DNA from insects that had fed on MLO-infected plants. Thus, for detection of MLOs in plants, DAPI provided the most sensitive test, whereas for insects, hybridization with the ^{32}P-labeled probe was the only suitable method.

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PRODUCTION OF MONOCLONAL ANTIBODIES AGAINST FLAVESCENCE DOREE MYCOPLASMA-LIKE ORGANISM. J.R. GUO, T.A. CHEN and N. LOI. Department of Plant Pathology, Rutgers University, New Brunswick, N.J. 08903.

Two hybridoma cell lines (FD-1 and FD-2) secreting monoclonal antibodies specific for the Flavescence doree mycoplasma-like organism (FD-MLO), were produced by fusions of murine myeloma cells (P3-NS1/1-Ag4-1) with the splenocyte of BALB/c mice immunized with partially purified preparations from infected periwinkle plants. Isotyping of the FD-1 and FD-2 indicated that they belonged to IgG2a and IgG3. Using ELISA, FD-1 and FD-2 reacted only with FD-MLO infected periwinkle preparation, but not to those of healthy and other nine MLO-infected periwinkles. Dilution end points of FD-1 and FD-2 in culture supernatant were 625 and 125 and in ascitic fluids were 100,000 and 10,000, respectively. Indirect immunofluorescence staining of diseased plant sections showed that FD-1 and FD-2 strongly reacted with FD-MLO located in the sieve tubes. Dot-blot immunoassay was as sensitive as ELISA and thus was suitable to detect FD-MLO in crude saps.

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EFFECT OF CROPPING SYSTEMS ON MYCORRHIZAL SPORE DISTRIBUTION AND INFECTION IN CORN AND SOYBEAN. S. Ananth and D. H. Rickerl, Plant Science Department, South Dakota State University, Brookings, SD 57007.

Mycorrhizal spores and infection of plant roots could be affected by cultural practices such as tillage and crop rotation. Field studies were conducted at the Southeast Experiment Farm near Beresford, South Dakota to determine the effect of five cropping systems on mycorrhizal spore distribution in the soil profile and infection in corn and soybean roots. The cropping systems were moldboard plow (MP) corn/oats, corn/corn, corn/fallow, ridgeplant (RP) corn/corn and corn/soybean. Mycorrhizal infection increased, levelled off and then decreased during the growing season in the moldboard system while the opposite was observed in the ridgeplant system. Previous crop did not affect infection in either corn or soybeans. Spore counts were not significantly affected by cropping system or phosphorus rate. However, the 12-18" depth had a very high concentration of spores irrespective of cropping system or phosphorus rate.

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POPULATION ECOLOGY OF VESICULAR ARBUSCULAR MYCORRHIZAE (VAM) IN IRON TAILINGS RECLAMATION. D. L. Stenlund, F. L. Pfleger, and E. L. Stewart, Department of Plant Pathology, University of Minnesota, St. Paul, MN 55108.

The occurrence and immigration of VAM fungi from undisturbed vegetated areas into recently deposited coarse and fine taconite (iron) tailings was studied in northern MN. Plant roots and soil were collected from plots every two weeks from May through September and analyzed for spores and percent root colonization. There was a low correlation between spore numbers and percent root infection. Average spore numbers in all plots ranged from 0.9 to 76 gm^{-1} of soil. There was a high correlation in spore numbers between permanent and adjacent containerized immigration plots. ***Acaulospora bireticulata***, ***Glomus aggregatum***, ***Gl. claroides***, ***Gl. intraradix***, and ***Gl. mosseae*** have been identified to date. ***Glomus intraradix*** represents the dominant spore recovered and has been isolated from both roots and soil from all plots examined.

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THE EFFECT OF MANAGEMENT AND EDAPHIC FACTORS ON VESICULAR-ARBUSCULAR MYCORRHIZAL (VAM) POPULATIONS IN A CORN-SOYBEAN ROTATION. James E. Kurle, F. L. Pfleger, and R. K. Crookston, Departments of Plant Pathology and Agronomy and Plant Genetics, University of Minnesota, St. Paul, MN 55108.

The effect of management and edaphic factors on VAM populations was examined in corn-soybean rotations established in two areas with the following different management histories; 1) no fertilizer or herbicide inputs and 2) conventional inputs. No input, organic input, minimum conventional, and conventional input management systems were established within each rotation. VAM populations were not correlated with N, P, soil type, or soil moisture. Spore numbers averaged 320 gm^{-1} and 260 gm^{-1} of soil in corn and soybeans, respectively. In no input, organic input, minimum conventional, and conventional input areas, spore populations were 315, 314, 275, and 263 gm^{-1} of soil, respectively. Spore number appeared to be influenced by weed and crop root density which was itself determined by management system and crop.

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RESPONSE OF KENAF AND ROSELLE TO MELOIDOGYNE INCOGNITA. J. R. Barillas, G. W. Lawrence and K. S. McLean. Department of Plant Pathology and Weed Science, Mississippi State University, Mississippi State, MS 39762.

Kenaf (Hibiscus cannabinus) cultivars Tainung 1, Tainung 2, Everglades 41, Everglades 71, Cubano and Roselle (H. sabdariffa) were evaluated for resistance to the root-knot nematode (Meloidogyne incognita). Cultivars were inoculated with a mixture of 5000 eggs and juveniles/500 cm^3 of soil and placed in the greenhouse for 60 days. Uninoculated plants served as controls. At harvest, plant heights, stem diameters and stem dry weights were significantly reduced by M. incognita in all cultivars compared with the controls. Conversely, root growth of each cultivar was stimulated by M. incognita. At harvest, plant growth parameters were significantly lower in Cubano and Tainung 2 compared with the other cultivars. The largest nematode populations were recovered from Cubano and Tainung 2. Nematode reproduction ("R = final population/ initial population) ranged from 4 to 25 on Roselle and Cubano, respectively. All kenaf cultivars and Roselle appear to be good hosts for M. incognita.

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RENIFORM NEMATODE MANAGEMENT IN A CORN-COTTON ROTATION SYSTEM. G. W. Lawrence, G. L. Windham* and K. S. McLean. Department of Plant Pathology and Weed Science and *USDA-ARS, Mississippi State University, Mississippi State, MS 39762.

Five cropping sequences of corn (Zea mays cv. Pioneer brand 3165) and cotton (Gossypium hirsutum cv. DPL-20) were evaluated for the management of the reniform nematode (Rotylenchulus reniformis). Cropping sequences consist of monoculture cotton, monoculture corn, and alternate year combinations of corn-cotton. Each treatment was planted with and without aldicarb at 1.18 kg ai/ha. R. reniformis population densities were significantly larger at harvest in plots monocultured for two years with cotton compared with corn-cotton and monocultured corn plots. Reniform populations at harvest in monoculture cotton, monoculture corn, and corn-cotton plots were 15,898, 4,649 and 74 nematodes/500 cm^3 soil, respectively. Seed cotton yields were 2,402 and 2,807 kg/ha in the monoculture cotton and corn-cotton plots, respectively. The addition of corn in a cotton production system reduced R. reniformis without the use of aldicarb.

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IN VITRO ROOT AND SHOOT REGENERATION OF HIBISCUS CANNABINUS. K. S. McLean and G. W. Lawrence. Dept. of Plant Pathology and Weed Science, Mississippi State University, Mississippi State, MS 39762.

In vitro root and shoot regeneration of kenaf was initiated for use in pathogenicity studies. Murashige and Skoog media salts served as the basis of two auxin/cytokinin matrixes where NAA/BAP and 2,4-D/ kinetin were prepared at concentrations of 0.1, 0.3, 1.0, and 3.0 mg/L in sixteen possible combinations. Kenaf cv. Tainung 1 stems were aseptically sectioned and plated on each media. The 2,4-D/kinetin matrix produced significantly larger callus on the 0.3/3.0 and 1.0/3.0 mg/L matrixes. The NAA/BAP matrix produced the largest callus at 1.0/1.0 and 3.0/1.0 mg/L matrixes compared to all other combinations. Adventive and/or de novo rhizogenesis was induced on all NAA/BAP media matrix combinations. The 2,4-D/kinetic matrix only produced roots on matrixes containing kinetin at 0.1 mg/L regardless of 2,4-D concentration. Subcultured root sections are being examined in vitro to culture three species of nematodes. Adventive shoot formation was observed in both matrixes at auxin/cytokinin concentrations of 0.3 mg/L or less. Adventive shoots transferred to rooting medias produced plantlets to be used in in vitro pathogenicity studies.

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REACTION OF SELECTED COTTON CULTIVARS TO ROTYLENCHULUS RENIFORMIS. J. D. Mueller and S. B. Martin. Clemson University, Edisto Research and Education Center, P. O. Box 247, Blackville, SC 29817.

Six cotton cultivars were evaluated in 1990 for host suitability and yield in a field infested with Rotylenchulus reniformis (Rr). Cultivars were grown in paired plots nontreated and treated with 28 L/ha 1,3-dichloropropene and 0.56 kg/ha aldicarb. Nematicide treatment significantly (P=0.05) increased mean yields (209 kg/ha treated vs 141 kg/ha nontreated) and midseason populations of Rr/100 cm^3 soil (Pm) (4,965 nontreated vs 3,128 treated). Nematicide treatment significantly reduced Pm and increased yields of each cultivar. Yields (kg/ha) and Pm for each nontreated cultivar were: Deltapine 90 (180-4,780), Coker 320 (170-5,403), Coker 315 (153-5,005), KC 380 (136-4,135), Deltapine 50 (118-4,152), and PD-3 (91-6,296).

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RELATIONSHIP OF POPULATION DENSITY OF HETERODERA GLYCINES AT HARVEST AND SOIL PHYSICO-CHEMICAL FACTORS AT PLANTING. L. J. Francl. Plant Pathology Department, Walster Hall #306, North Dakota State University, Fargo, ND 58105.

Experiments were conducted in 1988 and 1989 on a Norfolk loamy sand infested with Heterodera glycines in two different locations in the same field. Soil chemical and physical parameters were measured each year after planting Essex soybeans (susceptible to H. glycines). Principal components analysis grouped 12 parameters into fewer, uncorrelated factors. In 1988, there were five factors, of which "pH + Mg" was positively correlated ($P<0.001$) with cyst and egg populations at harvest. The factor "% fine texture + Cu" was negatively correlated ($P<0.05$) with egg population density. In 1989, there were three factors; "pH + Mg + (-Cu)" was positively correlated with levels of cysts ($P<0.05$) but not eggs. This factor, moreover, was positively correlated ($P<0.05$) with percent fungal-infected eggs. Soil pH and Mg consistently were positively associated, and Cu seemed negatively related, with cyst nematode population density.

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EFFECTS OF GLYCEOLLIN I ON THE MOTILITY OF THE SOYBEAN CYST NEMATODE. Y.-H. Yi, J.-S. Huang, and C. H. Opperman. Department of Plant Pathology, North Carolina State University, Raleigh, NC 27695-7616.

Phytoalexin glyceollins are known to inhibit the motility of the root-knot nematode, *Meloidogyne incognita*. The dominant isomer of these compounds in soybean roots, glyceollin I, was tested for its effects on motility of soybean cyst nematode (SCN), *Heterodera glycines*. One ml of water agar (1%) mixed with 0, 20, 40, or 60 µg glyceollin I previously dissolved in 0.5% dimethylformamide was delivered into the wells of a tissue culture plate. Fifty to 100 second-stage SCN juveniles were placed on the agar in each well. Each treatment was replicated four times. After 24-hr incubation at 28 C, the percentage of non-motile nematodes in each well was determined. The motility of SCN treated with 20 µg of glyceollin I was slightly increased, but higher concentrations of glyceollin I inhibited the motility of this nematode.

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REACTION OF A DIALLEL CROSS OF MAIZE TO MELOIDOGYNE INCOGNITA UNDER FIELD CONDITIONS. G. L. Windham and W. P. Williams, USDA, ARS, P. O. Box 5367, Miss. State, MS 39762.

Seven inbred lines of maize exhibiting varying levels of resistance to Meloidogyne incognita were selected as parents of a diallel cross. The diallel cross was grown at the Plant Breeding Unit, Auburn University, Tallassee, Alabama in 1989 and 1990 in a Cahaba fine sandy loam soil (70% sand, 7% clay, 23% silt) naturally infested with M. incognita. Root-knot nematode reproduction was determined at 14 weeks after planting by counting nematode eggs extracted from egg masses on roots using NaOCl. Egg counts for both years were analyzed, and variation among hybrids was partitioned into general and specific combining ability components using Griffing's Experimental Method 4, Model I. General combining ability was a significant source of variation in nematode reproduction. Estimates of general combining ability effects for Mp307, GA203, and GA215 were negative indicating that these inbred lines imparted resistance to their hybrids.

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EVALUATION OF THE BIOLOG SYSTEM FOR IDENTIFICATION OF PLANT PATHOGENIC BACTERIA. J. B. Jones, A. R. Chase, and G. K. Harris. GCREC, Univ. of Florida, 5007 60th St. E., Bradenton 34203

Analysis of 1055 strains of *Agrobacterium, Clavibacter, Erwinia, Pseudomonas, and Xanthomonas* was done using the MicroLog™ data base, Release 2.01, (Biolog, Hayward, CA) and a microplate reader. Of 63 *P. syringae* strains tested, correct identification to pathovar and species were 2 and 100%, respectively. Of 603 *X. campestris* strains from 20 pathovars, correct identification to pathovar and species were 20 and 97%, respectively. *Pseudomonas* spp. (297 strains) and *Xanthomonas* spp. (649 strains) were identified to genus with 86 and 96% accuracy, respectively. Only 1 of the 21 *A. tumefaciens* strains and 18 of 49 *Clavibacter* strains were identified correctly to species and subspecies, respectively. With *E. carotovora* (23 strains) and *E. chrysanthemi* (16 strains), 22% and 62% of the strains were correctly identified to species. Of the latter 3 genera, 90, 97, and 55 % of the strains were identified correctly to genus, respectively. This system is useful for identification of gram negative plant pathogenic bacteria.

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MULTIPLEX REVERSE TRANSCRIPTION/POLYMERASE CHAIN REACTION FOR THE DETECTION OF MIXED CITRUS VIROIDS IN A SINGLE REACTION. L. Levy[1], A. Hadidi[1], and S. M. Garnsey[2]. [1]USDA-ARS, NGRL, Beltsville, MD, [2]USDA-ARS, USHRL, Orlando, FL.

A Multiplex Reverse Transcription/Polymerase Chain Reaction (MRT/PCR) assay was developed to detect citrus exocortis viroid (CEV), and citrus cachexia viroid (CCaV = CV-IIb), a member of the hop stunt viroid group, from 1 ug of total nucleic acid in a single reaction from known mixed infections. MRT/PCR required the simultaneous addition of a CEV oligonucleotide primer pair corresponding to nucleotides (n) 71-90 and 91-114 of CEV-A, and a HSV oligonucleotide primer pair corresponding to n 61-79 and 80-104 of an HSV-variant. MRT/PCR products of appr. 297, 371, and 600 n were generated and observed in silver stained PAGE. The origin of amplified MRT/PCR products was confirmed by Southern blot hybridization analysis with SP6-generated ^{32}P-labeled HSVcRNA and CEVcRNA probes. HSVcRNA probes hybridized to the 297 and 600 n products. CEVcRNA probes hybridized to the 371 n product. No hybridization was observed in healthy samples. The potential for simultaneous pathogen detection will be discussed.

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CHARACTERIZATION OF MONOCLONAL ANTIBODIES FOR CONIDIAL ANTIGENS OF PYRICULARIA GRISEA SACC. J. Q. Xia, F. N. Lee, and K. S. Kim. Department of Plant Pathology, University of Arkansas, Fayetteville, AR 72701.

Several monoclonal antibodies (MAbs) prepared against crushed conidia of Pyricularia grisea Sacc. were characterized by using various immunological assays as well as chemical and enzymatic analyses. MAb 4G11 recognized two major proteins, one with an approximate molecular mass of 63 kilodaltons (kDa) in crushed conidial suspensions, and the other about 20 kDa in saline mycelial washings. Both proteins were present in sonicated mycelial suspensions. The MAb also bound to several minor proteins with molecular weights ranging from 23-31 kDa in saline conidial washings. Immunoelectron microscopy demonstrated binding of MAb 4G11 to the cytoplasm of conidial cells and cytoplasm and walls of hyphal cells. It is presumed that the 63 kDa protein was synthesized in the fungal cytoplasm and then excreted as a smaller polypeptide. The epitopes recognized by MAbs 8H1 and 3E4 were distributed mainly in conidial cytoplasm and on the surface of growing points of germ tubes, whereas the epitope to MAb 11C6 was present in both cell walls and cytoplasm of hyphae and conidia. Chemical and enzymatic analyses confirmed that the epitope which reacted with 4G11 is either a protein or glycoprotein, and the epitopes which reacted with 8H1, 3E3 and 11C6 are carbohydrates.

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AMPLIFICATION OF RIBOSOMAL DNA FROM A SMALL NUMBER OF TELIOSPORES OF THE WHEAT BUNT FUNGI. Yunling Shi and Hei Leung. Dept. of Plant Pathology, Washington State University, Pullman, WA 99164-6430.

Wheat export from the Pacific Northwest has been hampered by a quarantine on the dwarf bunt fungus (*Tilletia controversa*) imposed by China. Reliable identification of different *Tilletia* species is necessary to avoid erroneous rejection of wheat shipments. We aim to develop a diagnostic technique for the wheat bunt fungi based on analysis of a small number of spores. Teliospores were squashed between two siliconized slides and the cell extract was recovered in 5 μl of water. Ribosomal DNA was amplified by using two μl of the extract in a polymerase chain reaction with primers that flank the intergenic transcribed spacers of fungal ribosomal genes (White et al. 1990. PCR Protocol pp. 315-322). Discrete DNA fragments were amplified with extract from 10 teliospores from *T. controversa* and *T. tritici*. Weak amplification was also detected with extract from a single teliospore. Additional primers are being tested to identify *Tilletia* species based on a few teliospores.

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IMPROVED EFFICIENCY OF POLYCLONAL ANTIBODY CROSS ABSORPTION FOR DAS-ELISA WITH SAP FROM SENESCING PLANTS. J. S. Lamboy[1], C. J. D'Arcy[1], and W. F. Lamboy[2], Departments of [1]Plant Pathology and [2]Agronomy, Univ. of Illinois, Urbana 61801

Cross absorption of enzyme-conjugated polyclonal antibodies to plant viruses removes the antibodies to host compounds from the antiserum, thereby reducing background in double antibody sandwich ELISA. In an eight microtiter plate experiment, we compared the efficacy of cross absorption with leaf sap (1:10 w/v in PBS-Tween + nonfat dry milk) from uninfected oat plants harvested at 3 and 6 weeks after germination. Samples analyzed were from two oat cultivars, barley yellow dwarf virus strain MAV-NY infected or uninoculated, and sucrose- or salicylic acid-treated or untreated. Cross absorption with sap from senescing plants lowered the optical density in wells of uninoculated plant sap to 20% of the OD with sap from younger plants, doubling the sensitivity of polyclonal DAS-ELISA.

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CHARACTERIZATION OF MONOCLONAL ANTIBODIES TO BARLEY YELLOW DWARF VIRUS STRAIN MAV-NY.
J. S. Lamboy and C. J. D'Arcy, Department of Plant Pathology, University of Illinois, Urbana, IL 61801

Fourteen hybridoma cell lines that secrete antibodies to barley yellow dwarf virus strain MAV-NY (BYDV-MAV) were screened from 2 mouse spleen fusions with myeloma cells. Using cell culture medium or ascitic fluids, these cell lines were tested for antibody isotype and reactivity to the antigen, BYDV-MAV, under several conditions. Antibodies of all lines have kappa light chains; 10 cell lines produce IgG1 heavy chains, 3 produce IgG2a. Antibodies of three cell lines cross react with BYDV strain PAV-IL. Differences in affinity for antigen were observed in triple antibody sandwich ELISA, and in antigen coat indirect ELISA at pH 7.4, 8.0, and 9.6 with or without BSA as an effector. Conformation sensitive immunoassay results indicate that the epitopes to which the antibodies bind are conformational rather than sequential.

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SPECIFIC DETECTION OF *CLAVIBACTER MICHIGANENSIS* SUBSP. *SEPEDONICUS* VIA DNA/DNA HYBRIDIZATION. J. L. Drennan, L. M. Delserone, A. Westra, A. Oleson*, A. Collmer, and S. A. Slack, Dept. of Plant Pathology, Cornell University, Ithaca, NY 14853 and *Dept. of Biochemistry, North Dakota State University, Fargo 58105.

A specific DNA probe may provide for accurate detection of *Clavibacter michiganensis* subsp. *sepedonicus* (*Cms*) which is required to meet zero tolerance limits for bacterial ring rot in seed potato programs. A high-copy number, 1.092 kb repeated sequence (RS) had previously been isolated from *Cms* by Mogen et. al. (Phytopath. 80:90-96). Specificity of the ^{32}P-labelled RS was determined in high stringency slot-blot hybridization analyses of total DNAs from a variety of bacteria. All 37 *Cms* strains tested hybridized with the RS, while other related corynebacteria (*C. m.* subsp. *insidiosum*, *Curtobacterium flaccumfaciens* subsp. *violaceum*, and subsp. *poinsettiae*) and strains of *Erwinia carotovora* subsp. *carotovora* and subsp. *atroseptica* did not. Bacteria isolated from healthy potato stems and tubers, including seven unidentified bacterial strains known to cross-react in immunological assays with antisera prepared against *Cms*, did not hybridize. The sensitivity of the RS probe was 10^4-10^5 CFU. Additional tests are underway to assess detection reliability in plant samples.

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IMMUNODETECTION OF THE DIAPORTHE/PHOMOPSIS COMPLEX OF SOYBEANS. R. K. Velicheti, R. D. McClary, C. Lamison (E. I. du Pont de Nemours & Co., Inc.), and J. B. Sinclair, Dept. of Plant Pathology, Univ. of Illinois at Urbana-Champaign, Urbana, IL 61801.

An immunodiagnostic assay was developed for early detection of the Diaporthe/Phomopsis complex (DPc) of soybeans using polyclonal antisera developed against Phomopsis longicolla, which reacted with all members of DPc. Greenhouse-grown plants (cv Hack) were uninoculated or inoculated with Diaporthe phaseolorum var. sojae (Dps) at V1 growth stage. Stem samples collected at V1, V2, V3, and V4 were assayed by antigen-capture ELISA and by agar plating. Field tests using asymptomatic plants at R5, R6, and R7 from various geographic areas were assayed using ELISA for DPc in stem and pod tissues. Stem samples from inoculated greenhouse plants showed positive reactions. Dps was not observed on surface-sterilized inoculated plants, suggesting epiphytic growth. Field studies showed a decrease in DPc in stems and pods with maturity. Stem samples showed significantly higher levels of DPc than pod samples, differences decreasing with maturity.

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A Survey for Maize Chlorotic Mottle Virus in the U.S.A. and Six Additional Countries. X. Q. Jiang, J. A. Berry, D. R. Wilkinson, B. M. Anderson, and W. E. Dolezal. Pioneer Hi-Bred International, Inc., P. O. Box 1004, Johnston, IA 50131.

After the 1990 report of an outbreak of Maize Chlorotic Mottle Virus (MCMV) in Hawaii, a MCMV survey was conducted on maize throughout major corn growing states in the U.S.A., Puerto Rico and several additional countries. In the U.S.A., 826 samples that appeared to be virus infected were assayed by ELISA for MCMV. Of those samples, MCMV was detected only in samples assayed in Nebraska and Hawaii. An addition 136 samples were assayed in Italy, Chile, Mexico, Jamaica and Puerto Rico. All samples were ELISA negative for MCMV. A follow-up survey was conducted on maize in Hawaii during the winter of '90-'91 following a three month quarantine of growing maize in Kauai. No MCMV was detected. Additional surveys will be conducted in 1991.

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THE BUG SHOW--A COLORADO YOUTH URBAN IPM AND PESTICIDE AWARENESS EDUCATION PROGRAM. Pickett, Laura S., and William M. Brown, Jr., Jefferson County Cooperative Extension, Golden, CO 80523 and Department of Plant Pathology and Weed Science, Colorado State University, Fort Collins, CO 80523.

The "Bug Show" is an urban IPM educational program designed to complement existing elementary science curriculum in Colorado. This program features three large hand puppets (60 to 100 cm) that are used to teach children in Kindergarten through third grade about the environment, some of the organisms that inhabit it, and how best to live with and without pesticides in an urban setting. The program was evaluated by pre- and post-program questioning of children audiences. After attending the program children developed an enhanced awareness of their environment, beneficial organisms and pesticides.

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VIDEODISC TECHNOLOGY FOR TEACHING OF PLANT PATHOLOGY. T. A. Evans, G. L. Schumann, and F. H. Tainter. Dept. Plant and Soil Sciences, Univ. Delaware, Newark, DE 19717; Dept. Plant Pathology, Univ. Massachusetts, Amherst, MA 01003; and Dept. Forest Resources, Clemson University, Clemson, SC 29634.

A videodisc can store 54,000 still frames or slides or 30 minutes of video, or any combination of the two. The images are highly durable and rapidly available through random access for repeated use in different contexts. Thus, a videodisc offers an archival image library for nearly any aspect of plant pathology. Computer authoring systems allow instructors to prepare teaching modules using text overlays with the visual images. Modules may present simple introductions to symptoms and signs of diseases as well as more challenging and complex diagnostic problems. Expert systems and diagnostic keys may be linked with the images for use in both teaching and extension. A visual image glossary accessible from various modules is also planned. Photographs are being solicited to represent major commodities and pathogens as well as management practices and other special topics.

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IMAGE ANALYSIS FOR EVALUATION OF BEAN RUST SEVERITY. J. R. Venette and R. C. Venette. North Dakota State University, Department of Plant Pathology, Fargo, ND 58102-5012.

Image analysis (Skye-Probetech) was used to evaluate severity of rust (Uromyces appendiculatus var. appendiculatus) on field-grown pinto beans (Phaseolus vulgaris 'UI 111'). The number of uredia determined from digitized images compared favorably with manual counts when uredia were large. When pustules were small, they were undercounted by the imaging system. Analysis of pustule sizes showed that mean pustule size was not reduced when rust severity was high.

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THE ERGOSTEROL ASSAY AS A TOOL FOR MEASUREMENT OF RESISTANCE TO FUNGI IN PEA. M.A. Gretenkort, J.P.F.G. Helsper, Centre for Plant Breeding and Reproduction Research (CPRO), Postbus 16, 6700AA Wageningen, The Netherlands.

Ergosterol (a specific fungal lipid), determined by HPLC analysis, was used to quantify the disease reactions in in vitro grown plantlets and tissue cultures of Pisum sativum (pea) infected with Fusarium solani f.sp. pisi. Development of infection in in vitro grown plantlets of genotypes with known field resistance to F. solani and the corresponding callus tissue was monitored. A relationship between disease index scores and ergosterol content of infected plant material was found. The method appears to have potential to differentiate between resistant and susceptible cultivars. The use of ergosterol assay to quantify infection by other pea root rot pathogens Phoma medicaginis var. pinodella and Aphanomyces euteiches is under investigation.

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Comparison of ELISA with other assays for Phytophthora spp. in citrus orchards in Florida and California. L. W. Timmer,[1] J. A. Menge,[2] S. E. Zitko,[1] E. Pond,[2] and S. A. Miller,[3] Univ. Florida, CREC, Lake Alfred 33850,[1] Univ. Calif., Riverside 92521,[2] and Agri-diagnostics Associates, Inc., Cinnaminson, NJ 08077[3]

Two ELISA systems were used to assess levels of Phytophthora spp. in fibrous roots and in soil debris. They were compared to a standard propagule assay on selective media, to % root rot, and to % isolation of the fungus from roots in 19 citrus orchards in Florida. Propagule densities ranged from 0-68 propagules/cm^3. Root ELISA was significantly correlated with propagule densities ($r = +0.62$), to % root rot ($r = +0.60$) and to % isolation ($r = +0.62$). The ELISA of soil debris was not related to the root ELISA or any other parameter measured ($r = \leq 0.17$). In 21 California orchards with 0-39 propagules/g, root ELISA was significantly correlated with propagule counts ($r = +0.86$). Root ELISA appeared to be an effective tool for quantification in Florida where P. parasitica is the primary pathogen and in California where P. citrophthora is important.

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USE OF MAPPING SOFTWARE IN THE SOUTH DAKOTA PEST SURVEY AND DETECTION PROGRAM. K. L. Kloster and D. J. Gallenberg, Dept. of Plant Science, South Dakota State University, Brookings, SD 57007.

Surveys for diseases, weeds and insects provide data which are important in monitoring and controlling these plant pests. The South Dakota Pest Survey and Detection Program has placed emphasis on the use of computer mapping software to enhance plant pest data reporting, management and summarization. ATLAS*GRAPHICS™ and ATLAS*DRAW™ have been used to map first occurrence, presence/absence, distribution, beneficial release/recovery sites, and other data. Recently, SDSU personnel developed Grafer which can be used in conjunction with the commercial mapping software SURFER® to create detailed gradient maps of pest abundance or severity. These and other softwares have increased the usefulness of pest survey data and the overall visibility of the pest survey and detection program.

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GRAFER: A SOFTWARE THAT ENHANCES THE CAPABILITIES AND USEABILITY OF COMMERCIAL MAPPING SOFTWARE. L. G. Knutson, C. G. Carlson, K. L. Kloster and D. J. Gallenberg, Dept. of Plant Science, South Dakota State University.

The software Grafer was recently developed at SDSU for use in conjunction with the commercial mapping software SURFER®. Grafer is a series of batch files which manipulates location data into a format compatible with SURFER®. Township-range-section data can be transformed to latitude-longitude coordinates and ultimately to UTM coordinates which are used to create topographic and three-dimensional maps. Maps portraying gradients of pest severity or abundance are just one possibility. Grafer options are professionally presented in a menu-driven, user-friendly format. Various aspects of the maps can be modified, through SURFER®, to meet specific needs. The Grafer/SURFER combination represents an alternative to the more expensive Geographic Information Systems (GIS) presently available. Though presently designed for South Dakota, simple modifications in Grafer would make it suitable for any state.

605

INJURY INDUCED COMPOUNDS IN CITRUS FRUIT. R. Stange, Jr., S. L. Midland, J. W. Eckert, and J. S. Sims. Department of Plant Pathology, University of California, Riverside, CA 92521.

Both wound gum and lignin turn red in phloroglucinol/HCl [pg/HCl (+)], but are distinguished by other histochemical tests. The pg/HCl(+) reaction of lignin is due to CHO domains within the lignin polymer. No pg/HCl (+) compounds were extracted from control tissue, which contained vascular lignin. The reaction of wound gum is attributed to simple "aromatic aldehydes" in a carbohydrate matrix. During healing of injured citrus exocarp, deposition of wound gum, development of infection resistance, and the appearance of extractable pg/HCl(+) compounds were all synchronous. Four induced pg/HCl(+) compounds were purified by chromatography from extracts of healed tissue. Two of these compounds had antifungal activity. One antifungal compound was identified as a prenylated coumaral, 3-[4-hydroxy-3-(3-methyl-2-butenyl)phenyl]-2-(E)-propenal, by ^1H and ^{13}C NMR and mass spectrometry. This is a newly described compound not similar to any described plant defense compound.

606

ANTAGONISM OF SPOROBOLOMYCES ROSEUS AGAINST MAJOR POSTHARVEST PATHOGENS OF APPLE AND PEAR. W. Janisiewicz and B. Bors, USDA, ARS, AFRS, Kearneysville, WV 25430

A pink yeast, Sporobolomyces roseus, isolated from apple surfaces during screening of epiphtic microorganisms for antagonistic activity against Penicillium expansum (blue-mold) and Botrytis cinerea (gray-mold), exhibited antagonism against both diseases. Wounded Anjou pears protected with the yeast suspension at a concentration of 7.9×10^6 CFU/ml and inoculated with conidia of P. expansum and B. cinerea at concentrations as high as 1×10^5 and 1×10^4 conidia/ml, respectively, did not develop rot. Golden Delicious apples treated with the antagonist at concentrations of 6.3×10^5 and 7.9×10^6 CFU/ml and challenged with B. cinerea at 1×10^5 conidia/ml and P. expansum at 1×10^4 conidia/ml, respectively, also remained rot free. Population of the yeast at the wound site after 24 hr incubation at 24C increased almost one hundred-fold, indicating excellent colonizing ability.

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APPLICATION OF LEMNA MINOR (DUCKWEED) BIOASSAY FOR PHYTOTOXICITY SCREENING OF FUSARIA TOXINS. R. F. Vesonder, D. P. Labeda, and R. E. Peterson, NCAUR, USDA-ARS, 1815 N. University St., Peoria, IL 61604; T. Krick, University of Minnesota, 1479 Gortner Avenue, St. Paul, MN 55108; and H. K. Abbas, Delta States Res. Ctr., USDA-ARS, Stoneville, MS 38776.

Phytotoxicity and inhibitory effects on chlorophyll synthesis in the aquatic macrophyte Lemna minor L. of the fusarial toxins fumonisin B_1 (FB_1), fusaric acid, butenolide (4-acetamido-4-hydroxy-2-butenoic acid lactone), 9,10-dihydroxyfusaric acid, and moniliformin were examined. FB_1 proved to be most active, reducing the growth of L. minor fronds and their ability to synthesize chlorophyll by 48% at 0.7 ug/ml and by 95% at 5.3 ug/ml. The number of fronds produced was reduced 55% by 6.7 ug/ml fusaric acid, 60.5% by 66.7 ug/ml butenolide, and 29.5% by 66.7 ug/ml 9,10-dihydroxyfusaric acid. Moniliformin was the least phytotoxic to L. minor, with only a 22% suppression of frond growth and 53% reduction in chlorophyll at 66.7 ug/ml. Chemical modification of FB_1 for potential detoxification are being investigated using the L. minor assay system.

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VARIATIONS IN RESISTANCE TO POSTHARVEST AFLATOXIN CONTAMINATION AMONG INBRED MAIZE LINES. R. L. Brown, P. J. Cotty, T. E. Cleveland, USDA/ARS, Southern Regional Research Center, P.O. Box 19687, New Orleans, Louisiana 70179

Individual kernels of 12 inbred lines from two separate sources were inoculated with an aflatoxin-producing strain of Aspergillus flavus and examined for resistance to fungal growth and aflatoxin production. Prior to inoculation with the fungus, kernels were either halved, crushed, or left whole. Kernels that were pin-wounded, autoclaved, or decutinized prior to inoculation, were also tested. Whole kernels of two lines (MAS:gk and MAS:pw,nf) from one source were superior to all others in resistance to growth of A. flavus and to aflatoxin contamination. Resistance was lost when kernels were crushed or halved. Results from the testing of pin-wounded, autoclaved and decutinized kernels will be discussed. Factors responsible for resistance to fungal growth and aflatoxin production in the two resistant lines may be located in the kernel's outer layers.

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BIOCONTROL OF POSTHARVEST DECAY OF LEMONS WITH *PSEUDOMONAS SPP.* J. L. Smilanick, R. Denis-Arrue and D. J. Henson, USDA, ARS, 2021 South Peach Avenue, Fresno, CA 93727

Pseudomonas cepacia applied to lemons after harvest reduced decay by the pathogen *Penicillium digitatum* 85% or more. *P. cepacia* grew rapidly in wounds. Filter-sterilized, cell-free culture fluid from *P. cepacia* cultures did not control decay while washed cells did. *Pseudomonas cepacia* and *P. fluorescens* reduced the incidence of decay about equally when diluted or undiluted 3-day-old cultures were applied to wounds, although only *P. cepacia* produced large clear zones *in vitro* when co-cultured with *P. digitatum* on potato dextrose agar. The clear zones observed around *P. cepacia* colonies reportedly are caused by the antifungal antibiotic pyrrolnitrin. Isolates of *P. digitatum* resistant to pyrrolnitrin were selected from among spontaneous mutants. In pyrrolnitrin-amended potato dextrose broth, spores of the sensitive isolate would not germinate above 0.1 ug/ml, while spores of resistant isolates derived from it germinated in 60 ug/ml pyrrolnitrin. Clear zones did not develop *in vitro* around *P. cepacia* colonies co-cultured with pyrrolnitrin-resistant *P. digitatum* isolates. Although the resistant isolates decayed lemons, they were readily controlled by *P. cepacia*. Nutrient competition may be a factor in the action of *P. cepacia*.

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MOVEMENT OF GENETICALLY MODIFIED RHIZOSPHERE BACTERIA (P. AUREOFACIENS) INTO THE AERIAL PORTION OF PLANTS. T.G. Lamb, D.A. Kluepfel, and D.W. Tonkyn, Clemson University, Dept of Bio. Sci., Dept of Plant Pathology and Physiology, Clemson, SC 29634

The movement of genetically modified bacteria from the site of application presents a risk to non-target organisms. To determine the extent of this movement seeds of 15 different species of plants were inoculated with a lac ZY modified rhizosphere inhabiting bacterium, Pseudomonas aureofaciens (L11), and the roots, stems, leaves, and guttation drops were tested over a 21 day period. L11 moved into the stems and leaves of all plants tested. In corn, L11 maintained a density of 10^8 - 10^9 CFU/g fresh wt. However, in the part of the stem just above the soil line L11 attained a maximum density of 10^6 CFU/g fresh wt. 6 days after seed inoculation and then declined to 10^4 CFU/g fresh wt. at day 21. In the leaves L11 declined from 4.47×10^4 CFU/g fresh wt. (day 6) to 3.15×10^2 CFU/g fresh wt. (day 21). L11 was also compared with its non engineered parental strain (RN) in both monoculture and mixed culture inoculum.

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EFFECT OF LIGHT ON HORMONAL REGULATION OF SEXUAL REPRODUCTION IN PHYTOPHTHORA PARASITICA.
L. L. Chern and W. H. Ko, Department of Plant pathology, University of Hawaii, Hilo, Hawaii 96720

A^1 (P991) and A^2 (P6134) isolates of Phytophthora parasitica were paired on V-8 agar blocks and incubated under light or in darkness at different stages of development to determine the effect of light on hormonal regulation of sexual reproduction. Exposure to light during the whole process of sexual reproduction reduced the amount of oospores produced to only about 6% of that produced in darkness. Light was inhibitory to α hormones produced by both A^1 and A^2 isolates of P. parasitica. However, after being produced α hormones were stable under light. The amount of oospores produced was greatly reduced when A^1 or A^2 culture was exposed to light during hormone induction of sexual reproduction, but was not affected when the culture was exposed to light during oospore formation after hormone induction. Results suggest that the effect of light on oospore formation in P. parasitica was mainly through inhibition of hormone production and hormone induction of sexual reproduction.

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EFFECTS OF TILLAGE PRACTICES AND ROTATION CROPS ON THE RHIZOCTONIA DISEASE COMPLEX OF WHITE POTATO. S.S. Leach[1/], G. Porter[2/], R. Rourke[2/], and W.M. Clapham[1/]. [1/] USDA, ARS, N.E. Plant, Soil and Water Laboratory: [2/] Plant, Soils and Environmental Sciences Dept. University of Maine, Orono, Maine 04469.

Two tillage practices, chisel plowing (12") and deep mouldboard plowing (9"), and five rotation crops (oats, lupins, buckwheat, peas and broccoli) were studied for their effects on the soil population of Rhizoctonia solani AG3 and disease severity on potato. The plots were planted in a RCB split plot design with four replications. Crops were the whole plots and tillage sub plots. The results reported are for three years data (1988-1990). None of the crops had any effect on the incidence or severity of disease or soil populations of AG3. Chisel plowing significantly reduced (P=0.05) the severity of R. solani on potato stems and stolons and the soil population of AG3. No differences were observed on the amount of sclerotia formed on tubers for any treatment. There were no interactions observed between crops and tillage. In 1990 peas did increase the amount of R. solani isolated from the soil, but the increase was not AG3.

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FURFURALDEHYDE FOR CONTROL OF SCLEROTIUM ROLFSII. G. H. Canullo, J. W. Kloepper, and R. Rodríguez-Kábana, Department of Plant Pathology, Auburn University, AL 36849-5409.

The efficacy of 2-furfuraldehyde for control of Sclerotium rolfsii was studied in laboratory and greenhouse experiments. The mycelial growth of the fungus was reduced proportionally upon addition of varying concentrations (0.1-0.5 ml/l medium) of the compound in PDA. The viability of the sclerotia was diminished upon exposure to vapors of 2-furfuraldehyde. Populations of bacteria and fungi, including Trichoderma spp., were reduced in the presence of the aldehyde in soil dilution plates. However, repeated treatments of field soil with the fumigant in greenhouse conditions increased the populations of Trichoderma spp. and bacteria, while diminishing numbers of actinomycetes. These experiments suggest that a part of the activity of the compound in field soil could be due to changes in antagonistic microflora.

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RHIZOSPHERE COLONIZATION BY SELECTED SOIL BACTERIA AND EFFECTS ON GROWTH AND SOILBORNE DISEASES OF WHEAT. E. A. Milus, C. S. Rothrock and M. L. Rhoads, Dept. of Plant Pathology, University of Arkansas, Fayetteville, AR 72701.

Eight soil bacteria identified previously as rhizosphere competent or as biological control agents for soilborne diseases on wheat in other states were evaluated as seed treatments at two locations in Arkansas. Locations differed in soil texture, matric potential and oxygen diffusion rate. All bacteria contained antibiotic resistance markers and were detected in the rhizosphere by dilution plating at both fall and spring sampling times. Good rhizosphere colonizers included Pseudomonas fluorescens and Xanthomonas maltophilia. There was a high positive correlation between initial populations on seed and subsequent populations in the rhizosphere. Soil fumigation generally increased colonization by less than one log unit. No beneficial plant growth parameters or disease control were detected.

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S. T. Lam and N. R. Torkewitz. Soil-inoculated bacteria are better positioned than seed-inoculated bacteria to colonize plant roots. CIBA-Geigy Biotechnology Research, P.O. Box 12257, RTP, NC 27709.

Do soil resident microorganisms have a competitive advantage in colonization of plant roots? The question cannot be easily answered because of the unknown "competitiveness" of the soil resident microorganisms. To address the question, we eliminated the variable of relative competitiveness. P. fluorescens strain BL900 and its TnCIB100-containing derivative 900-3 were compared in pair-wise competitive colonization assays. When the strains were mixed and applied to the same location, i.e., either to seeds, which were then planted in autoclaved soil, or to autoclaved soil, in which was then planted surface sterilized seeds, the ratio of the two strains recovered from plant roots did not differ from that at the beginning of the experiment, indicating that the two strains were equally competitive. However, when one strain was applied to the seeds and the other to the soil, the soil-inoculated strain invariably predominated in the population of bacteria recovered from the plant roots. The same results were obtained regardless of which strain was applied to the soil. Thus, when two equally competitive strains were compared, the soil-inoculated strain was better positioned to colonize the growing plant roots.

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ROLE OF CARBOHYDRATES IN SPERMOSPHERE PROLIFERATION BY ENTEROBACTER CLOACAE EcCT501. D.P. Roberts. Biocontrol of Plant Diseases Laboratory, USDA-ARS, Beltsville, MD 20705.

A rifampicin-resistant mutant of Enterobacter cloacae EcCT501 (501R3) was added to cucumber seed at 10^4 cfu/seed and the seed was sown in autoclaved or natural sandy loam soil. Strain 501R3 reached 10^8 cfu/seed after 22 hr in autoclaved soil, while populations only reached 10^6 cfu/seed after 46 hr in natural soil. When 10^4 cfu of 501R3 was added to natural sandy loam soil on a sterile glass bead, 501R3 decreased to 10^3 cfu/bead after 29.5 hr. These results suggest that seed nutrients affect populations of E. cloacae EcCT501 (501). Since carbohydrates are known to be exuded from seed, studies were done to evaluate the effect of carbohydrate on 501 profileration in the spermosphere. Strain 501 grew on a wide variety of mono- and oligo- saccharides found in seeds when such carbohydrates were supplied as sole carbon and energy sources in vitro. In addition, corn and pea seed extracts induced four 501 glycosidases that have the potential to degrade seed carbohydrates suggesting a role for seed carbohydrates in spermosphere profileration of 501.

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SOYBEAN RHIZOSPHERE COLONIZATION BY BACTERIAL INOCULANTS IN MIDWESTERN SOILS. R.M. Zablotowicz, C.D. Bierle, A.H.Bosworth and J.C. Gates. Plant Science Research Inc., Sun Prairie, WI 53527.

Soybean rhizosphere colonization by seed inoculation with a Pseudomonas aureofaciens and a Serratia liquefaciens strains was studied in soils from WI, IA and IN in the greenhouse and WI field trials. Colonization of the upper 1 to 5 cm of root ranged from log 4.2 to 6.5 cfu / g. Maximum colonization was achieved when applied at log 5 to 7 cfu per seed, depending upon strain and soil. Colonization of the lower 5 to 9 cm of root was typically 1 to 2 log lower with variable recovery from root tips. The introduced strains represented from 10% to less than 1% of the total Gram-negative rhizosphere bacterial populations. A barrier to rhizosphere colonization distal to the zone of inoculation was observed for both strains and all soils.

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THE INTERACTION OF TEMPERATURE WITH VIRULENCE AND MANGANESE OXIDIZING POTENTIAL IN THE EPIDEMIOLOGY OF *GAEUMANNOMYCES GRAMINIS*. T.S. Roseman, R.D. Graham, H.J. Arnott, and D.M. Huber. Botany & Plant Pathology, Purdue University, West Lafayette, IN.

Virulence of *Gaeumannomyces graminis* is correlated with the Mn oxidizing ability of this fungus; however, both characteristics are conditioned by temperature. The Mn oxidation potential of 12 isolates of *G. graminis* grown 21 days on 4% PDA unamended and amended with 50 $\mu g\ g^{-1}$ reduced Mn at 8, 16, 24, and 30 C was conducted. Virulence of the isolates on the take-all susceptible wheat cultivar "Caldwell" grown in "cone-tainers" filled with Bloomfield loamy fine sand in growth chambers was determined after 4 weeks at either 15 or 25 C. Light and electron microscopy evaluation, combined with energy dispersive X-ray microanalysis, confirmed the presence of Mn oxides in the Mn-amended agar and on and near fungal structures. Temperature was a significant interactive factor with both Mn oxidation and virulence. Some strains of the pathogen were only virulent and able to oxidize Mn at either low or high temperatures, while other isolates were insensitive to temperature for these characteristics. These reactions appear highly important in the epidemiology and control of take-all.

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OCCURRENCE OF BOTH HOST-SPECIALIZED AND NON-SPECIALIZED FORMS IN *COCHLIOBOLUS SATIVUS*, CAUSE OF ROOT ROT OF WHEAT AND BARLEY. R. C. Koech and R. W. Stack. Dept. of Plant Pathology, North Dakota St. Univ., Fargo ND 58105

Monoconidial cultures of *Cochliobolus sativus*, four isolated from barley and six from wheat, were tested for pathogenicity to wheat and barley in three replicated factorial experiments. Wheat and barley plants were raised in individual plastic tubes containing a sand-conidia mixture (400 cfu / g). After five weeks, plants were scored for disease using the subcrown internode index. All cultures caused some disease in both wheat and barley and seven of the ten cultures ranked similarly for disease caused in the two hosts. Two cultures, derived from wheat, caused high disease levels in wheat but low levels in barley while one culture from barley showed the converse. Since all cultures originated from a single locality, natural populations of *C. sativus* may well exist as mixtures of specialized and non-specialized forms.

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INFLUENCE OF TILLAGE ON SOIL POPULATIONS OF *FUSARIUM* SPECIES IN A SPRING WHEAT CROPPING SYSTEM. B. Salas and R. W. Stack, Dept. Plant Path., ND St. Univ., Fargo, 58105.

In the fifth year of an integrated cropping system trial, 4793 isolates of *Fusarium* were recovered from soil by dilution plating. Fourteen species were identified, of which eight made up 98%. *F. solani (27.28%), F. equiseti (21.86%),* and *F. oxysporum* (18.46%) were the most common. The cereal root pathogens *F. culmorum, F. graminearum,* and *F. avenaceum* together accounted for 4.39% of all cultures. The total populations of fusaria were similar under conventional (3266 cfu/g) and reduced tillage (3124 cfu/g) but the composition was different. *F. equiseti* was just 16% of the total in reduced tillage plots while it comprised 27% under conventional tillage. Populations of most other species including

F. culmorum. F. graminearum, and *F. avenaceum* were higher under reduced tillage than under conventional tillage. Neither fertility level nor crop rotation appeared to affect populations of these three cereal root pathogens.

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SUSCEPTIBILITY OF TISSUE-CULTURED ASPARAGUS PLANTLETS TO FUSARIA IN VITRO. Youn Su Lee and W. J. Manning, Dept. of Plant Pathology, Univ. of Massachusetts, Amherst, MA. 01003.

Asparagus plantlets (*Asparagus officinalis* L.) (female clone, NJ 362M) were obtained via meristem tip culture, increased on multiplication medium, and placed on filter paper slants in tubes containing Hoagland's solution. Two agar discs (controls) or two agar discs bearing a *Fusarium* isolate, were placed in contact with roots, just below the crown. Three isolates of *F. oxysporum* and *F. moniliforme*, and one isolate of an avirulent *F. oxysporum* and one of *F. solani* were used as inoculum. Plantlets were evaluated for disease incidence after 4 weeks. All plantlets inoculated with all *F. moniliforme* isolates were killed. Isolates of *F. oxysporum* killed some plantlets, but caused only moderate disease in others. The avirulent *F. oxysporum* isolate caused moderate root discoloration, but no disease. *Fusarium solani* caused severe root discoloration, no disease, and appeared to mildly stimulate plantlet growth. The experiment was repeated twice.

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PURIFICATION AND CHARACTERIZATION OF AN ISOLATE OF TOBACCO STREAK VIRUS INFECTING ESCAROLE AND LETTUCE IN SOUTH FLORIDA. L.L. McDaniel, ATCC, Rockville, MD 20852; R.N. Raid, C. Elliott and R.T. Nagata, Univ. of Florida, Belle Glade 33430; and J.H. Tsai, Univ. of Florida, Fort Lauderdale 33314.

Isometric, virus-like particles (VLP) were associated with escarole necrosis, affecting Cichorium endivia and Lactuca sativa in the vegetable producing area of south Florida. Symptoms on infected escarole and lettuce leaves included chlorosis and necrotic lesions. Necrotic lesions formed on leaves, petioles, pulvini and stems of Phaseolus vulgaris. A close relationship was demonstrated between the VLP and strains of tobacco streak virus (TSV) using serological assays and one-dimensional peptide mapping. The VLP was identified as the bean red node strain of TSV. This is the first report of natural infection of escarole and lettuce by TSV.

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THE CAUSAL AGENT OF LIGUSTRUM RINGSPOT DISEASE. M. Lucia R. Z. da Costa Lima, V. C. Lima Neto and Vania B. V. de Souza. CNPq and Universidade Federal do Parana. 80.000-Curitiba, PR-Brazil.

Ligustrum ringspot disease (LRSD) was first noticed in 1976 in one plant of Ligustrum lucidum Ait. from a hedge; it took 14 yr to spread up to a distance of 20 m. Recently, bacilliform virus-like particles (VLPs) were identified in cells of symptomatic leaves. The VLPs were similar to rhabdoviruses in shape and cellular distribution, but were smaller (27x80-100 nm). VLPs were found exclusively in a 3 mm radius around the lesion center. This may explain the failure to transmit the virus by routine inoculation procedures and suggests that 3 mm disks of lesion tissue should be used for inoculum or for purification. Several ultrastructural characteristics indicate that the agent of the LRSD is a new virus which will be referred to as ligustrum ringspot virus (LRSV). This virus shows similarities to some of the ungrouped small bacilliform viruses such as citrus leprosis, striped chlorosis of mimosa, cacao swollen shoot, commelina yellows and orchid fleck viruses.

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DETECTION OF MAIZE CHLOROTIC MOTTLE VIRUS IN MAIZE EMBRYOS. S. G. Jensen, B. Doupnik, Jr. and E. M. Ball. USDA-ARS, and University of Nebraska, Lincoln, NE 68583.

Seed from Nebraska grown corn, infected with maize chlorotic mottle virus (MCMV), was harvested at weekly intervals during maturation. At harvest, and for several weeks afterward, the seed was tested for the presence of MCMV in the embryos by infectivity, ELISA, and seed transmission to the next generation. Infectivity tests detected virus in more than 50% of excised embryos harvested before physiological maturity (35% seed moisture). As the seed dried down in the field or the laboratory, virus recovery by infectivity declined. However, MCMV was still detected in embryos from mature seed at 11% moisture. Early ELISA tests correlated with infectivity tests but later ELISA tests were confounded by a high level of false positives in healthy control embryos as well as embryos from infected seed. No seed transmission of MCMV was found in more than 3000 seedlings grown from infected seeds.

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OCCURRENCE OF AGROPYRON MOSAIC VIRUS IN WHEAT IN COLORADO. D.L. Seifers, Kansas State University, Fort Hays Branch Experiment Station, 1232 240th Avenue, Hays, Kansas 67601.

Wheat collected in Colorado was determined to be infected with two viruses, wheat streak mosaic virus and Agropyron mosaic virus (AMV). The identity of the viruses was determined using host range tests and serology. The coat proteins of the AMV isolates were compared in native and denatured forms by electrophoresis. The AMV capsids were further compared using partial chemical proteolysis followed by electrophoretic analysis of cleavage products. This is the first report of AMV in Colorado.

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REPLICATION AND MOVEMENT OF WHEAT SOILBORNE MOSAIC VIRUS (WSBMV) IN HARD RED WINTER WHEAT. J. L. Sherwood, L. D. Myers, and R. M. Hunger. Department of Plant Pathology, Oklahoma State University, Stillwater, OK 74078-9947.

Cultivars (cvs) of hard red winter wheat (Triticum aestivum L.) resistant to WSBMV are available, but the mechanism of resistance is not understood. When susceptible (Sage and Vona) or resistant (Hawk and Newton) cvs were inoculated using root washings from WSBMV infected wheat, WSBMV was detected in the roots of all cvs, but lower WSBMV levels were detected in the foliage of resistant as compared to susceptible cvs. However, when foliage of resistant cvs was mechanically inoculated, replication of WSBMV was similar to susceptible cvs (Phytopathology 80:1033). Additional experiments have indicated that WSBMV replicates and moves into roots of both resistant and susceptible cvs following mechanical inoculation of foliage, and expression of resistance to WSBMV may involve an inhibition of virus movement from roots to foliage.

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EFFECT OF EXOGENOUS GA_3 APPLICATION ON ARROWLEAF CLOVER (*Trifolium vesiculosum* Savi) INOCULATED WITH CLOVER YELLOW VEIN VIRUS. I.J. Pemberton and G.R. Smith, Texas Agricultural Experiment Station, P.O. Drawer E, Overton, TX 75684.

Severe dwarfing is one of the typical symptoms of clover yellow vein virus (CYVV). The virus kills about 30% of inoculated 'Yuchi', but none of breeding line ALVT plants. Sixty-day old plants of both lines were inoculated with CYVV-Pratt. Plants were sprayed with 10 or 100 ppm GA_3 either 1 wk pre-or 3 wk post-inoculation. Leaf area of the youngest expanded leaf, stem height, and dry matter yield were measured and shoot numbers per plant counted 5 wk after inoculation. Inoculated and healthy non-inoculated controls were included for comparison. CYVV reduced yield 60%, leaf area 80%, stem height 30%, but had no effect on shoot number. GA_3 at 100 ppm restored stem height of inoculated plants to levels observed for healthy controls, but leaf area increased only 20%. Pre-inoculation application of GA_3 at 10 ppm increased survival compared to post-inoculation treatments, or plants not receiving GA_3. Yield was not affected by plant line, rate or time of GA_3 application. Our results suggest CYVV inhibits GA synthesis, resulting in dwarfed plants, and that exogenous application of GA_3 can partially restore some growth parameters and enhance survival of inoculated plants.

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EVALUATION OF *PRUNUS* FOR RESISTANCE TO NEMATODE TRANSMISSION OF TOMATO RINGSPOT VIRUS. E. V. Podleckis[1], J. M. Halbrendt[2], A. Hadidi[1], R. Scorza[3] and R. Welliver[4]. [1]USDA-ARS, NPGRL, BARC-West, Beltsville, MD 20705, [2]PSU Fruit Lab, Biglerville, PA, [3]USDA-ARS, Kearneysville, WV, [4]PDA, Harrisburg, PA.

Rooted cuttings of *Prunus* varieties Halford, Redhaven, Stanley, and Marianna 2624 were planted in soil containing approximately 38 tomato ringspot virus (TmRSV) infested *Xiphinema americanum* nematodes per 100 cc. Sap extracts were made from root, bark, and leaf tissues of test plants and controls after 10 and 22 weeks. Aliquots of these samples were assayed by inoculation to *Chenopodium quinoa* and by enzyme linked immunosorbent assay (ELISA). Nucleic acid extracts prepared from the remainder of each sample were analyzed by dot blot hybridization using a

cRNA probe for TmRSV. In both tests, ELISA failed to detect TmRSV in any test plant, and bioassay identified one Stanley and one Redhaven as positive. Dot blot results indicated 2/2 Stanley, 2/3 Halford, 4/5 Redhaven and 0/6 Marianna cuttings were infected. These results illustrate the sensitivity of dot blot for TmRSV detection in *Prunus* and indicate that Marianna 2624 may possess resistance to TomRSV or its nematode vector.

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INFLUENCE OF TIME AFTER INOCULATION OF SOURCE PLANTS ON TRANSMISSION OF BYDV-PAV-IL BY RHOPALOSIPHUM PADI. A. D. Hewings, USDA-ARS Crop Protection Reserarch Unit, C. E. Eastman, Illinois Natural History Survey and E. M. Bauske, Department of Plant Pathology, University of Illinois, Urbana, IL 61801

Don oats, moderately sensitive to barley yellow dwarf virus (BYDV-PAV-IL), were grown individually in pots in a growth chamber at 20-22C and 14L:10D. At the 2-leaf stage the seedlings(source plants) were infested for two days with viruliferous *Rhopalosiphum padi*, then fumigated. At 2, 5, 8, 11, 14, 17, 20, 23, 26, 29, and 40 days after infestation, non-viruliferous *R. padi* were allowed a 1-day acquisition access period on detached leaves from 3 randomly selected source plants. A total of 45 aphids (15/source plant) were transferred individually to Coast Black oat test seedlings for a 2-day inoculation access period. The plants were fumigated and moved to the greenhouse. Test plant infection was determined by symptoms and confirmed, when necessary, by enzyme-linked immunosorbent assay. The experiment was done 3 times. Transmission was lowest 2 days after inoculation (2%). A rise in transmission was observed on day 5 that peaked at day 23 (61%). After day 23, transmission rates dropped, but transmission continued until day 40 (22%). Similar experiments with other widely-grown oat cultivars differing in sensitivity to BYDV-PAV-IL are in progress.

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Detection of Maize Chlorotic Mottle Virus in Maize Plant Parts, Seeds of Varying Moisture, and Other grasses in Hawaii. X. Q. Jiang, D. R. Wilkinson, and J. A. Berry. Pioneer Hi-Bred International Inc., 7250 NW 62nd Avenue, P.O. Box 1004, Johnston, IA 50131.

Maize plants exhibiting symptoms of Maize Chlorotic Mottle Virus (MCMV) infection were separated into their various components and assayed for MCMV by ELISA. Leaves, leaf sheaths, stalks, roots, husks, silks, cobs, anthers, and seeds of 13%, 18.7%, 20%, 27%, and 30% moisture tested positive to the MCMV antibody. Sixteen grass species surrounding infected maize fields were assayed for MCMV infection. One sample, sour grass [Trichachne insularis (L.) Nees], was positive to MCMV antibody; all others were negative. The ELISA positive seed of 13% and 30% moisture and the positive sour grass sample were mechanically inoculated to caged healthy young maize plants. The sour grass sample did not mechanically transmit. The plants inoculated with 30% moisture seed all tested ELISA positive for MCMV. Of the maize plants inoculated with the 13% moisture seed, 33% tested weakly ELISA positive and 33% tested strongly ELISA positive.

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HIGH VELOCITY MICROPROJECTILE MEDIATED TRANSMISSION OF WHITEFLY-TRANSMITTED GEMINIVIRUS DNA OR PURIFIED VIRIONS TO INTACT PLANTS. J.K. Brown[1,2] and R. Ryan[2]. [1]Department of Plant Pathology, [2]Department of Plant Sciences; University of Arizona, Tucson, AZ 85721.

Partially purified nucleic acids extracted from plants infected with different whitefly-transmitted (WFT) geminiviruses, or purified virions of the watermelon curly mottle isolate of squash leaf curl virus (WCMoV/SLCV) or the chino del tomate virus (CdTV), were used to inoculate host plants by bombardment with high velocity microprojectiles. Tungsten microprojectiles were coated with nucleic acid or virion inoculum and used to bombard whole plants. Plant host species within the Cucurbitaceae, Euphorbiaceae, Leguminosae, Malvaceae, and Solanaceae were successfully infected when nucleic acid preparations from fifteen different WFT geminivirus isolates were tested. Virions purified from WCMoV/SLCV or CdTV-infected hosts were infectious in tomato and pumpkin, respectively, when mixed with microprojectiles in the presence of polythylene glycol and sodium chloride. Positive evidence for virus infection was based upon the development of characteristic symptoms, and on the ability to detect geminivirus DNA in extracts from symptomatic plants in hybridization assays. These experiments represent the first successful transmission of WFT geminiviruses using microprojectile-mediated inoculation.

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OCCURRENCE AND INHERITANCE OF DOUBLE-STRANDED RNA IN PEPPER. R. A. Valverde. Department of Plant Pathology and Crop Physiology, Louisiana Agricultural Experiment Station, Louisiana State University Agricultural Center, Baton Rouge, 70803.

Twelve distinct dsRNA banding patterns were obtained after screening 80 pepper cultivars. All 19 pepper cultivars belonging to the bell type had similar dsRNA patterns. DsRNAs among cultivars of other pepper types were variable. All 12 dsRNAs were transmitted through the seed after self-pollination. Inheritance studies were conducted with three dsRNA banding patterns designated b, f, and g. DsRNAs b and f were transmitted at a high rate maternally and at a low rate paternally. However, dsRNA g was transmitted at a high rate both maternally and paternally. Preliminary studies indicated a lack of correlation between pepper phenotype and presence or absence of particular dsRNAs.

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COMPARISON OF dsRNA PURIFIED FROM TWO HYPOVIRULENT ISOLATES OF *LEUCOSTOMA PERSOONII*. C. J. P. Jensen, G. C. Adams, and R. F. Allison. Department of Botany and Plant Pathology, Michigan State University, E. Lansing, MI 48824-1312

Extraction of dsRNA from a hypovirulent isolate of *Leucostoma persoonii* (14.4A) yields seven distinct bands on PAGE with estimated sizes of 7.9, 3.0, 2.8, 2.6, 2.4, 2.3, and 0.7 Kb. A second hypovirulent isolate (HT) derived from successive hyphal tipping of 14.4A, contains only the 7.9 Kb and 2.3 Kb dsRNA bands. Abundant virus-like particles (VLP) are visible in the hyphae of 14.4A but not in HT. The VLPs from 14.4A have been purified and partially characterized. Spectrophotometric analysis of sucrose gradients revealed two peaks with absorbances at 254 nm. Both peaks contained protein and dsRNA with 5 bands of dsRNA in the top peak and 6 in the bottom. All dsRNA bands found in the VLP comigrate with dsRNA present in 14.4A tissue except for one unique band of 3.5 Kb. In addition, the bottom peak contains a band of dsRNA that comigrates with the 7.9 Kb band found in 14.4A and HT. To determine the relationship between the dsRNA isolated from tissue of both isolates and the dsRNA of the VLPs, we are probing Northern blots of tissue-extracted dsRNA with individual end-labeled dsRNA bands electroeluted from agarose gels. We are most interested in determining which bands are unique to the VLPs.

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EXAMINATION OF SEROLOGICAL RELATIONSHIPS BETWEEN THREE FILAMENTOUS VIRUSES OF SWEET POTATO USING POLYCLONAL ANTISERA AND MONOCLONAL ANTIBODIES. J. Hammond[1], R. L. Jordan[1], R. C. Larsen[2] and J. W. Moyer[2]. [1]USDA-ARS, FNCL, Beltsville, MD 20705 and [2]Dept. of Pl. Path., N.C.S.U., Raleigh, NC 27695.

Sweet potato feathery mottle (SPFMV), sweet potato latent (SPLV), and sweet potato mild mottle (SPMMV) viruses all have filamentous particles and produce cytoplasmic inclusions typical of the potyvirus group. SPFMV is transmitted by aphids, SPMMV by whiteflies, and no vector is known for SPLV. SPFMV and SPMMV were reported to be related (Shukla et al., 1989, Arch. Virol. 105:143) using a potyvirus cross-reactive polyclonal serum. This relationship was re-examined with virus-specific antisera and several monoclonal antibodies (MAbs) by Western blotting and indirect ELISA. None of the MAbs reacted to SPMMV, but each reacted to distinct epitopes present on either SPFMV or SPLV, or other epitopes common to SPFMV, SPLV and other potyviruses. Polyclonal sera also showed a reciprocal relationship between SPFMV and SPLV, and a lack of relationship between these viruses and SPMMV.

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EVALUATION AND APPLICATION OF PRUNE DWARF VIRUS-SPECIFIC MONOCLONAL ANTIBODIES IN VIRUS DETECTION AND DISEASE DIAGNOSIS. Ramon Jordan[1], Tom Matsumoto[2] and Hei-Ti Hsu[1]. [1]USDA-ARS, FNCL, Beltsville, MD 20705 and [2]Calif. State Dept. of Food and Agric., Sacramento, CA 95814

A panel of 77 monoclonal antibodies (McAbs) previously developed by in vivo or in vitro immunization to Maryland and Wisconsin isolates of prune dwarf virus (PDV) were further evaluated. Most of the McAbs detected PDV isolates from California, Washington, Italy, Bulgaria, West Germany, Hungary, and France. Ascities fluids produced to nine of the most cross-reactive McAbs were tested individually and as specific admixtures for the detection of PDV in California stone fruit trees. These McAbs were able to detect and differentiate diverse cherry and peach isolates, including those from peach stunt-affected trees. At least two spatially distinct epitopes were identified with these nine McAbs; one on the virion surface and the other within intact virions.

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MORPHOLOGICAL VARIATION OF INCLUSIONS INDUCED BY ZUCCHINI YELLOW MOSAIC VIRUS ISOLATES. **M.A. Petersen, J.R. Edwardson, H. Lecoq and D.E. Purcifull. Departments of Plant Pathology and Agronomy, University of Florida, Gainesville, FL 32611, and Station de Pathologie Vegetale, INRA, Montfavet, France.**

Five isolates of the zucchini yellow mosaic potyvirus (ZYMV), including the type isolate from V. Lisa in Italy, an isolate from Reunion Island, and three Florida isolates which differ in symptom severity, were chosen for cytological comparison. Each isolate induced cylindrical inclusions (CI) which were visible by light microscopy. Symptomatic areas of leaf tissue from infected pumpkin (*Cucurbita pepo* L. 'Small Sugar') were prepared for transmission electron microscopy. All five isolates induced pinwheels and scrolls, but the mild Florida isolate produced noticeably fewer inclusions. Each of the other two Florida isolates also induced both straight and curved laminated aggregates. In immunodiffusion tests using antiserum to CI protein of the intermediate Florida isolate, precipitin bands without spur formation were obtained when the other four isolates were compared to the intermediate isolate. The cause of variation in types of inclusions detected among isolates in pumpkin is unknown, but the variations observed have implications for the assignment of ZYMV isolates into subdivisions on the basis of inclusion morphology.

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UI-114 PINTO BEAN IS HETEROGENEOUS FOR RESISTANCE TO NL-8 AND NY-15 STRAINS OF BEAN COMMON MOSAIC VIRUS. R.L. Forster, J.R. Myers, C.A. Strausbaugh, and K. Stewart-Williams, University of Idaho Research and Extension Center, Kimberly, 83341, and G.I. Mink and M.J. Silbernagel, IAREC, Prosser, WA 99350-9687.

Strain NL-8 of bean common mosaic virus (BCMV) which is reported to be non-infectious in UI-114 pinto bean (Phaseolus vulgaris L.) was detected in that cultivar in Idaho in 1989. The disease reactions of three breeder and foundation class UI-114 seedlots to the NL-8 (ID) and NY-15 (Zaumeyer) strains of BCMV (mechanical inoculation with Carborundum in sap; 100 plants of each lot, plus controls) were determined in the greenhouse in 1990. Systemic mosaic symptoms developed in 64-76 and 82-97% of the plants in the three seedlots inoculated with NL-8 and NY-15, respectively. All results were confirmed by ELISA. Results of another test with a fourth UI-114 seedlot (second generation increase of seed stored in the National Seed Storage Laboratory in Ft. Collins, CO, since 1966) indicated more than 90% of the plants were resistant to the two strains; thus, there are at least two populations of UI-114 in existence.

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EVIDENCE FOR BEETLE-SPECIFIC DIFFERENCES IN CIRCULATIVENESS OF PLANT VIRUSES. R. Y. Wang, R. C. Gergerich, and K. S. Kim. Department of Plant Pathology, University of Arkansas, Fayetteville, AR 72701.

The movement of ingested plant viruses into the hemolymph of Chyrsomelid beetle vectors has been demonstrated with several beetles in this family, including the bean leaf beetle (BLB; Cerotoma trifurcata [Forster]) and the spotted cucumber beetle (SCB; Diabrotica undecimpunctata howardii [Barber]). To determine whether plant viruses also move into the hemolymph of beetles in the family Coccinellidae, parallel experiments were conducted to study the movement of four plant viruses (two beetle-transmissible and two non-beetle-transmissible) in the Mexican bean beetle (MBB; Epilachna varivestis Muls.) along with the SCB and BLB. Beetles acquired virus either by feeding on infected plants or by drinking drops of concentrated purified virus. Hemolymph was assayed for virus by inoculation to appropriate local lesion hosts and by ELISA. No viruses were detected in the hemolymph of the MBB, regardless of the acquisition source, the type of virus, or the method of virus detection. The four viruses were not destroyed by the hemolymph of the MBB, as demonstrated by their survival in hemolymph up to four days after virus injection into the hemocoel of the MBB. These results contradict the assumption that plant viruses are circulative in all beetle vectors.

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GENOME SIZE AND REPETITIVE DNA CONTENT OF *PUCCINIA GRAMINIS* F. SP. *TRITICI*. J. E. Backlund and L. J. Szabo. USDA/ARS, Cereal Rust Lab., Dept. of Plant Pathology, Univ. of Minn., St. Paul, MN 55108

Little is known about the basic structure of cereal rust genomes. Since knowledge of genomic characteristics is essential for molecular biological research, we have investigated the genome of the wheat stem rust fungus using reassociation kinetics. In this method, genome size and repetitive DNA content are derived from an analysis of the rate at which fragmented, denatured DNA reanneals. We estimate that the total genome size is 58 ± 10 million base pairs (mbp) and consists of three classes of sequences: 1) 64% unique; 2) 30% repetitive; and 3) 4% foldback DNA. The overall genome size was corroborated using a slot-blot genomic reconstruction experiment (68 ± 16 mbp), in which the genome size was derived from the number of copies of a unique sequence in a known amount of genomic DNA.

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ISOLATION AND CHARACTERIZATION OF LIGHT-ENHANCED CDNA CLONES FROM CERCOSPORA KIKUCHII. M. Ehrenshaft, T.M. Callahan and R.G. Upchurch. USDA/ARS and Dept of Plant Pathology, NCSU, Raleigh, NC

Biosynthesis of the red, phytotoxic polyketide, cercosporin, is light-induced in the fungal soybean pathogen Cercospora kikuchii. A subtractive hybridization technique was used to isolate 6 light-enhanced cDNA clones. One of these clones is strongly light-regulated, while the transcripts corresponding to the other five cDNAs exhibit light enhancement ranging from slight to marked. In the wild-type strain, PR, transcript accumulation of two cDNAs, cLE6 and cLE7, strongly parallels toxin accumulation. Transcripts for the other 4 light-enhanced cDNAs did not show any marked changes in accumulation. Transcript accumulation for 5 of the 6 light-enhanced cDNAs is dramatically reduced in a medium-regulated mutant grown on non-inducing medium. Under conditions of low stringency, cLE7 hybridized to the acetyl-malonyl transferase domain of the 6-methylsalicylic acid polyketide synthase gene from Penicillium patulum. The characerization of the genomic clones corresponding to these cDNAs is underway.

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ISOENZYME POLYMORPHISMS AND GENETIC DIFFERENTIATION AMONG BINUCLEATE *RHIZOCTONIA* SPECIES. M. Damaj, P. M. Charest and S. Jabaji-Hare. Dépt. de Phytologie, Université Laval, St. Foy, Québec, Canada, G1K. 7P4.

Isolates of binucleate *Rhizoctonia* spp. are grouped by anastomosis similar to *Rhizoctonia solani*. Although their assignment to anastomosis groups (AGs) confers some degree of order, such a classification system can not delineate species. Electrophoretic profiles of 7 enzymes were used as taxonomic characters to asses genetic variations in 10 Japanese AGs and 5 North American CAGs of binucleate *Rhizoctonia* spp. The relative mobility values of the electrophoretic bands were subjected to UPGMA cluster analysis. Two main clusters were formed. The first cluster consisted of isolates belonging to CAG-1, CAG-3, AG-D, AG-G, AG-O, and AG-B and its subgroups. The second cluster was composed of AG-A, AG-K, AG-F, CAG-2 and CAG-4. Isolates of AG-D and CAG-1, whose hyphae are known to inter-anastomose, were genetically related and were grouped at a high level of genetic similarity followed by those of CAG-3 and AG-G. In the second cluster, the highest degree of similarity was observed for AG-K and CAG-4. Intragroup variation was observed in AG-B and CAG-2.

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TRANSFORMATION OF *FUSARIUM OXYSPORUM* F. SP. *NIVEUM* TO HYGROMYCIN B RESISTANCE. D. H. Kim, R. D. Martyn, and C. W. Magill, Department of Plant Pathology and Microbiology, Texas A&M University, College Station, TX 77843.

Fusarium oxysporum f. sp. *niveum* (FON) was transformed to hygromycin B (Hyg B) resistance using two heterologous vectors, pDH25 and CosHyg1. $5x10^7$ protoplasts were mixed with 3 - 5 µg of DNA in polyethylene glycol and Ca^{2+} and transformants were selected on 1.2 M sorbitol-regenerating media containing 120 µg/ml of Hyg B. Five and 15 transformed protoplasts, respectively, were obtained per µg of pDH25 and CosHyg1; however, 1-10% lost resistance during alternating transfers on Hyg B^+ and Hyg B^- media. Five pDH25-mediated and twelve CosHyg1-mediated transformants were assayed for Hyg B resistance stability through conidiation and all but two produced Hyg B resistant-microconidia. Of four transformants examined, none contained the autonomous replicating vectors. Probe analysis showed that the vector had integrated into the FON genome. Multiple integration of vectors was detected; however, the concatenate integration of the vector was not detected. No change in pathogenicity was observed among four transformants tested.

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CHROMOSOMAL POLYMORPHISM IN *FUSARIUM OXYSPORUM* F. SP. *NIVEUM*. D. H. Kim, R. D. Martyn, and C. W. Magill, Department of Plant Pathology and Microbiology, Texas A&M University, College Station, TX 77843.

Genome size and chromosome number in seven isolates of *Fusarium oxysporum* f. sp. *niveum*, the causal agent of Fusarium wilt of watermelon, representing six mtDNA RFLP groups (I - VI) and the three known pathological races were examined by transverse alternating field electrophoresis (TAFE). Chromosomes were separated on 0.8% agarose gel at 80 volts using five different switch intervals for 168 hr. Six different karyotypes were detected among the seven isolates. Two representative isolates from RFLP I divided into two different karyotypes while the representative isolates from RFLP IV and RFLP V had the same karyotype. Chromosome number varied from five to nine among the isolates and ranged

from approximately 950 kb to 4,600 kb. The two largest chromosomes (4.6 Mb and 3.4 Mb) were conserved in all isolates; however, smaller chromosomes varied both in number and size among isolates. The total genome size of *F. o.* f. sp. *niveum* ranged from 13.8 Mb to 23.6 Mb.

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GENETIC AND MOLECULAR DIVERSITY IN COLLETOTRICHUM GLOEOSPORIOIDES. J. C. Correll, G. J. Weidemann, D. O. TeBeest, and J. C. Guerber. Dept. of Plant Pathology, University of Arkansas, Fayetteville, AR 72701.

Isolates of Colletotrichum gloeosporioides from several diverse hosts (apple, northern jointvetch, lime, and pecan) were examined for vegetative compatibility, using nitrate nonutilizing (nit) mutants, and mitochondrial DNA restriction fragment length polymorphisms. Four to twelve isolates from each host were examined. VCG diversity was high (i.e. most isolates belonged to a unique VCG) among isolates from all hosts except those from northern jointvetch. Isolates from northern jointvetch all belonged to a single VCG. No isolates from different hosts were vegetatively compatible. Mitochondrial genome sizes ranged from approximately 53-56 kb among the isolates examined. Restriction fragment patterns of mtDNAs were similar or identical for isolates from any given host but were different for isolates from different hosts. These data indicate that restriction patterns of mtDNAs are correlated with host and not VCG. These data are in contrast to the pathogenic strains of the strictly asexually reproducing organism Fusarium oxysporum in which mtDNA restriction patterns are correlated with VCG. Similar or identical mtDNA restriction patterns among different VCGs of a common host may represent distinct populations within this single morphological species.

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SEPARATION OF CHROMOSOMES OF PHYTOPHTHORA SPECIES USING CONTOUR-CLAMPED HOMOGENEOUS ELECTRIC FIELD (CHEF) GEL ELECTROPHORESIS. P. W. Tooley and M. M. Carras, USDA-ARS, Frederick, MD 21702.

Protoplasts produced by treating young mycelia grown 3-5 days in pea broth with 0.5% Novozym 234 and 0.5% Driselase were embedded in 0.5% agarose and treated overnight at 50 C in NDS buffer (0.5 M EDTA, 10 mM Tris, 1% sodium lauroylsarcosine, 0.1% Proteinase K). Chromosomal DNAs were electrophoresed on 0.6% chromosomal grade agarose gels in 0.5X TAE buffer using a Bio-Rad CHEF gel unit. Ramped switching times of 3 to 45 minutes at 50 volts for 7 days at 14 C allowed resolution of 13 bands for P. megasperma isolate 63, in agreement with the chromosome number reported from cytological studies. At least six bands were resolved for P. cactorum, and four bands for P. bohmeriae. Chromosomes of P. capsici separated but were poorly resolved under these conditions. Chromosomes of P. infestans and P. parasitica failed to separate, migrating as a single high molecular weight band. Substantial variation in band intensity was observed within isolates of all species.

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MOLECULAR ANALYSIS OF GENOME ORGANIZATION IN USTILAGO HORDEI AND FATE OF TRANSFORMING PLASMID DNA. Kevin McCluskey and Dallice Mills, Department of Botany and Plant Pathology, Oregon State University, Corvallis, Oregon, 97331-2920.

Chromosome-length polymorphisms have been identified among strains representing the fourteen races of U. hordei. A probe for rDNA identifies variability of as much as 2,000 kilobases (kb). Chromosome-specific, random genomic, and heterologous gene probes were hybridized to ten unique chromosomes. In some strains several of these probes are apparently linked. This genomic variability was analyzed by using heat shock to generate a mutant that has lost a chromosome of 960 kb. The mutant has an altered cell and colony morphology and is apparently deficient in mating. To determine the ability of episomal DNA to interact with the genome, the replicating plasmid pCM54 was used to transform strain 8.1a. The plasmid is apparently present in both monomeric and concatameric forms, and replicates without integrating into the genome.

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PHYLOGENETIC AND STRUCTURAL RELATIONSHIPS OF THE RRNA GENES OF *EPICHLOË/ACREMONIUM* GRASS ENDOPHYTES AND OTHER CLAVICIPITACEAE. Jih-Shiou Liu, Alfred D. Byrd, Huei-Fung Tsai, Malcolm R. Siegel, and Christopher L. Schardl. University of Kentucky, Lexington KY 40546-0091.

Acremonium sect. *Albo-lanosa* (Fungi Imperfecti) includes beneficial, endophytic mycosymbionts of various grasses of the subfamily Pooideae, and also the anamorph of the grass choke pathogen, *Epichloë typhina* (Clavicipitaceae). The endophytes are seed disseminated, thus stably maintained for many host generations. Isolates of *Acremonium* spp. endophytes and *E. typhina* were obtained from several host grasses. The two internal transcribed spacer (ITS) sequences of the rRNA genes of these isolates and other Clavicipitaceae were determined and compared by maximum parsimony. The cladogram indicated that the *Acremonium* spp. have evolved from *E. typhina* on multiple occasions. Furthermore, these mycosymbionts do not appear to have coevolved with their host species. The primary and predicted secondary structures of the ITS RNA sequences from *Acremonium coenophialum* and *Neurospora crassa* (Sordariaceae) were compared. Although there was extensive sequence divergence, similar secondary structures were indicated. The ITS RNAs of other Clavicipitaceae were also predicted to possess these secondary structures. ITS sequences aligned by the structure models were useful in helping elucidate phylogenetic relationships among genera and species of the Clavicipitaceae.

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INTRASPECIFIC GROUPS OF RHIZOCTONIA SOLANI IDENTIFIED BY ISOZYME ANALYSIS. Zonglin Liu, and J. B. Sinclair, Dept. of Plant Pathology, Univ. of Illinois at Urbana-Champaign, 1102 S. Goodwin Ave., Urbana, IL 61801-4709.

Horizontal starch gel electrophoresis was used for isozyme studies of Rhizoctonia solani anastomosis groups: AG-1 and AG-2. Banding patterns of acid phosphatase (EC 3.1.3.2), aconitase (EC 4.2.1.3), esterase (EC 3.1.1.1), hexose kinase (EC 2.7.1.1), isocitric dehydrogenase (EC 1.1.1.42), leucine amino peptidase (EC 3.4.11.1), malate dehydrogenase (EC 1.1.1.37), phosphoglucoisomerase (EC 5.3.1.9), phosphoglucomutase (EC 2.7.5.1), and 6-phosphogluconate dehydrogenase (EC 1.1.1.44) were polymorphic for 118 isolates from various hosts and geographic origins. Preliminary results indicated that the isolates separated into seven subgroups with intragroup variations based on their relationship by numerical cladistic analysis. Variations between AGs were greater than within AG. These subgroups agreed with previously reported intraspecific groups based on anastomosis behavior and pathogenicity tests, with the exception of one subgroup in AG-2.

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ISOLATION OF FUNGAL DNA GREATER THAN 200 KILOBASES USING STANDARD EXTRACTION METHODS. P. T. Gieser and N. D. Young, Dept. of Plant Pathology, University of Minnesota, St. Paul, MN 55108.

Many questions in eukaryotic molecular biology require analysis of high molecular weight (HMW) DNA (100-1000 kilobases; kb). Pulsed-field gel electrophoresis and cloning into yeast with artificial chromosomes can be used to manipulate HMW DNA and examine long-range genomic organization. Preliminary tests have demonstrated that HMW DNA can be isolated from fungal tissue using standard detergent-based, chemical extraction methods under limited handling conditions to minimize shearing. We have isolated HMW DNA larger than 200kb from a variety of fungal isolates including; *Armillaria ostoyae*, various *Fusarium* species, *Rhizoctonia* species and *Uromyces appendiculatus* with some DNA isolates containing molecules larger than 1000kb. This DNA should be suitable to construct yeast artificial chromosome (YAC) libraries for molecular analysis at the megabase level.

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A METHOD FOR RAPID SMALL-SCALE PREPARATION OF FUNGAL DNA. C.-S. Yoon, D. A. Glawe, and P. D. Shaw. Department of Plant Pathology, University of Illinois, Urbana 61801.

Studies on restriction fragment length polymorphisms require DNA that is sufficiently free of contaminants to allow efficient digestion by restriction endonucleases. We tried a variety of published techniques for extracting DNA from Pyrenomycetes (Fungi, Ascomycetes), but found that all gave DNA preparations that included contaminants that interfered with endonuclease activity, or gave low yields of DNA. The technique described involves treating ground mycelium with a lysis buffer containing SDS, followed by precipitation of contaminants using CsCl. The procedure was tested using a range of fungi, and gives high yields of DNA that is digested efficiently by restriction endonucleases. The procedure is rapid, relatively inexpensive, does not involve phenol extraction, and is well-suited for studies on large numbers of individuals.

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REGULATION OF NORSOLORINIC ACID REDUCTASE, A KEY ENZYME CATALYZING AN EARLY STEP IN AFLATOXIN B_1 BIOSYNTHESIS BY ASPERGILLUS PARASITICUS. T. E. Cleveland, J. W. Cary, D. Bhatnagar, and N. P. Keller, USDA/ARS, Southern Regional Research Center, P.O. Box 19687, New Orleans, Louisiana 70179.

An antiserum probe was produced against a reductase, isolated from Aspergillus parasiticus mycelia, which catalyzes the first

known step in aflatoxin biosynthesis. The quantity of the reductase in developing mycelial shake cultures was determined at several times during a 3-day fermentation by enzyme activity measurements and by Western blotting. The enzyme was first detected after 24 hr when the first traces of aflatoxin B_1 were detected. The antiserum probe has been used to: (1) study regulation of a key biosynthetic step in aflatoxin biosynthesis and (2) screen a cDNA expression library for cDNA's coding for the reductase; the library was constructed using λZAP as an expression vector and A. parasiticus cDNA's synthesized from mRNA isolated from mycelia 20 h after inoculation.

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IN VITRO CLEAVAGE OF BARLEY YELLOW DWARF VIRUS RNA BY A SYNTHETIC RIBOZYME. J. D. Griffin and P. H. Berger. Dept. of Plant, Soil, & Entomological Sciences, Univ. of Idaho, Moscow, ID 83843.

A hammerhead ribozyme was designed based on the sequence of a portion of the coat protein gene of barley yellow dwarf virus (BYDV). A synthetic oligonucleotide containing the ribozyme sequence was cloned into the T7 transcription vector pTZ18. *In vitro*-transcribed ribozyme RNA was incubated at 50°C with a 576-base target RNA transcribed from a plasmid containing an *Eco*RI fragment of the BYDV coat protein gene. The ribozyme cleaved target RNA corresponding to the plus strand of BYDV RNA, resulting in products of the predicted size, but did not cleave minus-strand RNA. Incubation of the target RNA with a molar excess of ribozyme RNA resulted in cleavage of >90% of the target RNA.

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NUCLEOTIDE SEQUENCE AND GENOME ORGANIZATION OF THE SATELLITE VIRUS OF ST. AUGUSTINE DECLINE, STRAIN "N". U. B. Gunasinghe, P. J. Shiel, and P.H. Berger. Department of Plant, Soil and Entomological Sciences, University of Idaho, Moscow, ID 83843.

Complementary DNA (cDNA) was synthesized to the genomic RNA of the satellite virus of St. Augustine decline (sSAD, strain N) using random primers and reverse transcriptase followed by cloning into the plasmid vector pBluescript. Clones with inserts were selected and used for sequence analysis. Sequence of the 5'-end was confirmed by direct RNA sequencing. For determination of the 3' end sequence, genomic RNA was polyadenylated and primed with oligo d(T) to make cDNA followed by cloning into pBluescript. When the nucleotide sequence of this satellite virus was compared to the nucleotide sequences of the satellite viruses of panicum mosaic virus (sPMV) (Masuta *et al.*,. 1987) and maize white line mosaic virus (MWLMV) (Zhang *et al.*, 1991), it was closely related to sPMV but had no appreciable homology to the satellite virus of MWLMV. Comparisons of genome organization and structure of these satellite viruses will be presented.

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ISOZYME AND DNA POLYMORPHISMS IN *MYCOSPHAERELLA FIJIENSIS*, AND *M. MUSICOLA*, THE FUNGI CAUSING SIGATOKA LEAF SPOTS OF BANANA. A. Johanson, Natural Resources Institute, Chatham Maritime, Chatham ME4 4TB, United Kingdom.

Two forms of sigatoka leaf spots affect bananas and plantains. Yellow sigatoka is caused by the fungus *Mycosphaerella musicola* and black sigatoka by *M. fijiensis*. Both diseases are of economic importance, but black sigatoka develops more rapidly, and is more difficult to control than yellow sigatoka. It is often not possible to distinguish between the pathogens by symptoms or morphology alone.

The genetic variability of the two species is currently being investigated. Polyacrylamide gel electrophoresis (PAGE) of soluble proteins was used to compare isolates from different geographical areas. Of the sixteen enzymes initially tested, several, including esterases, acid phosphatase, alkaline phosphatase, and glucose phosphate isomerase were differential for the species. No differences have so far been observed within each species. The potential of genomic and mitochondrial DNA restriction fragment length polymorphisms, and Random Amplified Polymorphic DNA markers as a taxonomic tool, and as a means of studing the population dynamics of these

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REGULATION OF EXOPOLYSACCHARIDE SYNTHESIS IN ERWINIA STEWARTII BY rcsB AND rcsC. D. L. Coplin, K. Poetter, and D. R. Majerczak. Dept. of Plant Pathology, The Ohio State University, Columbus, OH 43210-1087.

In Escherichia coli, extracellular polysaccharide (EPS) biosynthesis is regulated by a two-component system consisting of a sensor, RcsC, and an activator, RcsB. EPS production was restored to several nonmucoid Tn5 mutants of E. stewartii by cosmid pES2006 from a genomic library. In reciprocal tests, pES2006 complemented E. coli rcsB and rcsC137 mutants and a cloned E. coli rcsB gene complemented the E. stewartii EPS-minus mutants. Transposon mutagenesis of pES2006 followed by gene replacement confirmed the presence of an rcsB gene in pES2006 and located an adjacent rcsC region that corrected the phenotype of E. coli rcsC137 mutants. Transcription of a cps::lacZ gene fusions were reduced >60% in rcsB mutants of both species. Functional domains of E. stewartii rcsB and rcsC were defined by deletion analysis and DNA sequencing. E. stewartii and E. coli rcsB genes had 75% nucleotide sequence homology. Partial sequence comparisons of the rcsC region also revealed conserved regions. As in E. coli, the E. stewartii rcsB and rcsC ORFs appear to be convergently transcribed.

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MOLECULAR CLONING AND CHARACTERIZATION OF A GEMINIVIRUS INFECTING TOMATOES IN MEXICO. E. J. Paplomatas[1], S. H. Hidayat[2], D. P.Maxwell[2], and R. L. Gilbertson[1]. [1]Dept. of Plant Pathology, University of California, Davis, CA 95616, [2]Dept. of Plant Pathology, University of Wisconsin, Madison, WI 53706.

Recent viral epidemics in tomatoes in the Southern United States and Central America have led to an effort to identify and characterize the viruses involved. A geminivirus (TGV-MX) from infected tomatoes from Queretaro, Mexico was sap-transmitted to *Nicotiana benthamiana*. In contrast to a tomato-infecting geminivirus from Florida (TGV-FL), TGV-MX was also sap-transmitted to *Phaseolus vulgaris* cv. Topcrop. Furthermore, TGV-MX did not hybridize under high stringency conditions with a full-length clone of the TGV-FL DNA-B component, indicating that these are distinct viruses. Viral DNAs were extracted from TGV-MX-infected *N. benthamiana* and putative full-length DNA-A and DNA-B clones obtained. Restriction maps and partial nucleotide sequences of these clones confirmed that TGV-MX is different than TGV-FL and other previously characterized geminiviruses. Efforts are underway to establish whether the cloned viral DNAs are infectious. These results indicate that several geminiviruses may be involved with these tomato virus epidemics.

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SEQUENCE ANALYSIS OF THE 5' END RNAs OF THE STRAINS OF COWPEA CHLOROTIC MOTTLE VIRUS. H. Z. Shang and J. J. Bujarski, Plant Molecular Biology Center, Northern Illinois University, DeKalb, IL 60115.

An agarose gel analysis of the RNA components from four cowpea chlorotic mottle virus (CCMV) strains has revealed size differences among the RNAs 2 and 3. In order to find out the nature of this variance, a primer extension protocol was used to determine sequences near the 5' end. It was found that the first 60 nt of RNA 1 in all strains were identical. In RNA 2, short insertions were present in S and R but not in D and N strains. In RNA 3, the identical sequences were found among D and N strains and among S,R and T strains, with 85% homology between them. We are generating transcribable cDNA clones of CCMV strains that will be used to localize the most significant interstrain sequence differences

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GENOME CHARACTERIZATION OF THE RICE TUNGRO BACILLIFORM VIRUS (RTBV) A new type of double stranded DNA virus infecting monocots. A.de Kochko[1], R.Qu[1], G.S.Laco[1], M.Bhattacharyya[1], B.L.S.Rao[1], M.Kaniewska[1], J.S.Elmer[2] D.E.Rochester[1], C. E.Smith[2] & R.N.Beachy[1], 1: Dept. of Biology, Washington University, St. Louis, MO 63130. 2: Monsanto, St. Louis, MO 63198

The Rice Tungro Bacilliform Virus, associated with the Rice Tungro Spherical Virus, produces severe disease in rice plants in Southeast Asia. The RTBV genome is a double stranded DNA of 7969 bp with five open reading frames. The predicted translational product of ORF3 is a polyprotein of 1665 amino acids which contains the 37 kDa coat protein (we confirmed its identity by expression in *E.coli*),the consensus sequences for proteinase, reverse transcriptase and ribonuclease H activities. A DNA fragment that contains a promoter has been found in the intergenic region which starts after ORF 5. Both 5' and 3' ends of a genome length transcript have been determined, and show an overlap of 267 bp.The products encoded by ORFs 1-2-4-5 are unkown. According to these results, RTBV is not a caulimovirus, it may be a possible member of the Commelina yellow mottle virus group.

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MUTATIONAL ANALYSIS OF POTYVIRUS HELPER COMPONENT ACTIVITY. C.D. Atreya, M.L. Fritzo, P.L. Atreya, D.W. Thornbury, and T.P. Pirone.

The potyvirus potato virus C (PVC) produces biologically inactive "helper component" (HC). In previous work, comparison of the amino acid sequence of PVC-HC with that of five aphid transmissible potyviruses revealed two amino acid differences specific to PVC. Two approaches have been used in an attempt to identify specific amino acid sequences involved in potyvirus HC activity using genome-length infectious tobacco vein mottling virus (TVMV) cDNA clones. In the first approach, we made site-directed mutations to change these amino acids in the HC region. We also constructed PVC/TVMV-as well as PVY/TVMV-hybrid genomes in order to assess the effect on HC activity. The results of the site-directed mutations and the chimeric nature of the virus on infectivity, symptomatology and transmissibility will be discussed.

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MUTATIONS NEAR THE N-TERMINUS OF THE COAT PROTEIN AFFECT APHID TRANSMISSIBILITY OF TOBACCO VEIN MOTTLING VIRUS (TVMV).
P.L. Atreya, C.D. Atreya and T.P. Pirone, Department of Plant Pathology, University of Kentucky, Lexington, KY 40546.

Coat proteins (CP) of naturally-occuring aphid transmissible (AT) and non-aphid transmissible (NAT) isolates of several potyviruses have been reported to differ in the second or third amino acid of an Asp-Ala-Gly (DAG) triplet sequence, located near their N-termini. To test the hypothesis that these specific changes in the DAG region result in loss of aphid transmissibility, we deleted the amino acids of this triplet in the CP of an aphid-transmissible (AT) isolate of TVMV, or changed them to amino acids that occur in NAT isolates of other potyviruses. Deletion of the DAG triplet completely abolished aphid transmission. A change in the first amino acid of the triplet had no effect, but changes in the second or third amino acids reduced or abolished aphid transmissibility. In addition, an amino acid change Lys to Glu adjacent to the DAG, which resulted in the sequence change DAGK to DAGE also drastically reduced aphid transmissibility. However, an amino acid change Gln to Pro several amino acid residues away from the DAG triplet had only a slight effect on aphid transmission.

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TRANSLATIONAL CONTROL OF BARLEY YELLOW DWARF VIRUS. R. Di, V. Brault, S. P. Dinesh-Kumar, W. A. Miller. Department of Plant Pathology, Iowa State University, Ames, IA 50011.

Barley yellow dwarf virus (BYDV) appears to use several unusual translational phenomena in the expression of its genes. These include a translational frameshift (polymerase gene), stop codon readthrough (a gene possibly involved in aphid transmission) and initiation at two sites on the same subgenomic mRNA (coat protein and putative VPg). Our results show that frameshifting occurs in vitro, in vivo. Frameshifting was quantitated in E. coli and oat protoplast by fusing the putative viral frameshifting sequence to reporter genes, lac Z and gus respectively. We will identify sequences and structures required for frameshifting in vitro and in vivo. We have precisely mapped the 5' end of the subgenomic mRNA from which coat protein, 17K and 50K proteins are translated, and will identify sequences and structures which facilitate translational initiation at two sites.

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ACCUMULATION PATTERNS OF CaMV STRAINS CM1841 AND W260 *IN PLANTA*: IMPLICATIONS FOR VIRAL GENE FUNCTION IN SYMPTOM EXPRESSION AND VIRION STABILITY. E.J. Anderson and J.E. Schoelz; Univ. of Missouri; Dept. of Plant Pathology; 108 Waters Hall; Columbia 65211.

CM1841, a mild isolate of cauliflower mosaic virus (CaMV), accumulated to high levels in turnips (*Brassica campestris* L. 'Just Right') when estimated serologically. The more severe isolate, W260, produced less viral antigen *in planta* but was purified consistently at significantly higher concentrations than CM1841. Through the use of wild type CM1841 and W260 strains and chimeric viruses, we have measured differences in disease severity as influenced by viral DNA, RNA, and coat protein accumulation patterns in turnips. These studies indicated that the CM1841 gene VI product enhanced the synthesis of the coat protein and possibly of the other proteins from the 35S RNA *in planta*. There was also an increase in viral DNA and RNA synthesis with a concomitant enhancement in disease severity when the coat protein was derived from strain W260. From the purification and stability characteristics of wild type and chimeric viruses, it was indicated that CM1841 coat protein caused the production of less stable virions. We propose a model of CaMV infection that may explain how viral gene function and virion stability influence virus accumulation and disease severity.

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ROLE OF GENE I IN STUNTING SEVERITY AND VIRUS ACCUMULATION OF CaMV IN TURNIPS. S.G. Qiu, E.J. Anderson, and J.E. Schoelz; Department of Plant Pathology; 108 Waters Hall; University of Missouri; Columbia, MO 65211.

Recent studies in our lab demonstrated that CaMV strains CM1841 and W260 induced different degrees of stunting in turnips (*Brassica campestris* L. 'Just Right'). CM1841 caused turnips to be mildly stunted whereas W260 caused a moderate to severe stunting. In addition, CM1841 and W260 differed in their abilities to accumulate in turnips. Even though CM1841 induced a much milder stunting of turnips than W260, it accumulated to a much higher level in turnips than W260. By exchanging segments between W260 and CM1841, we mapped several genes that are responsible for the stunting severity and/ or accumulation of the virus. Amongst the genes responsible for the two characteristics, genes VI and I are currently of interest to us. Chimeric viruses showed that gene I of W260, in combination with gene VI of CM1841, could severely stunt turnips and cause chimeric viruses to accumulate to very high levels. In contrast, gene I of CM 1841, in combination with gene VI of CM1841, affected stunting of turnips less severely and caused a much lower virus accumulation. At present, we are trying to determine the region(s) of gene I which might influence the stunting severity and virus accumulation.

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RESPONSE OF SOLANACEOUS PLANTS TO CAULIFLOWER MOSAIC VIRUS (CaMV) INFECTION IS INFLUENCED BY SEQUENCE VARIATION IN THE 3' PORTION OF THE CaMV GENE VI PRODUCT. W.M. Wintermantel and J.E. Schoelz; Department of Plant Pathology; 108 Waters Hall; University of Missouri; Columbia, MO 65211.

Research has shown that the 5' portion of gene VI of cauliflower mosaic virus (CaMV) is involved in determining the ability of the virus to systemically infect solanaceous hosts. Recombinant viruses constructed with three strains of CaMV have also demonstrated that sequences within the 3' half of gene VI, or the large intergenic region, further affect host-viral interactions. Current studies using recombinant viruses have determined that the 3' portion of gene VI is responsible for controlling the size of necrotic local lesions on *Nicotiana edwardsonii* and for contributing to the ability of the virus to move systemically in *Nicotiana bigelovii*. Nucleotide sequencing of the 3' portion of gene VI of the W260 strain and comparison of the deduced amino acid sequence to that of the other two strains used in recombinant construction has revealed significant variations within this region.

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CLONING, SEQUENCE ANALYSIS, AND E. COLI EXPRESSION OF THE COAT PROTEIN GENE OF THE YELLOWING STRAIN OF SOYBEAN DWARF LUTEOVIRUS. O. P. Smith[1], V. D. Damsteegt[1], K. F. Harris[2], and R. Vonder Haar[2]. [1]USDA-ARS, Frederick, MD 21702 and [2]Texas A&M University, College Station, TX 77843.

A cDNA library was constructed to the yellowing (Y) strain of soybean dwarf luteovirus (SDV). The strategy for selecting clones potentially encoding the coat protein gene used Acc I, which cleaves immediately downstream from the stop codon of three other luteoviruses, followed by testing for hybridization to a 3' viral subgenomic RNA. Clone pSDV-Y95 contained an open reading frame (ORF) of 600 nucleotides encoding a protein of 200 amino acids with a predicted mol. wt. of 22.2 kDa. The polymerase chain reaction (PCR) was used to express amino acid residues 7-200 of this ORF in E. coli as a ß-galactosidase fusion protein. Polyclonal antisera to SDV-Y virions reacted positively to crude bacterial lysates in a dot-blot immunoassay, identifying this ORF as the viral coat protein. Sequence comparisons to other luteoviruses will be presented

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cDNA SYNTHESIS OF PLANT VIRUSES WITHOUT FIRST ISOLATING RNA. S. D. Wyatt, Dept. Plant Pathology, Wash. State Univ., Pullman, WA 99164-6430 and P. H. Berger, Div. Plant Pathology, Univ. of Idaho, Moscow, ID 83843.

DNA copies of a wide range of RNA viruses can be made by the direct addition of appropriately treated, purified virus to a reverse transcription reaction. Viruses can be sufficiently destabilized so that RNA in sufficient quantity and physical condition is available for the synthesis of full length cDNAs. In the case of potyviruses, cDNA can be made by addition of reverse transcriptase and oligo d(T) primer to freeze\thaw destabilized virus. After brief SDS\phenol extraction and purification using a sephacryl 400 spun column, the amount of full length cDNA can be increased with a second incubation with reverse transcriptase. The second step does not require further addition of primer. Double stranded cDNA can be made using RNase H and *Taq* DNA polymerase to replace the RNA template. In the case of BCMV and PVY, authenticity of the cDNA was confirmed by Southern hybridization. Work is underway to optimize cDNA synthesis and cloning efficiency as well as authenticate the cDNA through sequencing.

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SEQUENCE OF THE COAT PROTEIN REGION OF A SOYBEAN DWARF-LIKE LUTEOVIRUS. A. Zipf†, R. French††, and M. R. McLaughlin†, †USDA-ARS, Crop Science Research Laboratory, Forage Research Unit, Mississippi State, MS 39762 and ††USDA-ARS, 406 Plant Sciences Hall, University of Nebraska, Lincoln, NE 68583.

Nucleic acid and corresponding amino acid sequences were obtained for the putative coat protein open reading frame (ORF) of a soybean dwarf-like luteovirus isolate from the southeastern U.S. (Phytopathology 78:1584). A small portion of the viral genome was amplified by the polymerase chain reaction (PCR) using degenerate primers based on common regions within published luteovirus coat protein ORF and polymerase ORF sequences. The PCR fragment was blunt-ended with T_4 DNA polymerase, ligated with Hinc II-cut pUC119, cloned and sequenced using the dideoxy sequencing method. This is the first coat protein ORF sequence data for any soybean dwarf-like luteovirus.

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SEQUENCE ANALYSIS AT THE 5' END OF THREE CUCURBIT POTYVIRUSES. Baker, C.A., Wisler, G.C., Marlow, G.C., Hiebert, E. and Lecoq*,H. University of Florida, Gainesville 32611 and INRA, Montfavet, France*

Nucleotide sequences of cDNA clones containing portions of the P1, P2 (helper factor), and P3 genes of single isolates of zucchini yellow mosaic (ZYMV-FL and -Reunion) and papaya ringspot type W (PRSV-W) viruses were compared. The P1 and a 5' portion of the P2 genes of ZYMV-FL and a ZYMV (-CA) isolate from California (Bälint, per. comm.) were 97% homologous. When ZYMV-FL and -Reunion were compared, respective homologies of 59 and 67% were noted for the 5' and 3' ends of the P1 genes, 78 and 80% for the 5' and 3' ends of the P2 genes, and 83% for the 5' end of the P3 gene. A 41% similarity between ZYMV-FL and PRSV-W was noted for the 3' end of the P1 gene. The P2 sequences of ZYMV-FL and PRSV-W were 53% homologous with respective homologies of 47 and 60% at the 5' and 3' ends of the P2 gene. A 49% homology was noted for these viruses at the 5' end of the P3 gene. While the homologies of P1, P2, and P3 from ZYMV-FL and PRSV-W are similar to those of either virus compared to tobacco etch virus, the homologies of ZYMV-FL and -Reunion fall in between those of ZYMV-FL/-CA and ZYMV-FL/PRSP-W.

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COMPOSITION OF THE *AGROBACTERIUM* POPULATION FROM A FALLOW FIELD H. Bouzar[1], D. Ouadah[1], Y. Dessaux[2], A. Petit[2], Z. Krimi[1], M. Trovato[2]. [1]Inst. Agronomie, Univ. Blida, Algeria and [2]Inst. Sci. Végét., C.N.R.S., Gif-sur-Yvette, France.

A large (10^7cfu/g) and diversified *Agrobacterium* population was recovered from eight soil samples collected randomly from a fallow field which had not been cultivated for five years. Of 140 isolates, 80 induced tumors and/or were sensitive to agrocin 84. Characterization of these isolates and nine additional nonpathogenic isolates revealed the predominance of biovar (bv) 1 (62) over bv2 (27) isolates. Characterization below the bv level revealed the presence of five different types of chromosome backgrounds (chr A, C, D, F & G) among bv1 and only one (chr B) among bv2. According to opine metabolism, chr A isolates harbored a nopaline Ti-plasmid (pTi), chr C isolates had an octopine pTi, and bv2 (chr B) catabolized nopaline but were nontumorigenic and agrocin-sensitive (apparently carrying a defective nopaline Ti-plasmid similar to pAtK84b). Four nontumorigenic isolates had unusual opine catabolism patterns.

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LOCATION OF PHYLLOSPHERE BACTERIA ON OR WITHIN LEAVES OF FIELD-GROWN SNAP BEAN PLANTS. J.D. Stock and *S.S. Hirano*, Department of Plant Pathology, University of Wisconsin, Madison, WI 53706.

The hydrogen peroxide leaf sterilization method of Kinkel & Andrews (*Trans. Br. Mycol. Soc.* 91:523-528, 1988) was modified and applied to bean leaves to determine the proportions of *Pseudomonas syringae* (Ps) and *Methylobacterium* spp. that are located on the leaf surface relative to those within the leaf or in protected sites on the surface. In growth chamber experiments, the percentage of either bacterial species that was killed by a 5-min treatment with 15% H_2O_2 was greater than 99.5% and less than 7.5% when bacteria were misted onto or infiltrated into bean leaves, respectively. Leaflets were collected from field-grown plants six times during the growing season. The percentage of leaf-associated bacterial cells that was exposed to H_2O_2 was greater for *Methylobacterium* spp. (range = 99.8 to 99.98%) compared to Ps (range = 84 to 99%). These two successful colonizers of bean leaves appear to differ in the extent to which they survived and/or grew within leaves. The proportions of Ps protected from peroxide increased as the duration between rain and sampling time increased.

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THE PROPORTION OF DIFFERENT PHYLLOSPHERE BACTERIA IN SITES ON OR WITHIN BEAN LEAVES PROTECTED FROM SURFACE STERILIZATION. M. Wilson[1], S.E. Lindow[1], and *S.S. Hirano*[2], Department of Plant Pathology[1], University of California, Berkeley, CA 94720, and Department of Plant Pathology[2], University of Wisconsin, Madison, WI 53706.

The number of bacteria on the surface of bean leaves relative to those not exposed to the surface was determined for pathogenic and nonpathogenic strains of *Pseudomonas syringae*, and other phyllosphere saprophytes (*Erwinia herbicola, Xanthomonas maltophilia*, and *Methylobacterium* spp.). Inoculum was sprayed onto growth chamber grown plants. Bacteria present in protected sites on or within leaves was estimated as the number of bacteria that were not affected by a 5-min treatment with 15% hydrogen peroxide. The proportion of bacteria in protected sites 2 or 3 days after application ranged from 2 to 22% for the pathogens, 0.4 to 2% for nonpathogenic *P. syringae* strains, and less than 0.6% for the saprophytes. For all strains, the proportion of bacteria in protected sites was greater on leaves kept dry as opposed to wet during incubation. The relative proportion of different bacterial strains in protected sites was correlated with their ability to grow internally when infiltrated into bean leaves.

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DIFFERENCES IN SUBSTRATE UTILIZATION AMONG ISOLATES OF XANTHOMONAS ORYZAE PV. ORYZAE AND X. CAMPESTRIS PV. ORYZICOLA FROM SEVERAL COUNTRIES. D. E. Griffin[1], W. M. Dowler[1], J. S. Hartung[2], and M. R. Bonde[1]. [1]USDA-ARS, Frederick, MD 21702 and [2]USDA-ARS, BARC-West, Beltsville, MD 20705.

Substrate utilization of 95 carbon sources was determined with Biolog GN MicroPlates™ for 18 isolates of Xanthomonas oryzae pv. oryzae (Xoo) (4 Chinese, 2 Indian, 6 Philippine, and 6 Texan) and 4 isolates of X. campestris pv. oryzicola (Xco) (1 Chinese and 3 Philippine). The Indian and 2 Chinese Xoo isolates utilized an average of 39 and 56 carbon sources, respectively. Other Xoo and Xco isolates utilized 18 to 22 carbon sources. Six substrates were used by all isolates tested. More isolates are being characterized by this method, and a database is being constructed based on the substrate utilization patterns. The data suggest that the Indian and some Chinese Xoo isolates differ significantly from the other isolates in their ability to utilize a greater number of substrates.

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DETECTION OF XANTHOMONAS ALBILINEANS IN SUGARCANE STALKS. M. J. Davis, J. L. Dean and C. J. Warmuth. University of Florida, IFAS, TREC, 18905 SW 280 Street, Homestead, FL 33031

Isolation on selective media was compared with the tissue-blot immunoassay (TBIA) (Harrison and Davis. 1988. Phytopathology 78:722-727) for detection of Xanthomonas albilineans, which causes leaf scald disease of sugarcane. Selective medium (Wilbrink's medium supplemented with 5 g/L of KBr, 100 mg/L of cycloheximide, 2 mg/l of benomyl, 25 mg/L of cephalexin and 30 mg/L of novobiocin) was inoculated with serial dilutions of stalk extracts obtained by centrifugation. This medium, further supplemented with 50 mg/L of kasugamycin and 200 mg/L of sodium deoxycholate, was also inoculated by blotting with the freshly cut cross-sectional surface of stalks. A total of 198 stalks, 100 asymptomatic and 98 symptomatic, from 11 cultivars were tested. X. albilineans was isolated from 123 stalks, and there was 91% agreement between the results of the two inoculation methods. There was 87% agreement between the combined isolation results and TBIA results; 92% of the disagreement being due to false-negative results in the TBIA.

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PECTATE LYASE PRODUCTION IN *ERWINIA RHAPONTICI* IS ACTIVATED DURING BACTERIAL GROWTH IN PLANT TISSUE. J.E. Choi, H. Murata, and A.K. Chatterjee; Department of Plant Pathology; 108 Waters Hall; University of Missouri; Columbia, MO 65211.

Erwinia rhapontici strains caused rotting of potato tuber tissue and Chinese cabbage leaves. Pectate lyase (Pel) activity was consistently detected in macerated tissue but not following bacterial growth in media supplemented with pectate. From extracts of rots caused by the strain ER1, we obtained Pel activity which caused electrolyte loss, cell separation, and cell death in potato tuber tissue. Multiple Pel species were detected in rots induced by ER1. The profiles of Pel isozymes were variable, depending upon the bacterial strain and the rot extract. Our findings indicate that *E. rhapontici* differs from other soft-rot *Erwinia* (*i.e., E. carotovora* and *E. chrysanthemi*) in its response to signals that activate Pel production.

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ENVIRONMENTAL AND NUTRITIONAL EFFECTS ON CORONATINE BIOSYNTHESIS IN PSEUDOMONAS SYRINGAE PV. GLYCINEA. D. A. Palmer and C. L. Bender. Department of Plant Pathology, Oklahoma State University, Stillwater, OK 74078-9947.

Pseudomonas syringae pv. glycinea (PSG), the causal agent of bacterial blight of soybeans (Glycine max), produces the polyketide phytotoxin coronatine. The effects of environmental and nutritional factors on the biosynthesis of coronatine and its precursor coronafacic acid (CFA) by PSG4180 were examined in a defined minimal medium. Organic acids were isolated from the supernatant of PSG4180 cultures and the levels of CFA and coronatine were quantified by HPLC analysis. Synthesis of coronatine and CFA was directly proportional to the mass of the culture. Coronatine and CFA levels were influenced by replacing the glucose or NH_4^+ in the medium with other carbon or nitrogen sources, or by varying the level of inorganic phosphate. Levels were also affected by growth temperature, with optimal production at 18°C and minimal production at 30°C within the temperature range examined.

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AGROBACTERIUM TUMEFACIENS CHRY5, A WILD-TYPE STRAIN THAT IS SUPER-VIRULENT ON SOYBEAN. L.G. Kovacs and S.G. Pueppke; Department of Plant Pathology; 108 Waters Hall; University of Missouri; Columbia, MO 65211.

Agrobacterium tumefaciens Chry5 is a wild-type strain originally isolated from chrysanthemum. The strain has an unusually high tumorigenic ability, especially on soybean. We developed a bioassay that employs axenically cultured soybean cotyledons and showed that the virulence of Chry5 is comparable to that of A281, the so-called "supervirulent strain". Using the sac cartridge, we cured Chry5 of its Ti plasmid. Subsequently, the Ti plasmid of strain T37 was conjugated into the Ti plasmidless derivative of Chry5. The transconjugant, that combines the Chry5 chromosomal background and pTiT37, incited seven to eight times more tumor tissue than does wild-type T37. A cosmid library of Chry5 has been constructed, and we are in the process of mapping the Ti plasmid.

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SELECTIVE ENHANCEMENT OF *PSEUDOMONAS PUTIDA* STRAIN PPG7 POPULATIONS IN SOIL AND ON TOMATO ROOTS WITH SALICYLATE. S. F. Colbert, and M.N. Schroth. Dept. of Plant Pathology, University of California, Berkeley, CA 94720.

Pseudomonas putida strain PpG7, containing the NAH7 plasmid that encodes the degradation of naphthalene and salicylate, was applied to tomato roots and surrounding soil through a drip irrigation system at the time of transplanting . One week later, and once every two weeks thereafter, salicylate (1000 ppm) was added to the irrigation water. Soil population sizes of PpG7 were constant at $\approx 10^5$ cfu/g soil in the salicylate treatments, whereas population size in the control soil declined more than ten fold. At the end of the season, amended soils had higher population sizes of PpG7 ($P \leq .05$) than the control. Population sizes of strain PpG7 on roots in amended and control soils declined during the season, but the treated root segments declined less and had significantly greater ($P \leq .05$) population sizes at the end of the season than the control. Transfer of the NAH7 plasmid to a biocontrol bacterium may be used as a strategy to maintain and increase bacterial inoculum in the field.

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GENETIC CHARACTERIZATION OF AN EXTENDED HOST RANGE LOCUS OF *RHIZOBIUM FREDII* USDA257. L.W. Meinhardt, H.B. Krishnan, and S.G. Pueppke; Department of Plant Pathology; 108 Waters Hall; University of Missouri; Columbia, MO 65211.

Inactivation of nolB with Tn5 extends the host range of *Rhizobium fredii* USDA257 to include improved soybean cultivars. Tn5 maps to a 4.2-kb BamHI fragment which has been mapped. There are five sequential open reading frames (ORFs): 495, 869, 626, 198, and 407 bp. A sixth overlapping ORF, of 654 bp, was identified in the opposite orientation. Computer analyses of the DNA sequence and the deduced amino acid sequence failed to find significant homology to any known genes. Genomic DNAs of 31 *Rhizobium* strains were probed with a 1044-bp SalI/BamHI fragment, containing most of ORF 3 and all of ORFs 4 and 5. Ten other strains of *Rhizobium fredii* all contain an 8.0-kb EcoRI fragment with homology to the probe. NGR234, a broad host range strain from New Guinea, also has homology to this fragment. Five strains each of *R. meliloti*, *R. leguminosarum* bv. *phaseoli*, *R. leguminosarum* bv. *viciae*, and *R. leguminosarum* bv. *trifolii* have no detectable homology to the nolB region.

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OUTCOME OF TRANSFORMING DNA IN *CLAVIBACTER MICHIGANENSIS* SUBSP. *SEPEDONICUS*. B.J. Schneider and C.S. Orser, Department of Bacteriology and Biochemistry, University of Idaho, Moscow, Idaho 83843.

We have previously detected transient expression of the chloramphenicol acetyl transferase gene from introduced DNA in *Clavibacter michiganensis* subsp. *sepedonicus (CMS)*, the causal agent of bacterial ring rot of potato. However, stable transformants have not been obtainable by selecting for Cm^r. In a continued effort to obtain stable transformants, we have screened many plasmid vectors, including gram-negative/gram-positive shuttle vectors and novel constructions from our lab containing random fragments from the endogenous *CMS* plasmid pCS1 cloned into pHV33, a *Staphylococcus aureus/ E. coli* shuttle vector. Transformation of *CMS* using the *Rhodococcus fascians* plasmid pRF29 or a population of plasmids containing pCS1 fragments in pHV33, have resulted in transient transformants as monitored by Southern hybridization analysis. Although the transformants exhibited resistance to Cm, we believe this resistance to be spontaneous rather than plasmid encoded. Transformants did not stably maintain or effectively express the incorporated DNA. Transformation was accomplished by freeze-thawing in liquid nitrogen.

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INDUCED RESISTANCE IN CUCUMBERS AND ALTERATIONS IN FATTY ACID METABOLISM. S. A. Avdiushko, X. S. Ye, D. Hildebrand*, and J. Kuc. Department of Plant Pathology, *Department of Agronomy, University of Kentucky, Lexington, KY 40546.

Infiltration of 100 µM linoleic or linolenic acids into cucumber leaves induced systemic resistance to *Colletotrichum lagenarium*. Arachidonic acid, not present in cucumber, was inactive for immunization. The 18:2 and 18:3 fatty acids decreased after immunization of cucumber plants both at the induction site and in systemically protected leaves. As determined by spectrophotometry and western blotting, lipoxygenase and lipid acyl hydrolase activities were higher in leaves inoculated with *C. lagenarium* as compared to the water control. Of several inositol-related compounds tested, myo-inositol and phytic acid were potent elicitors of systemic resistance to *C. lagenarium*. These compounds can be products of membrane lipid degradation.

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GENETIC ANALYSIS OF FUMONISIN BIOSYNTHESIS IN FUSARIUM MONILIFORME MATING POPULATION A. A. E. Desjardins and R. D. Plattner, NCAUR, USDA-ARS, Peoria, IL 61604; J. F. Leslie, Kansas State Univ., Manhattan, KS 66506; and P. E. Nelson, The Pennsylvania State Univ., State College, PA 16803.

Fumonisins are phytotoxic metabolites of Fusarium moniliforme mating population A and related species; they are implicated in a variety of mycotoxicoses of man and animals. Fumonisin-producing strains from corn in California were crossed with fumonisin nonproducing strains from corn in Nepal. Crosses were performed on carrot agar and ascospores were obtained after 2 weeks' incubation at 20-22 C with a 12-h dark/12-h light cycle using a mixture of fluorescent and black light. For fumonisin assays, cultures were grown on cracked corn for 30 days at 25 C in the dark and culture extracts were analyzed by combined gas chromatography-mass spectroscopy. Analysis of random ascospores from individual perithecia gave Mendelian segregation of fumonisin production. Tetrad analyses are in progress. This study demonstrates that fumonisin production in F. moniliforme mating population A is amendable to classical genetic analysis.

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PEROXIDASE AND SUBERIN OF NATURAL AND STREPTOMYCES INDUCED PERIDERM IN POTATO. K. Ludlam[1], R. Hammerschmidt[2], and D. Douches[1]. [1]Department of Crop & Soil Sciences and [2]Botany & Plant Pathology, Michigan State University, East Lansing, MI 48824

Resistance of potato tubers to Streptomyces has been attributed to the development of suberized barriers. To test this, the level of peroxidase (the last enzyme in the synthesis of suberin phenolic polymers) and suberin phenolics were determined in periderm samples from the apex of immature tubers. Differences in the level and types of peroxidases and in the amounts of suberin phenolics were found among the varieties tested, but no strict correlation was found with resistance. Chemical analysis of the phenolic domains of natural and pathogen induced suberin from scab lesions revealed some differences. Inoculation of wounded tuber tissue with pathogenic Streptomyces induced the lignification of host cell walls within 24 hours suggesting that the host has the ability to actively respond to the pathogen.

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SYSTEMIC INDUCTION OF SALICYLIC ACID IN CUCUMBER: RESPONSE TO ANOTHER SIGNAL? R. Hammerschmidt and J.B. Rasmussen, Department of Botany and Plant Pathology, Michigan State University, East Lansing, MI 48824

Inoculation of one leaf of cucumber with the HR-inducing bacterium Pseudomonas syringae pv. syringae (PSS) elicits systemic resistance within 24 hr. Examination of phloem exudates from the petiole of the PSS-inoculated leaf and the petiole of the leaf above demonstrated the presence of salicylic acid (SA) by 8 and 12 hr, respectively. Enhanced SA levels were also found in phloem exudates collected below the induced leaf. Detaching the inoculated leaf at intervals after PSS inoculation demonstrated that detaching the inoculated leaf as soon as 4 hr was sufficient to elicit a systemic increase in SA, even though increased levels of SA could not be detected in the exudates of leaf 1 at 4 hr. This suggests that SA is induced in response to another translocated signal. Timing studies also indicated that factors in addition to SA may be needed for maximum expression of induced resistance.

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A POSSIBLE ROLE FOR CALCIUM IN THE INDUCTION OF STRESS METABOLITE ACCUMULATION IN TOBACCO CELL SUSPENSIONS. C. L. Preisig, C. M. Schmitt, R. A. Moreau. USDA-ERRC, 600 E. Mermaid Lane, Philadelphia, PA

Tobacco cell suspensions were treated with cellulase elicitor and the accumulation of capsidiol, acylated sterol glycoside and two unidentified compounds were measured by HPLC-FID. Several classes of signal transduction antagonists were tested in this system. Where observed, inhibition of accumulation affected the four metabolites to the same extent, suggesting that inhibition was at an early step(s) in the signal transduction pathway(s). The Ca^{2+} chelator, EGTA, the inhibitors of extracellular Ca^{2+} uptake, nefidipine, verapamil and $LaCl_3$, of Ca^{2+} dependent protein kinase and calmodulin, W7, and of protein kinase C, staurosporine, all reduced the tobacco metabolites by 40 to 70%. Neomycin, a phospholipase C inhibitor, and LiCl, an inhibitor of inositol-1-phosphatase, both enhanced accumulation. With the exception of EGTA, the concentrations required to cause inhibition were all higher than those reported for other Ca^{2+}-mediated processes. EGTA (3 mM) inhibited accumulation by 50% when assayed in cell culture medium containing Ca^{2+} at 3 mM; $CaCl_2$ or $MgCl_2$ overcame this inhibition. EGTA was no longer effective if added 90 min after elicitor. EGTA, but not EDTA, at 0.1 mM enhanced accumulation of these compounds by 30% as did $MgCl_2$ alone. The results suggest the involvement of a multicomponent signal

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BIOSYNTHESIS OF THE PHYTOALEXIN PISATIN IN PEA (*PISUM SATIVUM* L.): REGULATION AND SUBCELLULAR LOCATION OF THE TERMINAL ENZYME Carol L. Preisig, Peter Cooke, William F. Fett, USDA - ERRC, 600 E. Mermaid Lane, Philadelphia, PA 19118

The terminal biosynthetic step for the isoflavonoid phytoalexin, pisatin, is catalyzed by the (+)6a-hydroxymaackiain 3-*O*-methyltransferase (HMKMT). Western blot analysis of this enzyme protein indicated that it is induced in pea from a low constitutive level by treatment with copper chloride, suggesting that the HMKMT is newly synthesized in response to stress. HMKMT mRNA translational activity also increased in peas with time after treatment with copper chloride. Peak translational activity occurred about 12 h after treatment, preceding peak enzyme activity by a few hours. Phenylalanine ammonia-lyase (PAL) mRNA abundance increased coordinately with that of HMKMT. The stress-induced increase in PAL mRNA translational activity has been shown to reflect transcriptional activation of PAL genes. Thus induction by stress of enzyme activity at both an early step and at the terminal step in the phenylpropanoid/isoflavonoid biosynthetic pathway appears to be at the transcriptional level. Several positive cDNA clones have been selected from an expression library using HMKMT antisera and are being characterized.

The HMKMT was present only in the 95,000*g* supernatant fluid after differential centrifugation. Immunolocalization by electron microscopy suggested that this enzyme was in the highly vesicularized regions of copper chloride treated pea tissue.

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EARLY PHYSIOLOGICAL RESPONSES ASSOCIATED WITH RACE/CULTIVAR SPECIFICITY OF SOYBEAN TREATED WITH PSEUDOMONAS SYRINGAE PV GLYCINEA. E. W. Orlandi[1,2], S. W. Hutcheson[2], and C. J. Baker[1]. [1]USDA, ARS, MPPL, Beltsville, MD 20705 and [2]Univ. of Maryland, Dept. of Botany, College Park, MD 20742-5815.

Pseudomonas syringae pv glycinea, race 4, (Psgr4) causes a compatible reaction on the soybean cultivar Mandarin while race 6 (Psgr6) causes an incompatible response. The *avrA* gene isolated from Psgr6 induces Psgr4 to cause an incompatible reaction on Mandarin leaves. Mandarin suspension cells were inoculated and monitored for the K^+/H^+ and active oxygen responses, two early physiological events associated with recognition. While Psgr4 failed to cause either response in Mandarin suspension cells, both Psgr6 and the transconjugant Psgr4(pAVRA) gave very strong K^+/H^+ and active oxygen responses by 3 h after inoculation. In addition, Mandarin leaf disks were inoculated with the three Psg isolates. Both Psgr6 and Psgr4(pAVRA) gave a K^+/H^+ response within 4 h while Psgr4 did not begin to increase until 15 h after inoculation.

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CORRELATION OF THE EARLY EVENTS OF THE HYPERSENSITIVE RESPONSE WITH THE INDUCTION OF SYSTEMIC RESISTANCE IN CUCUMBER. M. Zook and R. Hammerschmidt. Department of Botany and Plant Pathology. Michigan State University, East Lansing. 48824.

Cotyledons of 2-week-old cucumber plants were infiltrated with a 1×10^8 cfu/ml suspension of either wild-type Pseudomonas syringae pv. syringae (Pss) or a Tn5 hrp-mutant of Pss or water. Increased levels of electrolyte leakage were observed from cotyledons removed at 6 hr or at later times after infiltration with wild-type Pss as compared to cotyledons infiltrated with the Tn5 mutant or water. Peroxidase activity was systemically induced when cotyledons which had been infiltrated with wild-type Pss were detached 6 hr or at later times after infiltration, whereas no systemic induction occurred when cotyledons were detached earlier or following infiltration with the Tn5 mutant or water. Signals for systemically induced resistance may be associated with the early events of the hypersensitive response.

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PURIFICATION AND CHARACTERIZATION OF ARABILEXIN, A PHYTOALEXIN FROM ARABIDOPSIS THALIANA.
J. Tsuji[1], R. Hammerschmidt[2], and S. C. Somerville[1,2]. [1]MSU-DOE Plant Research Laboratory and [2]Dept. of Botany and Plant Pathology. Michigan State University, East Lansing. 48824

Leaves of Arabidopsis thaliana were found to accumulate a

phytoalexin following inoculation with the non-host pathogen Pseudomonas syringae pv. syringae. 2.5 mg of the Arabidopsis phytoalexin (arabilexin) was purified from 1.28 kg of elicited leaf tissue. A molar extinction coefficient of 14,800 M^{-1} cm^{-1} was calculated for the purified phytoalexin using the Beer-Lambert law. In leaves inoculated with P. s. syringae, arabilexin accumulated within 12 hours post inoculation and reached a maximum level of about 8 ug/g fresh weight by 36 hours The accumulation of arabilexin was negatively correlated with the in planta growth of P. s. syringae. The in vitro growth of both the fungus Cladosporium cucumerinum and the bacterium P. s. syringae was inhibited by arabilexin. A structure for arabilexin is proposed based on UV, MS, ^{1}H-NMR, and ^{13}C-NMR data.

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PEROXIDASES, PR PROTEINS, CHITINASES AND ß-1,3-GLUCANASES AS RELATED TO RESISTANCE IN TOBACCO INDUCED BY TMV TO *Peronospora tabacina* AND TMV AND BY ETHEPHON TO TMV. X. S. Ye, S. Q. Pan and J. Kuc. Dept. of Plant Pathology, University of Kentucky.

Inoculation of 3-4 leaves of cv Ky 14 with TMV induced systemic resistance to blue mold and TMV. Pricking with ethephon induced systemic resistance to TMV only. Changes in 14 acid-soluble proteins were detected in TMV-inoculated leaves. Most of the changes were induced locally and systemically by TMV and ethephon, but the accumulation of individual proteins was differential. Among 10 major acidic PR proteins, PR-2, N, and O were not inducible and PR-1a, b, c were weakly inducible by ethephon. Two new acidic proteins, EI1 and EI2, were induced in ethephon-pricked leaves. Chitinase and peroxidase activities were systemically enhanced by TMV and ethephon. Seven acidic proteins with chitinase activities were detected. One chitinase(PR-P) was systemically enhanced. Two highly anionic peroxidases were systemically enhanced and two moderately anionic peroxidases were induced in the TMV-inoculated leaves. Inoculation with TMV systemically induced two major ß-1,3-glucanases (PR-N and PR-O) and one minor unknown ß-1,3-glucanase. Ethephon did not enhance ß-1,3-glucanase activity.

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EFFECTS OF FLAVONOIDS ON AND THEIR METABOLISM BY PHYTOPHTHORA MEGASPERMA F. SP. GLYCINEA (PMG). L. Rivera-Vargas and T. L. Graham, Dept. of Plant Pathology, The Ohio State University, Columbus, OH 43210-1087.

The effects of different flavonoid compounds from soybeans and other sources on Pmg were determined using four criteria: fungal growth measurements, macroscopic and microscopic observations and HPLC chromatographic profiling. None of the compounds tested showed significant growth reduction of Pmg races, except naringenin. Macroscopic observations showed that aerial cottony growth was induced with some compounds. Changes in pigmentation and reduction of sporangium and oogonium production were observed. Some compounds also caused hyphal swelling, branching and twisting. At a molecular level, HPLC profiles showed that many of these compounds were partially or completely metabolized by Pmg.

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PATHOGENIC DETERMINANTS OF SCLEROTINIA TRIFOLIORUM. F.E. Callahan and D.E. Rowe, Crop Science Research Laboratory, USDA-ARS, Mississippi State, MS 39762.

A host-pathogen interaction system(Tomaso-Peterson and Krans, Crop Sci. 30:226)was utilized to test the hypothesis that oxalic acid is the sole pathogenic determinant in the exudate of Sclerotinia trifoliorum. Germinating alfalfa seedlings(Medicago sativa)were exposed to continuously produced fungal exudate without physical contact with the fungus. Blockage of diffusion of macromolecular components(>3500mw)of the exudate without alteration of oxalic acid levels reduced the observed inhibition of alfalfa radicle length by 40 to 50 per cent. Conversely, addition of oxalic acid in the media of noninoculated plates in amounts sufficient to mimic the pH of inoculated plates did not inhibit radicle lengths to same extent as whole exudate. We conclude that oxalic acid is not the sole pathogenic determinant for this fungus. Extracellular protein components observed in the fungal exudate probably share a codeterminant role in the pathogenesis; although, our results do not exclude the involvement of other, yet unidentified, exudate components.

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SUGAR COMPOSITION OF GUM VESSEL PLUGS IN BLIGHT-DISEASED CITRUS. S. Nemec and E.J. Mitcham, USDA-ARS, 2120 Camden Rd., Orlando, FL 32803.

Gum vessel plugs occur uniformly in trunk wood of healthy citrus trees, but increase in outer wood of blight-diseased trees. Plugs were excised from diseased trees with a needle and hydrolyzed in 2N trifluoroacetic acid for 1 hr at 121C. Alditol acetate derivatives were prepared and analyzed by GC. Neutral sugars detected were (µg/mg): galactose (48.90), glucose (11.59), mannose (0.78), xylose (44.0), arabinose (23.55), and rhamnose (3.46). Uronic acids (356.27 µg/mg) were solubilized in 78% H_2SO_4 and quantified by the carbazole method. Sugars and uronic acids accounted for 13 and 44% of the plugs, respectively; cellulose was not detected. In outer wood of diseased trees, gum plugging coincides with increased respiration and depletion of starch (Can. J. Bot. 53:2712). We suggest these plugs are not the result of cell wall breakdown, but may have starch as a precursor. Staining with iron hematoxylin revealed gum leakage from contact parenchyma through pits into vessels. Release of gum into vessels may be similar to release of sucrose into vessels of Acer saccharum (Can. J. Bot. 51:1).

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β-1,3-GLUCANASE ACTIVITY IN OOSPORES AND MYCELIUM OF *PHYTOPHTHORA CACTORUM*. J. Jiang, N. T. Keen, and D. C. Erwin, Dept. of Plant Pathology, University of California, Riverside, CA 92521.

β-1,3-glucanases were found in extracts from germinating oospores and mycelium of *Phytophthora cactorum* but dormant oospores yielded little or no activity. The activity increased with the increase of percentage oospore germination. Reducing sugars and proteins were very low in dormant oospores but increased with the increase of oospore germination. Fractions precipitated with 0-35% and 35-70% ammonium sulfate from both oospore and mycelium extracts had enzyme activity, but the major portion was retained in the 35-70% fraction. The crude glucanases from both extracts had an optimal pH of 7.0 and an optimal buffer concentration at 20.0 mM (citrate-phosphate). Gel filtration of the enzymes from both extracts on a Sephacryl S-200 column yielded three peaks with glucanase activity. Peak 1 corresponded to a molecular weight of 500,000-600,000. Peak 2 contained the major enzyme activity in both extracts but the mycelium extract yielded a higher amount of activity in peak 3 than the oospore extract.

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Effects of Exposure of Protocorms and Protocorm-like Bodies of Cattleya Hybrid Orchids to Agrobacterium tumefaciens. H.L. Saxon, D. Tzalis, C.M. Hutchinson, and C.N. Vann. Biology Department, Ball State University, Muncie, IN 47306-0440

Agrobacterium tumefaciens, a dicot pathogen, is widely used to transform dicots. Until recently, it was not considered a suitable transformation vector for monocots since it was not believed to infect them. We report that undifferentiated and dedifferentiated orchid (monocot) tissue exposed under various experimental regimes to Agrobacterium tumefaciens produces alterations in growth and development consistent with infection. Optimal exposure time was one hour. Acetosyringone, a dicot

wound signal molecule that initiates transfer of T-DNA from A. tumesfaciens, was found to be effective in increasing the virulence of A. tumesfaciens, but the enhanced virulence resulted in the killing of host tissue especially when the length of exposure was increased.

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PLANT CELL WALL DEGRADING ENZYMES OF SEPTORIA NODORUM. U. Lehtinen, and M. Romantschuk, Department of General Microbiology, University of Helsinki, Mannerheimintie 172, 00300 Helsinki, Finland

Ability of Septoria nodorum to form plant cell wall degrading enzymes was studied in batch cultures with a wheat cell wall preparate as the carbon source. Xylanase was the most abundant secreted enzyme activity and increased to 10 nkat/ml after growth of two weeks. Polygalacturonase activity (2-3 nkat/ml) occurred in the beginning of cultivation, but pectin lyase or pectin methyl esterase activities could not be demonstrated. Laminarinase activity reached the level of 2 nkat/ml after a week. Only little of β-1,4-glucanase activity could be detected. Filter paper or protein degrading activities were not observed. A putative cutinase activity was detected using p-nitrophenyl-butyrate as a substrate. Also activities cleaving side chains of pectin and xylan (α-arabinosidase, β-galactosidase and acetyl esterase) as well as β-glucosidase and β-xylosidase activities could be detected in the culture supernatant.

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CYTOCHEMICAL ASPECTS OF FUNGAL PENETRATION, HAUSTORIAL FORMATION AND INTERFACIAL MATERIAL IN ROSE LEAVES INFECTED BY *SPHAEROTHECA PANNOSA* VAR. *ROSAE*. M. R. Hajlaoui, N. Benhamou, and R. R. Bélanger. Dép. de phytologie, Université Laval, Québec (Qc), Canada, G1K 7P4.

Fungal development and host reactions induced in rose leaf epidermal cells by the powdery mildew fungus, *Sphaerotheca pannosa* var. *rosae*, were examined on the basis of ultrastructure and of cytochemistry of chitin, pectin, and cellulose subunits. Fungal growth in the epidermis was associated with the formation of large structures, the haustoria, which appeared multilobed and delimited by an extrahaustorial membrane. The extrahaustorial matrix was free of chitin following incubation with the WGA/ovomucoid-gold complex. Similarly, neither pectin nor cellulose were detected in this matrix, whereas these compounds were abundantly present in host cell walls. The formation of papillae was often associated with a restricted development of the infection peg. The most striking reaction was the occurrence of successive layers of a fibrillar material, the collar, around the haustorial neck. Our observations demonstrated that the collar was made of an amorphous material surrounded by fibrillar layers, the outermost ones being cellulose-rich.

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IMMUNOFLUORESCENT DETECTION OF PR-PROTEINS IN SYSTEMICALLY RESISTANT TOBACCO SAMSUN NN, CHALLENGED WITH PHYTOPHTHORA NICOTIANAE F. SP. NICOTIANAE. M. B. Sela-Buurlage, C. P. Woloshuk, E. J. S. Meulenhoff, B. J. C. Cornelissen and P. J. M. van den Elzen. Mogen Int. NV. Einsteinweg 97, 2333 CB Leiden, the Netherlands.

Inoculation of the lower leaves of tobacco (Samsun NN) with tobacco mosaic virus induces systemic resistance to Phytophthora nicotianae f.sp. nicotianae (PNN). Concomitantly, PR-proteins are produced both locally and systemically. In the present study, we used light microscopy to examine host/pathogen interactions in systemic leaves of induced and noninduced tobacco plants. When leaf disks from noninduced plants were inoculated with zoospores of PNN, infection occurred within 24 hours. The fungus spreads rapidly throughout the entire disk. In contrast, in leaf disks from induced plants, spread was restricted to the area underneath the inoculum droplet. PNN grew intercellularly and formed haustoria in both types of leaf disks, however only in induced leaves papillae were formed in response to the haustoria. We will present these data as well as immunofluorescence labelling of several PR-proteins in the systemic leaves of induced and noninduced tobacco plants, challenged with PNN.

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Natural infection of *Arabidopsis thaliana* by *Albugo candida* and *Peronospora parasitica*. E. B. Holub[δ], P. H. Williams[*], and I. R. Crute[δ]. [δ]Horticulture Research Int'l, East Malling, Kent, ME19 6BJ, UK; [*]Univ. of Wisconsin, Madison, 53706, USA.

Natural infection of *A. thaliana* by the oomycete fungi *A. candida* (*Ac*) and *P. parasitica* (*Pp*) was observed in Kent, UK in spring 1987. In 1990, twenty-six populations of *A. thaliana* from Kent were examined. Of these populations, 8% had plants infected with both parasites, 19% with *Pp* alone, and 12% with *Ac* alone. Neither parasite was found in the remaining 61% of populations. Two Kent isolates of *Pp* and one isolate of *Ac* were used to inoculate twenty-two ecotypes of *A. thaliana* (originating from ten European countries, USA, Japan, Libya, Kashmir, and Cape Verde Is.). The ecotypes were classified into four phenotypic groups according to asexual sporulation of the *Pp* isolates. Both isolates sporulated on 18% of the ecotypes (+/+), and neither sporulated on 55% (-/-). The remaining ecotypes reacted differently to the two isolates (18% -/+; 9% +/-). Three phenotypic groups of reaction to the *Pp* isolates (+/+, +/-, and -/+) were also found among progeny of *A. thaliana* plants collected from East Malling. The *Ac* isolate sporulated asexually on all 22 ecotypes of *A. thaliana*. However, the *Ac* isolate was unable to sporulate on *A. thaliana* collected from four locations in the UK. Genetic analysis of these differential responses has been initiated, and resistance loci will be mapped relative to RFLP and morphological markers available in *A. thaliana*.

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COLONIZATION OF SPEARMINT STEMS BY VERTICILLIUM DAHLIAE. D.A. Johnson and E.R. Miliczky, Washington State University, Rt 2 Box 2953-A, Prosser WA 99350.

Propagules of Verticillium dahliae were quantified in stems of Scotch spearmint and three wilt resistant mutants that exhibited a range of wilt symptoms in the field. Agar containing sodium polypectate, water extract from peppermint, and antibiotics was poured over sap expressed from stem sections. Colonies of microsclerotia originating from presumably individual spores were counted. Colonies per cm of stem increased logarithmically in each spearmint genotype as stems exhibited increasingly severe stunting ($P < 0.01$; R^2 ranged from .43 to .85). Colony numbers did not differ in stems of the four genotypes exhibiting the same relative degree of stunting. More colonies were recovered from the base and middle sections than from the top one third section of lightly and moderately stunted stems. The base and top sections of severely stunted stems usually did not differ in colony numbers. Nodes and internodes did not differ significantly in colony numbers.

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FUNGAL DEVELOPMENT AND HOST RESPONSE IN CUCUMBER PLANTS INFECTED WITH *PYTHIUM ULTIMUM*. M. Chérif, N. Benhamou, and R. R. Bélanger. Dép. de phytologie, Université Laval, Québec (Qc), Canada, G1K 7P4.

Long English cucumber plants cv. Corona were inoculated with a virulent strain of *Pythium ultimum*, and root samples were collected at different times for electron microscope observations. Initial phases of infection occurred from 2 to 7 hr after inoculation. During the first 48 hr, colonization of the vascular stele proceeded via the infection of the endodermis, the pericycle, the parenchyma cells and the vascular elements. Direct host wall penetration was presumably accomplished by mechanical and enzymatic action. Hyphal penetration of host cells was usually associated with collapse of the protoplasm and disintegration of the different organelles. Some parenchyma cells were filled with an amorphous electron-dense material devoid of pectic and cellulosic substances. Hyphae colonizing such reacting host cells suffered from serious damages and were prevented in their growth by the surrounding amorphous material. Another reaction was the deposition of a strongly electron-opaque material along secondary thickenings and pit cavities of xylem vessels neighboring colonized vascular parenchyma cells.

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THE AVIRULENCE GENE *AVRB6* FROM *XANTHOMONAS CAMPESTRIS* PV. *MALVACEARUM* AFFECTS PATHOGENICITY IN COMPATIBLE INTERACTIONS WITH COTTON, BUT NOT BACTERIAL GROWTH *IN PLANTA*. Y. Yang, R. DeFeyter, S. Swarup, and D. W. Gabriel. Plant Pathology Department, University of Florida, Gainesville, FL 32611.

The avirulence gene *avrb6*, cloned from a 90-kb plasmid in *Xanthomonas campestris* pv. *malvacearum* strain H (Xcm H), was found to have a pleiotropic pathogenicity function as indicated by watersoaking ability on cotton leaves. Marker exchange mutagenesis of *avrb6* in Xcm H greatly reduced its watersoaking ability on the susceptible cotton line Ac44, whereas a marker exchange mutation in *avr*BIn, another structurally similar *avr* gene on the same plasmid, resulted in almost no change in watersoaking ability. Despite the reduction in watersoaking ability, growth *in planta* of marker exchange *avrb6*$^-$ mutant was not significantly different from that of Xcm strain H and *avr*BIn$^-$ mutant. Similarly, a spontaneous *avrb6*$^-$ mutant of Xcm strain H was reduced in watersoaking ability, but not in its growth on Ac44. Introduction of *avrb6* into Xcm strain N conferred increased watersoaking ability, but the growth *in planta* remained the same as with strain N and the transconjugant containing *avr*BIn. The gene *avrb6*, therefore, has both avirulence and pathogenicity functions, but seems to be gratuitous to bacterial growth *in planta*. A *Xanthomonas citri* gene (*pth*A), which is structurally similar to 12 *avr* genes in Xcm H, was also found to have avirulence as well as pathogenicity functions. We have cloned an *npt-sac*B-*sac*R cartridge into *Xanthomonas citri pth*A gene in order to carry out marker exchange-eviction mutagenesis in Xcm H. A series of directed, unmarked *avr* mutants will be generated to study the possible functions of Xcm avirulence genes.

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COAT PROTEIN MEDIATED PROTECTION IN TRANSGENIC TOBACCO PLANTS EXPRESSING THE TOBACCO MOSAIC VIRUS COAT PROTEIN IN A TISSUE-SPECIFIC PATTERN
U.Reimann-Philipp & R.N. Beachy, Department of Biology, Washington University, St. Louis, MO 63130

Transgenic tobacco plants expressing the TMV coat protein are protected against TMV infection. This has been described as "coat protein mediated protection". The mechanism of the protection is not yet known. The results of previous studies suggest that the initial infection as well as the systemic spread of the virus are affected. Tissue-specific expression of TMV coat protein has been used in this work to study the two events separately in transgenic plants.

In order to achieve tissue specific coat protein expression, two promoter fragments have been used for the construction of plasmid expression vectors. The PAL promoter (Phenylalanin Ammonia Lyase gene 1 from Phaseolus vulgaris) has been shown to direct the constitutive expression of the reporter gene GUS (B-Glucuronidase from E. coli) in the upper leaf epidermis and xylem in transgenic tobacco. The rol c promoter from Agrobacterium rhizogenes controls the expression of GUS in the phloem. In most of the previous experiments, the 35 S promoter from CaMV (Cauliflower Mosaic Virus) has been used. It drives an almost uniform expression of foreign genes all tissues of transgenic tobacco plants.

Nicotiana tabacum cv. Xanthi plants of two genotypes (Sx and NN) have been transformed with the different plasmid vectors and used for infection experiments. Non transformed Sx-plants develop systemic mosaic symptoms upon inoculation with TMV, whereas NN-plants form local necrotic lesions at the infection site. The degree of protection in the transgenic plant lines has been determined and the possible mechanisms are discussed.

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DETECTION AND CHARACTERIZATION OF PUCCINIA SP. INFECTIONS IN LEAVES OF DYERS' WOAD (ISATIS TINCTORIA L.) BY WHOLE-LEAF STAINING. Karen M. Shotwell and Sherman V. Thomson, Department of Biology, Utah State University, Logan, Utah, 84322-5305.

A whole-leaf clearing and staining technique developed by Bruzzese and Hasan (Plant Pathology 32:335-338, 1983) was adapted for use with dyers' woad (*Isatis tinctoria L.*) to study infection by a microcyclic, and apparently systemic, *Puccinia* sp. being studied as a biological control of the weed. Cotton blue was substituted for aniline blue, and several chloral hydrate rinses were utilized to achieve satisfactory destaining. With this technique, it was found that asymptomatic woad plants, which had previously exhibited symptoms of rust infection, had a fungal mycelium ramifying the tissue of newly-emerged leaves. Plants of similar age and from the same source, which had never shown symptoms of rust infection, had no hyphae in their leaf tissue. In plants with developing or erumpent teliosori, intact hyphae were not evident, although structures which appeared to be hyphal remnants were present.

707

A New Method to Formulate Biocontrol Fungi for Seed Treatment or Foliar Application. G. R. Knudsen and D. J. Eschen. Plant Pathology Division, Department of Plant, Soil, and Entomological Sciences, University of Idaho, Moscow 83843.

Ease of manufacture, storage, and application are desirable attributes of biocontrol formulations. Trichoderma harzianum isolate ThzID1 was grown for 1 wk in potato dextrose broth, cultures were blended, and 1% (w/v) sodium alginate and 20% (w/v) polyethylene glycol 8000 (PEG) were added. The mixture was added by drops to aqueous $CaCl_2$ (0.25 M), forming pellets. After 48 hr drying, pellets were milled to mean particle sizes of 275, 500, or 700 μm. PEG enhances rapid growth from formulations (Knudsen et al., Phytopathology 80:1050), and addition of PEG before pelletizing facilitated the milling process. After more than 2 months storage (25 C), ThzID1 grew rapidly from >99% of rehydrated particles. Similar results were obtained with the fungal entomopathogen Beauveria bassiana. These methods are suitable to formulate biocontrol fungi for seed or foliar treatments.

708

GROWTH OF VERTICILLIUM LECANII ON UROMYCES APPENDICULATUS. J.R. Stavely and L.R. Batra, Microbiology and Plant Pathology Laboratory, USDA, ARS, Beltsville, MD 20705

Verticillium lecanii is a reported parasite of the bean rust fungus, Uromyces appendiculatus, and of other rust fungi. Among 17 isolates tested at Beltsville, an authentic V. lecanii isolate from Puccinia coronata produced most rapid and profuse growth over bean rust uredinia. Such growth occurred only when V. lecanii spores were applied after uredinia had erupted. When rusted bean plants were sprayed with V. lecanii and incubated under 12 hrs dew with 12 hrs dryness per 24 hrs at 25 C, rust uredinia became visibly overgrown in 72 hrs. At 20 C, 96 hrs was required. This isolate of V. lecanii grew well on media containing chitin or cell free urediniospore washes. Among other nutrients, the washes contained, maltose, sucrose and glucose. The conditions for growth of this virulent isolate of V. lecanii on U. appendiculatus suggest that field application of V. lecanii could reduce uredinial sporulation and secondary spread in humid climates.

709

SPORE GERMINATION AND PATHOGENICITY OF COLLETOTRICHUM TRUNCATUM A POTENTIAL MYCOHERBICIDE FOR BIOCONTROL OF HEMP SESBANIA (SESBANIA EXALTATA). G. H. Egley, B. J. Johnson, R. N. Paul, Jr., and C. D. Boyette. USDA-ARS, Stoneville, MS 38776.

We studied factors influencing spore germination to optimize use of Colletotrichum truncatum for biocontrol of S. exaltata. In studies on water agar, buffer type and pH influenced germination but pH values of 6 to 9 were generally more favorable than 3 to 6. Regardless of pH, germination decreased as buffer concentrations were increased from 1 to 100 mM and as spore density was increased from 10^5 to 10^7 spores/ml. Washing of spores improved germination. Temperatures of 15 to 28° C were more favorable than 35 to 40° C for both spore germination and lesion formation on S. exaltata seedling stems. Additions of 10 mM glycine to the agar or 1 to 40 u/L ethylene to the air enhanced germination. Agar additions with either intact stems or water extracts of stems enhanced germination. Microphotographs of disease formation in stems revealed that mycelia penetrated between cells resulting in stem collapse and plant death.

710

PREVENTION OF AFLATOXIN CONTAMINATION OF COTTONSEED BY QUALITATIVE MODIFICATION OF ASPERGILLUS FLAVUS POPULATIONS. Peter J. Cotty, Southern Regional Research Center, USDA, ARS, New Orleans, LA.

Populations of Aspergillus flavus are highly variable. This variability is reflected both in differences in ability to produce aflatoxins among isolates from a single field and in average toxigenicities of populations from different fields. Aflatoxin producing ability does not determine the ability of A. flavus to infect and multiply in developing cotton bolls and atoxigenic strains are capable of preventing other strains from contaminating developing cottonseed with aflatoxins. Therefore, we are developing techniques to modify A. flavus populations to increase the proportion of strains which are atoxigenic in an effort to reduce aflatoxin contamination of cottonseed. In experiments in the Yuma Valley of Arizona, an applied atoxigenic strain successfully displaced resident strains and displacement resulted in reduced crop contamination. Thus, this strategy has potential for success.

713

BIOLOGICAL CONTROL OF RHIZOCTONIA SOLANI ANASTOMOSIS GROUP 4 (AG 4) USING BINUCLEATE RHIZOCTONIA SPP. AND HYPOVIRULENT ISOLATES OF AG 4. J.R. Washington and F.N. Martin. Plant Pathology Department, Univ. of Florida, Gainesville, FL 32611.

Biological control of damping-off of tomato transplant seedlings caused by Rhizoctonia solani anastomosis group 4 (AG 4) with isolates of binucleate Rhizoctonia spp. and hypovirulent isolates of AG 4 was investigated. Preliminary screening trials consisted of inoculating 10-day-old seedlings with a mycelial homogenate of each isolate tested. Three days after inoculation, seedlings were transplanted to microwave treated field soil amended with a highly virulent AG 4 isolate at a inoculum density of one sclerotium per gram of soil. Isolates which resulted in the best control were retested in nonpasteurized field soil amended at a rate of two sclerotia per gram of soil, which resulted in 70-100% disease incidence in the untreated control. Many isolates which resulted in 90-100% reduction in disease incidence were identified; field trials with these isolates are in progress.

714

DEVELOPMENT OF A BIOASSAY SYSTEM TO COMPARE QUANTITIES OF AMMONIA PRODUCED BY STRAINS OF PSEUDOMONAS SPP., POTENTIAL BIOCONTROL AGENTS AGAINST SOILBORNE FUNGI. M. Baligh, K. E. Conway and M. A. Delgado, Department of Plant Pathology, Oklahoma State University, Stillwater, OK 74078-9947.

Various concentrations of NH_3 were used in a bioassay system to compare inhibition of eight fungi by volatile ammonia to inhibition caused by strains of Pseudomonas cepacia and P. aeruginosa. NH_4OH dilutions or bacterial isolates (grown on Czapek's agar with 20 g/l peptone) were placed in opposite quarters of quard-petri dishes. Other quarters contained fungi growing on Trypticase Soy agar. Fungi differed in sensitivity to ammonia. Sclerotium rolfsii was most sensitive, being completely inhibited by < 3 ug/ml, while Trichoderma harzianum was stimulated by this amount. Ammonia production differed slightly among bacterial strains and was equivalent to concentrations of NH_4OH ranging from 6 to 18 ug/ml. Production of ammonia by rhizophere bacteria should be considered as one of the mechanisms limiting soilborne fungi.

715

PRODUCTION AND GENETIC CHARACTERIZATION OF PYOVERDINE-DEFICIENT MUTANTS OF PSEUDOMONAS PUTIDA N1R. E. Noli, C. A. Ishimaru, and R. Baker, Department of Plant Pathology and Weed Science, Colorado State University, Fort Collins, CO 80523.

Siderophore-deficient mutants of Pseudomonas putida N1R were derived to assess the role of pyoverdine in biological control of Fusarium wilt. A total of 1170 Km^R mutants were obtained from several independent matings and screened for lack of a halo on chromazurol S (CAS) medium. Nineteen putative pyoverdine-deficient (Pvd^-) mutants were characterized further. Eight size classes of EcoR1 fragments containing Tn5 were identified by Southern hybridization experiments. None of the mutants were fluorescent on sucrose-asparagine medium (SA) or grew on solid SA medium containing 1.6 mM 2,2'dipyridyl or 1 mM EDDHA. In contrast with wild type N1R, conidia and chlamydospore germ tube elongation of Fusarium oxysporum f. sp. cucumerinum was not inhibited by any of the Pvd^- mutants on low iron medium. Pyoverdine is associated with in vitro inhibition of F. oxysporum f. sp. cucumerinum.

716

PHENOTYPES OF BENOMYL-RESISTANT TRANSFORMANTS OF THE BIOCONTROL FUNGUS *GLIOCLADIUM VIRENS*. Sue Mischke, USDA, ARS, Biocontrol of Plant Diseases Lab, Beltsville, MD 20705.

Transgenic *G. virens* strains were created by integration of a cloned mutant β-tubulin gene from *Neurospora crassa*. If insertion of the foreign DNA interrupted an active gene, then traits dependent upon this gene would be mutant. Comparisons were made between transformants having more than one insertion site and the parent Gl-21 strain for ability to control damping-off of cucumber by *Rhizoctonia solani* under controlled conditions. At least one transformed strain performed as well or better than the parent strain. Since the transformant is also resistant to benomyl, it may be superior to the sensitive parent for use in integrated pest management applications where this fungicide is in use. Other aspects of the phenotype which were compared included antibiotic and enzyme production and performance during *in vitro* bioassays. This is the first report of improvement of this biocontrol agent by genetic engineering.

717

CITRUS BLIGHT DISEASE IN NORTHERN CALIFORNIA AND ITS REMISSION BY JAI-TC-922. M.J.Thirumalachar, Jeersannidhi Anderson Institute, POB-506, Locust street, walnut Creek, CA 94596.
Citrus blight inciting much damage to crop in Florida, once thought to be soil borne, later shown to be graft-transmissible now is shown to be hopper transmitted xylem inhabiting fastidious bacterial disease caused by Xylella fastidiosa. Amorphous plugs in xylem tract, with high zinc levels resulting in reduced water uptake cause blighting. In citrus trees in Walnut Creek, Northern California disease was detected by isolation of X.fastidiosa from stem cores. Diseased trees treated with 2 to 2.5 grams pellets of JAI-TC-922 implanted in holes made in stem, similar to that reported earlier in Pierce Disease of grapes and almond leaf scorch and recently in citrus greening and die-back control in India which is psyllid-borne phloem inhabiting fastidious bacterium. Remission of symptoms of blight was observed after 3 to 4 months and treated trees have remained healthy and productive.

718

CONTROL OF BACTERIAL FRUIT BLOTCH OF WATERMELON WITH CUPRIC HYDROXIDE. D. L. Hopkins, Central Florida Research and Education Center, University of Florida, Leesburg, Fl 34748.

Bacterial fruit blotch of watermelon, caused by a non-fluorescent pseudomonad resembling Pseudomonas pseudoalcaligenes subsp. citrulli, produces both foliar symptoms that result in minor damage to the plant and fruit symptoms that render the fruit unmarketable. In a field test, 69% of the fruit in unsprayed plots were unmarketable; whereas, only 10% were unmarketable in plots receiving weekly applications of cupric hydroxide at 1.7 kg/ha from anthesis. Streptomycin and fosetyl-Al did not provide any significant control of the disease. Marketable yields were 19.9 metric tonnes/ha in the cupric hydroxide plots versus 4.2 metric tonnes/ha in the control plots. Slight stunting was observed in the cupric hydroxide plots.

719

EFFECT OF FUNGICIDES ON GROWTH, PYCNIDIA FORMATION, AND MATURATION OF *SEPTORIA NODORUM*. S. Leath and K. L. Everts, USDA-ARS, Department of Plant Pathology, North Carolina State University, Raleigh 27695-7616.

The effect of carbendazim, flusilazole, myclobutanil, propiconazole, tebuconazole, and triadimenol on hyphal growth of ten isolates of *Septoria nodorum* was studied by examining rate of growth on fungicide amended PDA. The effect of carbendazim and propiconazole on formation and maturation of pycnidia, pycnidial size and percent of mature pycnidia also was examined using fungicide resistant and susceptible isolates. Analyses of variance revealed that hyphal growth was significantly affected by a fungicide by isolate interaction. Propiconazole reduced formation of pycnidia, whereas carbendazim resulted in decreased pycnidial size of the susceptible isolate. The carbendazim resistant isolate formed small pycnidia; however, pycnidial size did not decrease as rate of carbendazim increased to 5.0 μg/ml.

720

CONTROLLING PHYTOPHTHORA AND NEMATODE ON CITRUS SOIL WITH ENZONE. Mani Skaria and Gloria Gonzalez-Ruiz, Texas A&I University Citrus Center, P.O. Box 1150, Weslaco, Texas 78596.

Enzone, (a.i., sodium tetrathiocarbonate) is a nematicide that has been developed by Unocal Chemicals Division, Los Angeles, CA. Enzone releases carbon disulfide (CS_2) when mixed with water. In the Texas Lower Rio Grande Valley, a nematicide/fungicide that controls both nematodes and Phytophthora would be ideal. A citrus orchard infested with nematode, *Tylenchulus semipenetrans* and fungus, *Phytophthora parasitica* was selected for drench treatment with Enzone. In

one experiment, forty drench sites were treated with 2700, 900, 300, and 0 ppm CS_2. Citrus nematode and Phytophthora were assayed pretreatment and monthly beginning March 1990. Nematode and Phytophthora in the 300 ppm CS_2 treated soil were initially higher than any other treatments. Soil treated with the 900 and 2700 ppm CS_2 showed reduced levels of citrus nematode and Phytophthora. Young 'Rio Red' grapefruit trees were planted in the treated soil, and to-date, trees planted in 2700 ppm CS_2 treated soil show an increase in trunk diameter compared to other trees.

721

PRODUCTION OF SPORANGIA AND CHLAMYDOSPORES BY METALAXYL-RESISTANT AND -SENSITIVE ISOLATES OF *PHYTOPHTHORA PARASITICA* FROM ORNAMENTAL HOSTS. D. M. Ferrin and M. L. Wadsworth, Dept. of Plant Pathology, Univ. of California, Riverside 92521.

The in vitro production of sporangia and chlamydospores by field isolates of *Phytophthora parasitica* resistant (P-015F) and sensitive (P-068F, P-012F and P-048F) to metalaxyl was compared in the presence and absence of the fungicide. At 0 μg/ml, sporangium production by P-015F was equal to, less than and greater than P-068F, P-012F and P-048F, respectively. Sporangium production by P-015F increased 45.9 and 62.5% at 1 and 10 μg/ml, respectively, and decreased 21.6% at 100 μg/ml. In contrast, sporangium production by P-068F decreased 39.1, 52.8 and 84.8% at 1, 10 and 100 μg/ml, respectively. Sporangium production by P-012F and P-048F at 10 μg/ml decreased 99.4 and 99.0%, respectively. At 0 μg/ml, chlamydospore production by P-015F was greater than both P-068F and P-012F. Chlamydospore production decreased 12.1, 54.8 and 88.4% for P-015F and 26.4, 70.7 and 88.7% for P-068F at 1, 10 and 100 μg/ml, respectively.

722

FUNGICIDAL CONTROL OF DAMPING OFF AND ROOT ROT OF VINCA CAUSED BY RHIZOCTONIA SOLANI AND PHYTOPHTHORA PARASITICA. Sharon L. von Broembsen, Department of Plant Pathology, Oklahoma State University, Stillwater, OK 74078-9947.

Five fungicides were evaluated for control of damping off and root rot of vinca (Catharanthus roseus) caused by R. solani or P. parasitica in growth chambers. Fungicides were applied as soil drenches immediately after planting seeds in peat-lite mix artificially infested with corn meal-vermiculite cultures of either fungus. Emergence, damping off and deaths were recorded every other day for three weeks. Metalaxyl and an experimental formulation of benomyl plus metalaxyl gave significantly greater control (97.9 and 87.2% respectively) of P. parasitica than etridiazole (6.4%) or a formulation of etridiazole plus thiophanate-methyl (no control) at the rates and inoculum levels tested. Benomyl and the formulation of benomyl plus metalaxyl gave significantly greater control of R. solani (98.0 and 94.1% respectively) than etridiazole plus thiophanate-methyl (74.5%).

723

The Influence of Cysteine and Related Compounds on Germination of Alternaria cassiae. D. J. Daigle and P. J. Cotty. South. Reg. Res. Ctr., USDA, ARS, New Orleans, LA 70179.

The influence of amino acids on spore germination of Alternaria cassiae was investigated in vitro. Most amino acids had little effect on germination. However, aspartic and glutamic acids stimulated germination while serine, tryptophan, cysteine, and phenylalanine were inhibitory. Cysteine was most inhibitory and was active in the presence of a normally stimulatory nutrient. At 1% cysteine reduced germination 96% after 4 h. Derivitization of either the mercaptan or amino group but not the carboxylic acid group of the cysteine molecule reduced the inhibition. Cysteine also inhibited A. crassa and A. macrospora and reduced alternaria leaf spot on cotton plants inoculated with A. macrospora. Thus, amino acids have diverse effects on spore behaviour and suggest cysteine or similar compounds may be useful in preventing diseases caused by Alternaria species.

724

SCREENING BARLEY GERMPLASM FOR RESISTANCE TO BARLEY LEAF STRIPE (PYRENOPHORA GRAMINEA). J.A.G. van Leur, International Center for Agricultural Research in the Dry Areas (ICARDA), P.O. Box 5466, Aleppo, Syria.

A set of 200 barley lines, consisting of newly bred varieties, single head progenies of Syrian and Jordanian landraces and lines with reported resistance, were tested for resistance to barley leaf stripe (Pyrenophora graminea) under natural and controlled environment conditions. Screening in the field was done by planting the lines next to a heavily infested spreader, originating from seed harvested in farmer's fields in Syria. For the test under controlled environment an artificial seed inoculation was used. Results from both methods were highly correlated. A large difference in disease resistance was observed, both in the local germplasm and in exotic material. The high level of resistance in the varieties 'Betzes' and 'Vada' was confirmed. Some other varieties with a reported high level of resistance showed susceptibility against Syrian isolates.

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RFLP MAPS IN FUNGUS STAGNOSPORA NODORUM, A CAUSAL AGENT OF WHEAT GLUME BLOTCH DISEASE. P.P. Ueng[3], R.M. Slay, E.A. Geiger, [1]G. Shaner, [2]A.L. Scharen and [3]G. Bergstrom. USDA-ARS, Plant Molecular Biology Lab, Beltsville, MD 20705, 1. Purdue Univ., W. Lafayette, IN 47907, 2. USDA-ARS, Montana State Univ., Bozeman, MT 59717. 3. Cornell Univ., Ithaca, NY 14853.

In order to understand the genetic variability, virulence and possible sexual and parasexual recombinations in the fungus Stagnospora nodorum, which causes wheat glume blotch disease, nine isolates from different regions of the state of New York were used in RFLP mapping comparisons. Two other isolates, one from Montana and one from Indiana, were included in the study. The isolate SN2O9NY-88 was used to construct genomic clones based on its high tolerance to propaconazol at 0.1 ppm. Thirty-five clones were randomly chosen as probes. Of these, fourteen revealed variable restriction patterns. Furthermore, two of these fourteen probes gave three different restriction patterns and one showed five different profile. The data indicated a high degree of genetic variation in this fungal pathogen.

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VIRULENCE CHANGES OF PUCCINIA HORDEI IN THE U.S.A. Y. Jin, and B. J. Steffenson, Department of Plant Pathology, North Dakota State University, Fargo, ND 58105.

Populations of barley leaf rust (Puccinia hordei) in the U.S.A. have changed considerably in the last decade. Races 19, 22 and 30 are considered to possess the greatest number of virulence genes. Additional virulence genes within races 22 and 30 were identified on previously resistant barley genotypes Cebada Capa and La Estanzuela (both possess Rph 7) and/or Tunisia 16 and Tunisia 26 (unknown Rph genes). These virulence types were collected from California, Pennsylvania and Virginia. By the addition of Cebada Capa, Tunisia 16 and Tunisia 26, five of the eight possible virulence combinations were found in the race 30 group, and three in the race 22 group. This range in virulence within the populations of P. hordei may not be due to the deployment of resistance genes since most commercial cultivars in the U.S.A are susceptible to leaf rust.

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DETECTION OF THE T GENE FOR RESISTANCE TO PUCCINIA GRAMINIS F. SP. TRITICI IN BARLEY SEEDLINGS. B. J. Steffenson, J. D. Miller*, and Y. Jin, Department of Plant Pathology, North Dakota State University and *USDA-ARS Northern Crops Science Laboratory, Fargo, ND 58105.

In barley, the T gene (Rpg 1) confers resistance to many races of the wheat stem rust pathogen (Puccinia graminis f. sp. tritici [Pgt]); however, the identification of this gene in the seedling stage has been difficult because most genotypes exhibit a mixture of high and low infection types (ITs). Seedlings of cultivars Chevron and Glenn (with T gene); Steptoe and Klages (without T gene); and Heitpas-5, Black Hulless, and PI 382313 (different resistance genes) were inoculated with races Pgt-MCC and -HPH. ITs were assessed on plants after 12 days incubation in a growth chamber at 25-28C. With both races, Chevron and Glenn exhibited low ITs (0;1) and Steptoe and Klages intermediate to high ITs (3-2 to 33+). Heitpas-5, Black Hulless, and PI 382313 could be differentiated from genotypes with the T gene since they gave ITs of 23- to 33+ to race HPH. These data demonstrate the usefulness of races MCC and HPH in differentiating genotypes with and without the T gene.

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EFFECT OF LEAF RUST, POWDERY MILDEW, SEPTORIA, AND TAN SPOT ON WINTER WHEAT GRAIN YIELDS IN OKLAHOMA. E. Williams, Jr., K. E. Jackson, and P. W. Pratt. Plant Pathology Department, Oklahoma State University, Stillwater, OK 74078-9947.

Foliar fungicide testing on wheat at Stillwater and Haskell, OK, from 1985-1990 provided 6 years of data showing relationships of disease severity and grain yield. Treatments providing the lowest AUDPC values were compared with the highest control AUDPC values on effects of grain yield for each disease, each year, and each location (6 years X 2 locations = 12 evaluations). This was an opportunity for a comprehensive study on the influence of foliar fungus diseases on wheat production. Significant ($P>0.05$) negative correlations between AUDPC and yield occurred in 5 of 12 possible comparisons involving Septoria leaf blotch ($r=-0.70, r=-0.79, r=-0.61, r=-0.72; r=-0.81$), twice with powdery mildew ($r=-0.90; r=-0.76$), twice with leaf rust ($r=-0.69$ both times), and twice with tan spot ($r=-0.70; r=-69$).

730

SCREENING WHEAT FOR RESISTANCE TO SCLEROTIUM ROLFSII. D.A. LAWN, AND B. SKOVMAND. CIMMYT, APDO. POSTAL 6-641, DELEG. CUAUHTEMOC, 06600 MEXICO D.F., MEXICO.

In many tropical and subtropical environments, Sclerotium rolfsii infections on wheat result in seedling blights or premature plant senescence. Triticum aestivum lines Lerma Rojo 64A, UP 301, Napo 63, Frontana, Hybrid 65 and Trigo 3 were inoculated at seeding with either 1g of air-dried sclerotia or 10g of fungus colonized dead barley seed. Barley line Gloria/Comanche was sown as a susceptible check. The field test site (Morelos, Mx.) was irrigated and the soil is a calcareous clay with pH 8. Plots, 2-m in length, were inoculated and number of spikes and prematurely ripened spikes were compared. Seedling reactions were further tested in a growth chamber with alternating 12 hr cycles of 30 C and 20 C. Inoculation techniques included i) incubating pregerminated seed in plates of actively growing mycelia and ii) planting pregerminated seed with a fungus colonized dead barley seed. Significant differences among genotypes in seedling emergence and survival after 10 days was observed.

731

SENSITIVITY OF SELECTED MAIZE INBREDS TO THREE STRAINS OF BARLEY YELLOW DWARF VIRUSES A. D. Hewings, USDA ARS, C. J. D'Arcy and W. L. Pedersen, Department of Plant Pathology, University of Illinois, Urbana IL 61801

Ten maize inbreds, A619, A632, A634, B68, B73, B84, CM105, Mo17, Pa91, and Va22 were tested for sensitivity to barley yellow dwarf viruses (BYDV). In the greenhouse at the 3-leaf stage, inbred seedlings were infested with *Rhopalosiphum padi* that had been reared on Hudson barley infected with BYDV-PAV-IL or BYDV-RPV-NY, or *Sitobion avenae* reared on Hudson barley infected BYDV-MAV-NY. The same inbreds were grown and inoculated in the field at the 7-leaf stage. Roots and shoots of 5 greenhouse-grown plants and shoots of 5 field-grown plants from each inbred line were assayed for the three virus strains using triple-antibody sandwich enzyme-linked immunosorbent assays developed at Illinois. No inbreds were infected with MAV-NY or RPV-NY. PAV-IL was found in 21-70% of A619, A634, B73, and B84 shoot and root samples. About 20% of the Pa91 root samples were positive. Preliminary studies to test PAV-IL transmission from barley to maize, maize to maize, and maize to barley suggest that *R. padi* transmits the virus with difficulty, if at all, from maize to maize but relatively easily from barley to maize and maize to barley.

732

YIELD LOSSES IN SPRING WHEAT LINES DUE TO AN MAV-like ISOLATE OF BARLEY YELLOW DWARF VIRUS. P.A. Burnett, R. Ranieri and M. Mezzalama. International Maize and Wheat Improvement Center. Lisboa 27, Col. Juarez, Apdo. Postal 6-641, 06600 Mexico, D.F., Mexico.

Wheat lines that have had low scores for the symptoms of infection of barley yellow dwarf viruses (BYDVs) for several cycles of field screening were yield-tested in small plots for two years at CIMMYT's, El Batan station (19°31'N 98°50'W 2249 masl) in Mexico. The treatments were: a) artificially infested with greenhouse-reared Metopolophium dirhodum transmitting an MAV-like virus isolate at the three-four leaf stage and b) a control protected with insecticides. In these experiments the cultivar Anza was used as a resistant check and Bobwhite as a susceptible check. A resistance index was obtained by dividing the yield of the insecticide-sprayed plots by the yield of the virus-infected plots. The lines Vee#5/Trap #1, PF79765, Trap#1, PRL produced ratios that were lower than the ratio for Anza, indicating that the above-mentioned lines were less susceptible than Anza.

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BARLEY YELLOW DWARF VIRUS CONTENT IN SPRING BARLEYS. R. Ranieri, P.A. Burnett and H.E. Vivar. Wheat Program, International Maize and Wheat Improvement Center. Lisboa 27, Col. Juarez, Apdo. Postal 6-641, 06600 Mexico, D.F., Mexico.

Quantifiable enzyme linked immunosorbent assay (ELISA) was used to assess the titres (ELISA values) of barley yellow dwarf viruses (BYDVs) in the shoots and roots of barley seedlings, 11 days after infection. Seedlings of the isogenic barley cultivars, California Mariout (Yd_2-) and CM67(Yd_2+) and eight advanced lines from the ICARDA-CIMMYT Mexican barley breeding program were infected singly with either PAV-, MAV-, RPV- or RMV-like isolates of BYDV. The plants were grown in a greenhouse. An index was developed to evaluate susceptibility of the genotypes to BYDV isolates. Some of the eight advanced lines tested exhibited low ELISA values and indices to a number of virus isolates. The lines Zarza and Agave had low indices when infected with PAV-, MAV- and RPV-like isolates of BYDV. The lines Shyri and Laurel had low indices when infected with PAV-, MAV- and RMV-like isolates.

734

HOST SPECIFICITY OF AGROBACTERIUM TUMEFACIENS ISOLATES FROM MUSCADINE (VITIS ROTUNDIFOLIA) IN MISSISSIPPI. S. V. Diehl and C. H. Graves. Dept. of Plant Pathology & Weed Sci., Mississippi State Univ., Mississippi State, MS 39762.

Pathogenicity of Agrobacterium tumefaciens isolates from muscadine was evaluated on nine hosts. Kalanchoe, sunflower, jimsonweed, castor bean and tomato were tested using whole plant inoculations. A detached leaf assay was used to test muscadine, grape, blackberry and chrysanthemum. All isolates produced galls on muscadine. Thus far, 7 of 24 isolates (five biovar 3 and two biovar 1) produced galls on blackberry and four (all biovar 1) produced galls on chrysanthemum. Absence of galling obtained on all other hosts confirms the hypothesis that both biovar 1 and biovar 3 isolates from muscadine have narrow host ranges. Additional testing of muscadine isolates and blackberry, blueberry and rose isolates from Mississippi is in progress.

735

EVALUATION OF THE APPLE SCAB PREDICTOR AND THE ENVIROCASTER FOR POSTINFECTION CONTROL OF APPLE SCAB. W.H. Shaffer, R.H. Thiesen, and M.M. Shaffer; Department of Plant Pathology; 108 Waters Hall; University of Missouri; Columbia, MO 65211.

Three fungicide spray schedules were compared on 'Starkrimson Red Delicious' apple trees for control of apple scab, fly speck, and sooty blotch. Fourteen standard protective sprays of Nova 40W (1.25 oz/100 gal) plus Captan 50W (32 oz/100 gal) were applied during the 1990 season. Postinfection (PI) fungicide sprays were made 72-96 h after the start of an apple scab infection period, as predicted by either an Apple Scab Predictor (Reuter-Stokes; Twinsberg, OH) or an Envirocaster (Neogen Corp.; Lansing, MI). Sprays were not repeated until 72-96 h after the next infection period, following the standard schedule-timing interval. Nine PI sprays of Nova 40W (2 oz/100 gal) plus Captan 50W (32 oz/100 gal) were applied using either the Apple Scab Predictor or Envirocaster to time sprays. All three spray schedules provided significant ($P=0.05$) control of apple scab on spur and terminal leaves and apple scab, fly speck, and sooty blotch on fruit, when compared to an unsprayed check. Disease control was equally good with all three fungicide schedules.

736

PROTECTION OF FRUIT TREE PRUNING WOUNDS OF PEACH AND NECTARINE TREES FROM CHONDROSTEREUM PURPUREUM USING CHLOROTHALONIL AND CAPTAFOL FUNGICIDES. Luis E. Sanchez, Melvin D. Grove, Casilla 154, Santiago 30, Chile.

Peach and nectarine trees had their pruning cuts treated with chlorothalonil and captafol at 12.5, 2.0, 2.5% a.i. concentrations by mist spraying and hand brush applications against silver leaf disease. The fungicides were applied within 48-72 hours after pruning. In one experiment the fungicides were applied by a mist sprayer and in another experiment by hand brush using a latex-fungicide solution. Both experiments were carried out under commercial orchard conditions. After 3 yrs., 12.5% and 15.0% of the unsprayed control trees showed silver leaf (Chondrostereum purpureum) symptoms. All fungicide treatments gave statistically significant control of the silver leaf disease, compared with the control trees. There were no symptoms of fitotoxicity in any of the fungicide treatments.

737

ASPERGILLUS SPECIES ASSOCIATED WITH PISTACHIO NUTS IN CALIFORNIA ORCHARDS. M. A. Doster and T. J. Michailides. University of California Berkeley/Kearney Agric. Center, Parlier 93648.

The following *Aspergillus* species were isolated from pistachio nuts collected from orchards in 1990: *A. amstelodami, A. flavus, A. japonicus, A. melleus, A. niger, A. ochraceus, A. oryzae, A. parasiticus,* and *A. wentii*. In addition, extensive isolations were made from pistachio nuts gathered in December after harvest from eight commercial orchards in Madera Co. A total of 1062 nuts were surface sterilized and plated on a medium of 6% NaCl and 0.5% agar. The following *Aspergillus* species were isolated (with the percentage of nuts with that species): *A. niger* (4.6%), *A. japonicus* (0.2%), *A. flavus* (0.1%), *A. wentii* (0.1%), and *A. amstelodami* (0.1%). *Aspergillus* spp. were isolated more frequently from nuts with visible insect damage (15.4%) than from nuts with no such damage (2.4%). However, approximately the same percentage of nuts had *Aspergillus* whether the nut was collected from the tree (2.9% and 15.1%, for nuts with and without insect damage, respectively) or from the ground (1.8% and 15.8%, with and without insect damage, respectively). The growth of *Aspergillus* species in nuts left in the orchard after harvest could be important for the increase of mold levels in harvested nuts in the following year.

738

STEM DIEBACK CAUSED BY AUREOBASIDIUM IN THREE VACCINIUM SPECIES. F.L. Caruso and J.S. Mika, University of Massachusetts, Cranberry Experiment Station, East Wareham 02538

Two strains of Aureobasidium pullulans were isolated in the field from stems showing dieback on cranberry (light strain) and blueberry (dark strain) plants. Both strains were used in a series of experiments to determine whether A. pullulans is a causal agent of dieback in three species of Vaccinium. Three highbush blueberry cultivars were inoculated with both strains; half were wounded. In 'Berkley' and 'Collins', percent recovery of A. pullulans from symptomatic stems increased greatly over controls for those infected with the light strain. The light strain appeared to be more virulent than the dark strain and did not require a wound for infection. 'Early Black' cranberry plants were susceptible to the light strain of A. pullulans only when inoculated during flowering. The dark strain, however, was recovered from diseased stems of both vegetative and reproductive uprights. Experiments are in progress using two clones of lowbush blueberry.

739

Susceptibility of hazelnut , *Corylus avellana,* cultivars to eastern filbert blight exposed to different inoculum densities of *Anisogramma anomala.* J. N. Pinkerton[1], K. B. Johnson[2], K. M. Theiling[2], and J. W. Pscheidt[2]. USDA/ARS Horticultural Crops Research Laboratory, Corvallis 97330, and Department of Botany and Plant Pathology, Corvallis 97331-2902

Common hazelnut cultivars displayed a range of susceptibility to eastern filbert blight (EFB) in a survey of infected orchards in Oregon. In 1989, the susceptibility of 9 major cultivars were evaluated under 3 inoculum densities by placing potted trees below wire-supported hazelnut branches that contained sporulating stromata. Ten additional cultivars were screened at the highest inoculum density. Inoculum density was determined by trapping spores throughout the study and disease was rated in July 1990. Number of cankers per tree, number of cankers x canker length, and proportion of dead branches differed significantly among cultivars. Willamette, Casina, Halls Giant, Tondi di Giffoni, and OSU 49-73 had low disease severities and the cultivar Gasaway was disease-free. Barcelona, Ennis, Butler, Daviana, and Duchilly, the predominant cultivars in Oregon, had significantly higher disease ratings. For most disease parameters, inoculum density had a significant effect, but inoculum density x cultivar interactions were not significant. Forty-two cultivars and breeding lines were exposed under high inoculum in 1990 and will be evaluated in early summer 1991.

740

INFECTION PERIOD OF *BOTRYOSPHAERIA DOTHIDEA* ON NONWOUNDED PEACH BARK. P. L. Pusey[1] and P. F. Bertrand[2]. [1]USDA-ARS, P. O. Box 87, Byron, GA 31008 and [2]University of Georgia, Cooperative Extension Service, Tifton, GA 31794

One-yr-old budded peach trees were planted outside in January 1987 and 1989. At 3-wk intervals from February through December, six trees were artificially inoculated by applying a spore suspension of *Botryosphaeria dothidea* onto the nonwounded stem. Surface wetness was maintained for 7 da. To determine the period of infection from natural inoculum, stems of newly planted trees in two commercial orchards were covered using plastic pipe and caulking. From April 1988 to December 1989, the stems of six trees in each orchard were uncovered monthly for 30 da. Compared to uninoculated check trees, the number of lesions at lenticels and total bark necrosis were statistically greater for trees artificially inoculated during the period from March to August. The peak period of infection occurred from May to July. With orchard trees periodically exposed to natural inoculum, infection appeared to be dependent on the availability of waterborne spores within temperature limits.

741

PECAN ANTHRACNOSE DISEASE CYCLE. C. C. Reilly, P. O. Box 87, Byron, Georgia 31008.

Glomerella cingulata (Ston.) Spauld and Schrenk, the causal agent of pecan anthracnose, overwinters in pecan orchards on peduncles of the previous year's crop. Peduncles remain on branches for 2 or more years and the fungus continues to sporulate. Conidia were released during rain showers from bud break in early April through late July. The optimum temperature for sporulation of *G. cingulata* on peduncles was 20 C. Initial infections of the developing pecan fruit occurred during pollination in early May. A latent period appears associated with pecan anthracnose. Symptomless fruit, surface sterilized and plated on PDA throughout the growing season, had *G. cingulata* associated with it. Large black, shiny sunken lesions with salmon-colored spore masses appeared on the shuck in mid-August, through harvest. The kernel filling process was adversely affected, reducing both yield and quality. Infection rates approaching 100% were detected in some orchards.

742

SUSCEPTIBILITY OF PECAN FRUIT, LEAVES, AND ROOTS TO *PHYTOPHTHORA CACTORUM*. M.W. Hotchkiss and C.C. Reilly, P.O. Box 87, Byron, Georgia 31008.

Detached fruit of ten pecan (*Carya illinoensis* (Wang.) K. Koch) cultivars were spray inoculated with a mycelium and zoospore suspension of *Phytophthora cactorum* (Leb. & Cohn) Schroet. (isolate B-1 from pecan) to compare suceptibility. Leaves of 'Cherokee' pecan seedlings were spray inoculated with *P. cactorum*. The fungal suspension was applied as a dip or a drench to roots of 'Cherokee' pecan seedlings. Fruits of all cultivars tested were equally susceptible to the B-1 isolate of *P. cactorum*. Inoculated leaves developed dark irregular lesions. Younger leaves appeared more susceptible than mature leaves. Roots of inoculated pecan seedlings became necrotic, plants wilted, and seedlings died within 3 months. The effects on pecan roots and foliage were the first observed occurrences of *P. cactorum* as a pathogen on these tissues.

743

EFFECT OF NEEM OIL ON WOUND ETHYLENE AND DECAY IN APPLE FRUIT INOCULATED WITH BOTRYTIS CINEREA. Harold E. Moline, USDA, ARS, PQDI, HCQL, Beltsville, MD 20705.

Seed and leaf extracts from the neem tree, Azadirachta indica, have been known to be beneficial to humans since the fourth century B.C.; the insecticidal properties of triterpenoid-containing extracts are well documented. An uncharacterized "neem oil" preparation was recently shown to inhibit growth of several genera of plant pathogenic fungi. As part of our continuing search for natural compounds with antifungal

properties, neem oil was tested against Botrytis rot of apples. Fruit dips of emulsifiable neem oil at 0.5, 1.0, and 2.0% (v/v) were tested on apple fruit that had been wound-inoculated with Botrytis cinerea. Decay reduction was demonstrated at the 1.0 and 2.0% application rates. Wound ethylene was also significantly reduced by these treatments. Since wound ethylene stimulates the infection of apple fruit by Botrytis cinerea, neem oil may be useful in reducing Botrytis rot.

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RESISTANCE INHERITANCE IN STRAWBERRY TO PHYTOPHTHORA FRAGARIAE. W. E. Van de Weg, S. Giezen, G. J. Galletta, and J. L. Maas. Center for Plant Breeding Research, PO Box 6700 AA, Wageningen, The Netherlands, and Fruit Laboratory, USDA-ARS, Beltsville, MD.

Strawberry (Fragaria x ananassa Duch.) is an allo-octoploid (x=7, 2n=56) which shows strong bivalent pairing at meiosis. Resistance to red stele root rot, caused by P. fragariae, was thought to be inherited quantitatively. Resistance breeding is complicated by the presence of pathogenic races of the fungus. Intercrossing of highly resistant clones shows variable percentages of resistant individuals, and specific combining patterns, in addition to the expected additive accrual of resistance. Recent applications of a proposed host-pathogen gene-for-gene model, with 5 host resistance and 5 virulence genes, has been helpful in interpreting frequency distributions. The model should help in determining whether inheritance is disomic or tetrasomic, and in determining expected progeny genotypes. The model also should facilitate prediction and characterization of new races of P. fragariae.

745

A SCANNING ELECTRON MICROSCOPE STUDY OF BLACKBERRY FLOWERS INFECTED WITH CERCOSPORELLA RUBI. B. J. Smith, USDA-ARS, Small Fruit Res., Poplarville, MS 39470, and S. V. Diehl, Dept. of Plant Pathology & Weed Sci., Miss. State U., Miss. State, 39762

Rosette disease, caused by the fungus Cercosporella rubi, often limits blackberry production in the southeastern United States. Flower buds with and without rosette symptoms were collected at four stages of development from four cultivars of erect thorny blackberry (Rubus sp.). Scanning electron microscope observations revealed that C. rubi hyphae did not penetrate host cells but grew appressed against the surface cell walls of both anthers and pistils and into natural openings of these organs. Hyphal strands often grew parallel forming rope-like structures covered with a gelatinous appearing substance. Mycelial growth was most abundant on the stigma around the mouth of the stylar canal. At advanced flower stages cells beneath this mycelial mat were shriveled and collapsed, while cells of uninfected buds at the same stage were not.

746

A LEAF DISK INOCULATION TECHNIQUE FOR EXAMININING THE INFECTION PROCESS OF ARTHRUIOMYCES PECKIANUS ON BLACK RASPBERRY WITH SEM AND LIGHT MICROSCOPY. D. L. Truxall, J. W. Travis, and K. D. Hickey. Department of Plant Pathology, The Pennsylvania State University, University Park, PA 16802

Orange rust, caused by Arthuriomyces peckianus, causes permanent loss of black raspberries (Rubus occidentalis). A unique leaf disk inoculation technique was used for documenting the infection process of orange rust with both light microscopy and SEM, along with the time needed for infection. Disks (10 mm dia.) were cut from fully expanded raspberry leaves, and dusted with aeciospores. A 40 µl drop of water was placed centrally on each disk. Disks were incubated on wet filter paper in petri dishes at 20°C for a period ranging from 6 to 72 hours. Successful infection can be determined immediately by light microscopy when cleared and stained, in contrast to the 21 day period required for normal visual symptom expression of orange rust. The use of small leaf disks also minimizes specimen processing time required for SEM. This leaf disk technique served as a practical method for rapid examination and for easily identifying sites of infection.

747

A STUDY OF THE DISTRIBUTION OF XYLELLA FASTIDIOSA WITHIN THE ROOTS OF PEACH. J. H. Aldrich, University of Florida, AREC, Monticello, FL 32344, A. B. Gould, Dept. of Plant Pathology, Rutgers University, New Brunswick, NJ 08903, and F. G. Martin, Dept. of Statistics, University of Florida, Gainesville, FL 32611.

The distribution of Xylella fastidiosa, the bacterium which causes phony peach disease, was studied in the roots of ten Flordaking peach scions on Nemaguard rootstock. Four cm sections of root were taken at 15 cm intervals long the length of randomly selected roots from each tree. Bacterial populations in the xylem fluid extracted from each root piece were determined. Bacteria were detected in all trees, whether or not they were symptomatic for phony peach disease. Distribution of bacteria along the length of each root was fairly even for all trees. In asymptomatic trees, the distribution of infected roots among the quadrants of the rootball was variable. The minimum sample size required to successfully detect X. fastidiosa in the roots of asymptomatic peach trees was calculated ($P < 0.01$, 0.05, and 0.10).

748

ASSOCIATION OF PHELLINUS NOXIUS WITH DECLINE OF LONGAN (EUPHORIA LONGAN) TREES. P. J. Ann and W. H. Ko, Chia-yi Agricultural Experiment Station, Chia-yi, Taiwan, ROC and Department of Plant Pathology, University of Hawaii, Hilo, Hawaii 96720

Discoloration of leaves followed by gradual defoliation and death of longan trees was noticed in central and southern Taiwan in the early 1970s. Extensive root rot was found on all the diseased trees examined. The infection frequently extended into the main trunk. The wood tissues of infected roots eventually became white and soft with brown lines. The outer surface of the infected bark appeared rough because of being covered with a layer of adhering soil particles. The inner surface of the infected bark was covered with white mycelial mats and brown network of lines. Hard and flat fruiting bodies of dark brown color identified by T. T. Chang as Phellinus noxius were frequently found on stem base of the diseased trees. A fungus which produced similar fruiting bodies on artificial medium was consistently isolated from diseased tissues. When the fungus grown on wheat-oat medium was used to inoculate roots of longan seedlings, 50% of the inoculated seedlings died within 3 months. All control seedlings remained healthy. P. noxius was reisolated from all diseased seedlings.

749

ASSOCIATION OF PHYTOPHTHORA CAPSICI WITH QUICK DECLINE OF MACADAMIA TREES. W. H. Ko and R. K. Kunimoto, Department of Plant Pathology, University of Hawaii, Hilo, Hawaii 96720.

Fungal fruiting bodies were not found on several macadamia (Macadamia integrifolia) trees with quick decline symptoms. However, about 30 cm above the ground the trunk of declining trees showed symptoms of canker and bleeding. Under this area the bark became blackish brown and the wood tissues appeared grayish brown. When pieces of diseased tissues were placed on a selective medium, Phytophthora capsici similar to the one causing a raceme blight of macadamia was consistently isolated. P. capsici was isolated from xylem tissues 80 mm away from the bark indicating that the fungus can also invade woody tissues. Fungi grown on a wheat-oat medium were used to inoculate branches of healthy macadamia trees. Three months after inoculation, the percentages of macadamia branches killed by trunk and raceme isolates of P. capsici were 70 and 50%, respectively. Control branches remained healthy during the period. P. capsici was reisolated from artificially infected branches.

750

EFFECT OF INOCULUM CONCENTRATION ON SEVERITY OF BACTERIAL SPOT AND THE RELATIONSHIP OF SEVERITY TO YIELD COMPONENTS IN TOMATO. G. L. Hartman and W. F. Hong, Asian Vegetable Research and Development Center, P. O. Box 42, Shanhua, Tainan, Taiwan 74199, ROC.

Six entries of tomato plants were inoculated with four concentrations of Xanthomonas campestris pv. vesicatoria (Doidge) Dye under controlled conditions. Disease severity ratings averaged over the four inoculum concentrations increased from 7 to 21 days in all entries, although the rate of increase was significantly less for two entries. There was no significant difference in the average severity of the six entries when inoculated at 5×10^8 or 1×10^8 cfu/ml; however, both of these concentrations caused significantly higher severity values than 10^6 or 10^4 cfu/ml. Regressions of percent leaf area infected ($\log_{10}$) to bacterial concentration ($\log_{10}$) were significant for each entry, but the rate of increase varied. In the field, one susceptible line was used in plots that were either not inoculated or inoculated at several different intervals during the season. Plants that were heavily infected had 90% fewer marketable fruits than plants in noninoculated plots. There was a significant decrease in fruit number, yield, and weight/fruit to disease severity.

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CYTOLOGY OF WHITEFLY-INDUCED SQUASH SILVERLEAF. D. R. Jimenez, R. K. Yokomi, and J. P. Shapiro. USDA, ARS, 2120 Camden Road, Orlando FL 32803.

Squash silverleaf (SSL) is a disorder of many cucurbits in which the upper leaf surface of plants colonized by Florida biotypes of the sweetpotato whitefly, *Bemisia tabaci*, become irreversibly silvered. Light and electron microscopy of thin sections from silvered pumpkin leaf tissue exhibited altered cell development in the adaxial palisade parenchyma. In lieu of normal palisade cells the subepidermal mesophyll of silvered leaves contained numerous small and disorganized cells surrounded by extensive intercellular spaces. Whitefly-induced distortion of the pumpkin mesophyll was more severe than that associated with stress or genetic causes. Anatomically, SSL was analogous to nonwhitefly associated silvering reported in tomato and pea, in which differential rates of cell division have been associated with disruption of the mesophyll.

752

The effect of temperature on Pyrenochaeta terrestris microsclerotia production, growth, and pathogenesis. C. Biles, (Entomology, Plant Pathology and Weed Science) M. Holland, M. Ulloa-Godinez, and J. Corgan (Agronomy and Horticulture). New Mexico State University, Las Cruces, NM 88003.

Experiments were conducted to determine optimum temperature for microsclerotia production, growth, and pathogenesis of Pyrenochaeta terrestris. Isolates were placed on agar with 1 cm sterile onion roots. Microsclerotia were observed after 20 days at 25 and 30 C. Microsclerotia were not observed at 15 and 20 C. Only a few were observed at 35 C. Optimum temperatures for growth on PDA for the isolates tested was 25 to 27 C. An isolate from Weslaco, TX appeared to be more aggressive at 27 and 32 C when considering root biomass, while a New Mexico isolate (Franzoy) displayed the characteristic pink root symptoms to a greater degree when compared to the Weslaco isolate. Variation was shown among the isolates ability to form microsclerotia, to grow a different temperatures, and cause pink root symptoms.

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PATHOGENICITY OF FUSARIUM SPP. IN SECTION LISEOLA ON ASPARAGUS. W. H. Elmer, The Connecticut Agricultural Experiment Station, P. O. Box 1106, New Haven, CT 06504.

Within the Fusarium section Liseola, F. moniliforme (FM), F. subglutinans (FS), and F. proliferatum (FP) have been implicated as pathogens on asparagus; however, different mating groups (MG) A-F within these species have not been tested. Pathogenicity on asparagus was determined by inoculating seedlings grown in test tubes filled with agar, and by growing greenhouse transplants in infested soils. Noninoculated plants served as controls. Pathogenicity among MG was determined by comparing disease ratings and fresh weights with control plants. Repeated trials indicated that isolates within MG A in FM and MG D in FP were the most virulent. Isolates in MG (B and E) within FS were also virulent, but variation was greater between isolates. MG C in FM contained isolates that were less virulent than those in MG A while isolates in MG F were avirulent on asparagus. These findings suggest that virulence on asparagus is a trait specific to certain MG in FM.

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CHARACTERIZATION AND PATHOGENICITY OF PHYTOPHTHORA CAPSICI ON PEPPER AND TOMATO. G. L. Hartman and Y. H. Huang, Asian Vegetable Research and Development Center, P. O. Box 42, Shanhua, Tainan, Taiwan, 74199, ROC.

Phytophthora capsici Leonian isolated from infected tomato foliage has not been characterized or compared to isolates from pepper. Isolates from pepper stems and tomato foliage were inoculated on cabbage, cucumber, pepper, tobacco, and tomato by atomizing foliage or drenching soil with zoospore suspensions under growth room conditions. Only tomato and pepper developed symptoms following inoculation with P. capsici, but isolates varied in their virulence on these hosts. On foliage of pepper and tomato, isolates varied from causing no symptoms to complete blighting and death of plants. Drench-inoculated pepper plants, but not tomato plants, developed collar rot symptoms. Isolates inoculated on foliage of eleven tomato lines (some previously reported as resistant to P. infestans) caused over 50% foliar infection with no differences in disease ratings among isolates or lines. The size of sporangia from tomato and pepper isolates was compared. Pedicel length was 33.5-98.9 µm and the sporangia sizes were 43.1-65.8 µm long and 30-50.4 µm wide. Isolates from tomato or pepper could not be distinguished.

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EFFECT OF METHAM SODIUM FUMIGATION ON PRATYLENCHUS PENETRANS, VERTICILLIUM DAHLIAE, AND YIELD AND QUALITY OF POTATO IN WISCONSIN. Rouse, D. I. and MacGuidwin, A. E., Department of Plant Pathology, University of Wisconsin, Madison, WI 53706.

Potato early dying has become an increasingly more severe problem in Wisconsin. Soil fumigation has become the primary means of control. The long term effectiveness of soil fumigation was not known. Fumigated and nonfumigated field plots were established in commercial fields and on the University of Wisconsin Hancock Research Station. Before plots were fumigated soil assays were taken for Verticillium dahliae and Pratylenchus penetrans. Fumigation reduced soil populations of these pathogens for periods of 4 to 5 years encompassing two crops of potatoes in the plots. Yield, size grade and specific gravity were each improved significantly by fumigation treatment in 2-3 subsequent crops of potatoes. Potato growers should not need to fumigate before each crop of potatoes in Wisconsin.

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A NEW DISEASE OF LETTUCE INCITED BY *FUSARIUM OXYSPORUM*. J. C. Hubbard, and J. S. Gerik, USDA/ARS, 1636 East Alisal St., Salinas, CA 93905

In the fall of 1990, a commercial field of lettuce (*Lactuca sativa* 'Empire') in Huron, CA (Fresno Co.) was observed to contain plants showing symptoms of a disease characterized by the death of some plants in the seedling stage, showing a red streak through the cortex, and a tan to yellow tip burn of older heads, with black streaks in the vascular system of some affected leaves, brown streaks in the vascular system of the crown, and a reddish-brown rot in the cortex. *Fusarium oxysporum* of an identical vegetative compatibility group (VCG) was isolated from all symptomatic plants, and from lettuce debris in the affected area of the field, but not from other areas of the field or from other fields. When roots of lettuce seedlings of the variety 'Empire' were inoculated with this *Fusarium*, plants showed wilting followed by yellowing of some leaves, and stunting or death of plants, accompanied by reddish-brown coloration in the cortex and brown streaks in the vascular system of the crown. *Fusarium* of the same VCG was reisolated from cortical tissue of affected plants.

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INFLUENCE OF TEMPERATURE ON THE ABILITY OF UREDIOSPORES OF *UROMYCES DIANTHI* TO CAUSE DISEASE ON CARNATIONS. M. Polek and D. M. Ferrin, Department of Plant Pathology, University of California, Riverside 92521.

Disease severity on carnations (cv. Improved White Sim), incubated at 10, 15, and 20 C, was assessed after inoculation with suspensions of urediospores of *Uromyces dianthi* produced at 10, 15, and 20 C in a 3 X 3 factorial experiment. Plants were assessed 2 wk after the first appearance of erumpent pustules, and disease severity was expressed as the number of pustules per plant. For all three types of inoculum, disease was most severe on plants incubated at 15 C. Disease severity at all three temperatures was greatest on plants inoculated with urediospores produced at 20 C, whereas disease severity was lowest when urediospores produced at 15 C were used as inoculum. Disease severity of plants inoculated with urediospores formed at 10 C was approximately midway between that at 15 and 20 C. The temperature at which urediospores were produced had no effect on the latent period.

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CHARACTERIZATION OF INFECTION PERIODS OF *BREMIA LACTUCAE* ON LETTUCE UNDER CONTROLLED ENVIRONMENTAL CONDITIONS
H. Scherm and A.H.C. van Bruggen. Department of Plant Pathology, University of California, Davis 95616.

Lettuce plants (cv. Salinas) were inoculated with *B. lactucae* pathotype III, incubated at seven different leaf wetness durations (LWDs) from 2 to 24 h at each of six fixed temperatures (5 to 30 °C), and then transfered to standard growth conditions for two weeks. Percent leaf area infected (Y) increased with increasing LWDs at all temperatures (T) except 30 °C, where no significant infection occured. Y > 30 % was obtained for LWD > 6 h at 5 °C, 4 h at 10 - 20 °C, and 24 h at 25 °C. Heavy infections (Y > 70 %) occured at 10 °C when combined with LWD > 18 h, and at 15 °C with LWD > 8 h. For the development of a preliminary infection model, data were fitted to second- and third-order polynomals of T and LWD, and to a three-dimensional extension of the Gompertz-function. Our results indicate that wet periods as short as 4 h may be sufficient for downy mildew development in the coastal regions of California.

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POSITIVE CORRELATION OF FIG SMUT IN CALIMYRNA FRUIT WITH AMOUNTS OF DUST AND PROPAGULES OF *ASPERGILLUS NIGER* ACCUMULATED ON THE TREES. Themis J. Michailides and D. P. Morgan, University of California, Berkeley/Kearney Agric. Center, Parlier 93648.

During 1986-1990 the California fig industry suffered increased levels of smut (caused by *Aspergillus niger*) and mold (caused by other fungi) which affected up to 17% of the fruit. To determine whether discing or noncultivation in fig orchards affected levels of fig smut, ten leaves each from six random trees in seven orchards were sampled periodically and the amounts of dust deposited on them were determined. In addition, the incidence of smut at the end of the season on fresh and dried fruit was determined. Using data from five out of seven orchards, the percentage of smut (Y) in dried figs was linearly ($\hat{Y}$ = 1.66 + 92.7X) correlated (r^2 = 0.947) with the weight (g) of dust (X) accumulated per ten leaves from late July through mid August. The levels of smut determined on Calimyrna fruit in late August in all seven orchards was linearly ($\hat{Y}$ = -1.06 + 0.0075X) correlated (r^2 = 0.986) with the number of propagules of *A. niger* deposited per ten leaves. However, data from six out of seven orchards showed that the relationship between the incidence of smut in dried Calimyrna figs and the number of propagules of *A. niger* per ten leaves was better described by $\hat{Y} = -13.35 + 0.035X - 0.0000118X^2$ (r^2 = 0.863; $P < 0.01$).

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FORECASTING DEVELOPMENT OF GRAPE DOWNY MILDEW. E.W. Park*, R.C. Seem, R.C. Pearson, and D.M. Gadoury. *Dept. of Agricultural Biology, Seoul National University, Suwon 441-744, South Korea; and Dept. of Plant Pathology, Cornell University, NYSAES, Geneva, NY 14456.

A weather-driven system of models that mimic the processes of oospore maturation, host development, primary infection, incubation, sporulation, spore survival, and secondary infection was evaluated for forecasting the progress of grape downy mildew epidemics in New York. Oospore maturation of *Plasmopara viticola* was forecasted based upon the amount and distribution of precipitation from Sept.-Jan. When predicted oospore maturity reached 3%, and 5-6 leaves of the host had unfolded, primary infection was forecasted by a second model based upon temperature and rainfall. Following a period of incubation, cycles of secondary infection were forecasted by a third model based upon temperature, rainfall, leaf wetness, humidity, and darkness. Observations of oospore maturation at Geneva and Riverhead, NY in 1990, and examination of weather and epidemic records from 1985-90, indicate that the system of models may be useful both for initiating control programs for grape downy mildew, and for timing subsequent fungicide sprays to control secondary infection.

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DISEASE PROGRESS RATES UNDERESTIMATED WITH MAXIMUM DISEASE INTENSITY LESS THAN 100 PERCENT. Deborah Neher and C. Lee Campbell, Department of Plant Pathology, North Carolina State University, Raleigh, NC 27695.

Most applications of disease progress models assume maximum levels of disease intensity (K_{max}) of 100% or 1.0, but this is not true for many diseases. Disease progress rates (r_*) calculated for the monomolecular, logistic, and Gompertz models with K_{max}=1.0 were compared to r_* calculated from actual K_{max} values for published data on soilborne, foliar, fungal, bacterial and viral epidemics. Residuals and r^2 values from linear regression analyses were assessed for selection of an appropriate model. Then, estimates of r_* were calculated for assumed K_{max}=1.0 and actual K_{max} using nonlinear regression. Disease progress rates calculated with assumed K_{max}=1.0 were lower and decreased linearly with decreased actual K_{max}. Degree of underestimation of r_* with decreasing K_{max} was greatest for the logistic and least for the monomolecular model. The results demonstrate the importance of including actual K_{max} in disease progress models.

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ROOT COLONIZATION OF SNAPBEAN BY BACILLUS SUBTILIS STRAIN GB07A. W. F. Mahaffee, P. A. Backman and J. W. Kloepper, Department of Plant Pathology, Auburn University, Alabama 36849.

Root colonization of a rifampicin-resistant mutant of Bacillus subtilis (GB07A) (Gustafson, Inc., Dallas, TX) on snapbean (Phaseolus vulgaris) was monitored over a growing season. GB07A was applied as a seed treatment at log 6 cfu/seed. Plants were sampled from planting to harvest based on morphological growth stage. Plants were sectioned from cotyledons downward to root tips, and populations of GB07A for each section were determined by dilution plating on tryptic soy agar amended with rifampicin and cycloheximide. GB07A was distributed along the developing root system decreasing towards root tips before increasing in the final 10 cm before the root tip. Populations were generally >Log 4 cfu/g on all sections through second node. At flowering, populations had become patchy and reduced to <Log 3.5 cfu/g, and were nondetectable at harvest. GB07A colonized roots in both vertical and horizontal planes and colonized above-ground parts through flowering, though colonization was affected by morphological growth stage.

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COMPARISON OF EXTRACELLULAR DECAY ENZYMES OF POSTIA PLACENTA ISOLATED FROM WOOD OR ARTIFICIAL MEDIA. C.A. Clausen, F. Green III, and T.L. Highley. U.S. Dept. of Agric., Forest Service, Forest Products Laboratory, One Gifford Pinchot Drive, Madison, WI 53705-2398.

Extracellular enzymes from the brown-rot fungus P. placenta, grown in liquid culture, malt agar, or wood, were solubilized with nonionic detergents and analyzed for differences in enzyme activity and antigenic affinity. Availability of low MW sugars in artificial media resulted in excess production of glucan, which complicated enzyme purification. Despite increased enzyme activity from artificial media, enzyme distribution and antigenic affinity were altered as determined by affinity chromatography using monoclonal antibodies specific for xylanase derived from wood. This study demonstrates that enzyme characteristics of P. placenta are substrate dependent and in vitro culturing does not mimic fungal metabolism in wood.

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A TELEOMORPH FOR THE BIRCH ANTHRACNOSE FUNGUS. S. C. Redlin, USDA-ARS, Systematic Botany and Mycology Laboratory, Beltsville, MD 20705-2350.

Birch anthracnose, caused by the coelomycetous fungus Cryptocline betularum (Ellis & Martin) Arx, has been known since 1882 and causes leaf spotting and premature defoliation of four North American Betula spp. Recently a pyrenomycete with immersed ascocarps containing asci with conspicuously thickened light refractive apical annuli and apiosporous ascospores was observed in fallen overwintered leaves of river birch, B. nigra L., in Iowa, Maryland and Minnesota. Cultures derived from single ascospores matched those derived from conidia on living leaves. The teleomorph is being formally described in the genus Apiognomonia Höhnel (Gnomoniaceae).

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RELATIONSHIP BETWEEN EXTERNAL AND INTERNAL SYMPTOMS CAUSED BY DISCULA SP. ON FLOWERING DOGWOOD. R. L. Anderson, and C. H. Walkinshaw, Pathologists. USDA, Forest Service, Rt. 3, Box 1249-A, Asheville, NC 28806.

Dogwood anthracnose was first reported in northeastern United States in 1978. In 1987 the disease was found in the northern mountains of Georgia. The disease has spread rapidly throughout the Appalachian chain. Leaf symptoms appear as yellow-haloed spots, large necrotic blotches, or reddened veins. In some instances, the fungus advances into the shoot or the dogwood stem, causing cankers. To understand the process of how the fungus affects host tissues, histological observations were made of host changes and fungal development that were associated with external symptoms. This report describes external symptoms on leaf and stem tissues, and illustrates internal responses of the host and the development of Discula sp. in relationship to the observed external symptoms. Healthy tissue is also presented for comparison.

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A LEAF DISC BIOASSAY FOR DETECTING RESISTANCE TO DOGWOOD ANTHRACNOSE CAUSED BY DISCULA SP. TAMMY M. HUNTER and JAMES L. SHERALD. Center for Urban Ecology, National Park Service, Washington, D.C. 20242

Discs (1.5 cm) excised from leaves of *Cornus florida, C. mas, C. kousa, C. stolonifera,* and *C. nuttallii* were disinfested in 10% Chlorox then rinsed 3 times in sterile water. Discs were placed into wells cut into 2% water agar plates. The center 4mm (8%) of each disc was wounded with a heated metal rod and inoculated with 10 μl of inoculum (2.3 x 10^7

conidia/ml). Wounded controls were treated with sterile water. Discs were incubated at 23-25 C under 6 h light/day. Percent green leaf tissue was measured every 3-7 days. Necrosis developed beyond the wound in *C. stolonifera, C. florida, C. nuttallii* but not in *C. kousa, C. mas* or the control. In 2 assays necrosis developed first in *C. stolonifera* at 9 & 14 days after inoculation, compared to *C. florida* at 12 & 27 days and *C. nuttallii* at 19 & 27 days. Green leaf tissue remaining after 5 wks was: *C. stolonifera*, 12%; *C. nuttallii*, 55%; *C. florida*, 63%; *C. mas*, 92%; and *C. kousa*, 92%. Results correspond to field susceptibility of *C. florida* and *C. nuttallii* and resistance of *C. kousa*. Field susceptibility of *C. stolonifera* and resistance of *C. mas* has not been reported.

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ASH YELLOWS ENDEMIC IN ZION NATIONAL PARK, UTAH. W. A. Sinclair, H. M. Griffiths, M. Treshow[1], and R. E. Davis[2]; Dept. Plant Pathology, Cornell Univ., Ithaca, NY 14853; [1]Biology Dept., Univ. of Utah, Salt Lake City 84112; and [2]USDA-ARS, Beltsville, MD 20705

Mycoplasmalike organisms (MLOs), which cause ash yellows, were detected by means of the DAPI (4',6-diamidino-2-phenylindole•2HCl) fluorescence test in root phloem of 38% of 174 indigenous velvet ash (*Fraxinus velutina*) but not in any of 34 singleleaf ash (*F. anomala*) that ranged in appearance from healthy to severely debilitated. MLO populations were generally low and often difficult to perceive. Few infected trees had witches'-brooms. Incidence of MLO infection was greater in trees with dieback than in trees without this symptom, but many declining ash lacked detectable MLOs. Many ash had been damaged by water shortage and defoliating insects. Growth ring analyses showed slower growth of MLO-infected than noninfected trees but did not reveal when trees became diseased. Dot hybridizations of DNA from diseased trees with biotinylated probes specific for DNA of ash yellows MLOs revealed sequence homology of the ash MLOs in Zion Park with New York strain AshY1, from which the probes were derived.

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INHIBITION OF AERIAL HYPHAE BY SPENT CULTURE FILTRATE OF AN ABERRANT STRAIN OF THE BROWN-ROT FUNGUS POSTIA PLACENTA. J. A. Micales, U. S. Forest Service, Forest Products Laboratory, One Gifford Pinchot Dr., Madison, WI 53705.

ME20, a wild-type, haploid strain of the brown-rot wood-decay fungus Postia placenta does not cause significant weight losses in standard soil-wood block decay tests and fails to form aerial hyphae in liquid and agar culture. Two-week-old culture filtrates of ME20 contain elevated levels of the autolytic enzymes laminarinase and protease. Aerial hyphae formation and total mycelial growth are inhibited in floccose strains of P. placenta when grown in fresh media that contain spent media from ME20. This suppression does not appear to be caused by extracellular autolytic enzymes since commercial preparations of protease and chitinase have no effect on colony morphology. Commercial laminarinase actually stimulates aerial hyphae formation. The suppressive agent is resistant to boiling and has a molecular weight of less than 10,000. Additional research is needed to characterize its nature, thus identifying a potential biorational inhibitor of wood-decay fungi.

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NITRATE NONUTILIZING MUTANTS OF HYPOXYLON ATROPUNCTATUM. S-C. Chun and P. Fenn, Department of Plant Pathology, University of Arkansas, Fayetteville, AR 72701.

Latent infections of oaks by H. atropunctatum complicate field studies with this canker rot fungus. Mutants with unique and easily scorable phenotypes would aid field inoculation studies. Of 26 wild-type isolates cultured in liquid medium containing nitrate, 18 did not utilize NO_3, six utilized it well and two were intermediate. Nitrate nonutilizing (nit) mutants were selected on chlorate-amended minimal NO_3 medium from two of the six nitrate utilizing isolates. Phenotypes of nit mutants were determined on minimal medium supplemented with NO_3, NO_2, NH_4, hypoxanthine, or urea. Of 98 mutants isolated, 58% were NO_3 nonutilizing mutants (Nit 1) and 27% failed to utilize NO_3 and NO_2 (Nit 3). Several other chlorate resistant mutants were unable to utilize any of the tested nitrogen sources. Complementation occurred between certain nit mutants. Several nit mutants grew well and were easily recovered after inoculation into detached oak stem segments indicating that they may be useful genetic markers to study the pathology and ecology of this fungus within trees.

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CONIDIAL MORPHOLOGY OF SPHAEROPSIS SAPINEA. W. J. Swart and M. J. Wingfield, Departments of Plant Pathology and Microbiology, University of the Orange Free State, Bloemfontein 9300, South Africa

Conidia of Sphaeropsis sapinea have been placed in two groups having smooth (Type A) or pitted (Type B) surfaces. Fifty monoconidial isolates of S. sapinea originating from eleven countries were examined using scanning electron microscopy. Conidial walls of 20 isolates were consistently smooth. Between 6 and 38% of conidia from the remaining thirty isolates had small indentations, or pits, distributed unevenly over the exterior of mature conidia. Authenticated type A and type B isolates from the north central USA grown on agar media supplemented with selected amino acids or sugars, each produced both pitted and smooth conidia. Tricyclazole, a melanin inhibitor added to growth media resulted in lack of conidial pigmentation, but in preliminary observations, no differences between the two isolate types could be discerned. Our results thus suggest that conidial wall morphology is unsuitable as a taxonomic criterion.

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Identification of Armillaria spp. in California. K.A. Jacobs [1], J.D. MacDonald [1], F.W. Cobb [3], and K. Wells [2]. [1], Dept. of Plant Pathology, [2], Dept of Botany, University of California, Davis 95616, and [3] Dept. of Plant Pathology, University of California, Berkeley 94544.

Armillaria root rot in California has historically been attributed to Armillaria mellea sensu lato, even though several species are now recognized as belonging to this group. We obtained basidiocarps and infected host tissue from11 counties in northern and coastal parts of the state. Pathogenic isolates came from several hosts including Quercus species, Prunus amygdalus, Abies concolor, Pinus species, Libocedrus decurrens and others. Collections were made in forested, rural and urban sites. Twenty-six monosporous isolates, and several vegetatively-derived isolates were paired with haploid tester strains representing the 9 North American biological species (NABS). All but four isolates were compatible with A. mellea s.str.(NABS VI). One isolate from the northern coast was compatible to A. bulbosa (NABS VII), and three others were compatible to NABS IX. Thus, the "oak root fungus" found on native oaks, low to mid-elevation forest trees, and some orchard crops in California is A. mellea. However, at least two other species were present and may be important in more northern regions or at higher elevation forests.

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GROWTH LOSSES IN EUCALYPTUS DUE TO EUCALYPTUS CANKER CAUSED BY CRYPHONECTRIA CUBENSIS. L.E.Camargo, A.Bergamin Filho, T.L. Krugner, R.A.B.Chaves, and H.T.Z. do Couto. Dept.Fitopatologia/ ESALQ. Cx.P.9, 13400-Piracicaba-S.P. Brazil.

We examined the association between canker age and a reduction in the growth rate (GR) of Eucalyptus grandis (Coff's Harbour/ Australia) trees in southeastern Brazil. Three different canker ages were compared by studying trees that became infected at the age of 2,4, and 7 yrs. GR was calculated using linear regression analysis according to the model LOG(cylindrical volume)= b0 + LOG(age) applied to each group, as well as for healthy trees. We found a positive association between canker age and a reduction in GR for trees with diameter at breast height (DBH) between 5-10 cm. Trees that were infected at the age of 2 or 4 yrs. showed significant reduction in GR relative to healthy trees of the same DBH class (45% and 38% reduction, respectively). No significant associations were found for trees with DBH above 10 cm.

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LOCALIZATION OF CHITIN IN WALLS OF THE WHITE ROOT ROT FUNGUS *RIGIDOPORUS LIGNOSUS*. M. Nicole[1] and N. Benhamou[2]. (1) Orstom/Forêts Canada C.P. 3800, Sainte Foy, (Québec) G1V4C7; (2) Université Laval G1K7P4, Sainte Foy, (Québec).

Localization of chitin in cell walls of *R. lignosus* was studied by using (WGA)-colloidal gold conjugates. Ultrastructural studies on *R. lignosus*-infected roots of rubber trees, revealed that fungal cell walls undergo modification throughout the whole infection process. Labeling did not occur over walls of hyphae achieving root penetration and host cell wall degradation suggesting that the alteration of chitin may be associated to excretion of host cell wall degrading-enzymes. Variation in labeling was observed over hyphal cell wall in host colonized-cells. Release of GlcNAc residues in host cells suggests the involvment of lytic enzymes in fungal cell wall alteration. These chitin oligosaccharides may play a role in root defense against the fungus.

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INFLUENCE OF NICOTIANA LEAF SURFACE COMPOUNDS ON GERMINATION OF PERONOSPORA TABACINA. B.S. Kennedy, M.T. Nielsen and R.J. Severson. University of Kentucky, N212 Ag Science North, Lexington, KY 40546-0091

An in vivo bioassay was used to evaluate the effects of leaf surface compounds, including α and β duvatriene-diols (DVTS), 15-OH abienol, cis-abienol, manool, 2-OH manool, sclareol and various glucose and sucrose esters, on germination of P. tabacina. Spore germination was not inhibited completely when exposed to any concentration of the chemicals tested. Exposure to >0.75 $\mu g\ cm^{-2}$ of α and β DVTS significantly inhibited sporangia germination. A slight stimulation in germination was observed when spores were exposed to 0.375 $ng\ cm^{-2}$. β DVT-diol was more inhibitory than the α isomer. Spore germination was significantly less than control treatments when spores were exposed to 30 $\mu g\ cm^{-2}$ of 15-OH abienol or sclareol. Cis-abienol, manool and 2-OH manool had little or no effect on spore germination at any of the concentrations tested. Glucose and sucrose esters partitioned from leaf washes of twelve different Nicotiana species were also evaluated for antimicrobial activity. Spore germination was significantly lower when exposed to 30 $\mu g\ cm^{-2}$ of several of the sugar esters. These results imply that a number of compounds may influence resistance to blue mold in tobacco.

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AGGRESSIVENESS OF ISOLATES OF *GIBBERELLA FUJIKUROI*, MATING POPULATION "F", ON GRAIN SORGHUM. Jardine, D. J. and Leslie, J. F. Dept. of Plant Pathology, Kansas State University, Manhattan 66506.

Strains of *Fusarium* (=*Gibberella fujikuroi* mating population "F") that are morphologically similar to but reproductively isolated from *Fusarium moniliforme* were isolated in Kansas. Isolates from 11 genetically-distinct vegetative compatibility groups were toothpick-inoculated into sorghum plants two weeks following anthesis. Lesion development was scored 18 days later, and some variation for aggressiveness was seen between the isolates. In a second experiment, aggressive and non-aggressive strains were inoculated into either *Fusarium*-resistant or -susceptible cultivars. Cultivar resistance appears to be general across the species rather than specific for a particular strain(s). *Fusarium* spp. were recovered from lesions in the inoculated plants. These plants were grown under greenhouse conditions from heat-treated seed and in pasteurized soil. In some cases, up to 45% of the recovered isolates were genetically distinct from the isolate with which the plant was inoculated.

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SMALL HOP CLOVER AS A NEW HOST OF SCLEROTINIA TRIFOLIORUM IN MISSISSIPPI. R. G. Pratt, USDA, ARS, P.O. Box 53657, Miss. State, MS 39762.

Symptoms of a Sclerotinia disease were observed in volunteer stands of small hop clover (Trifolium dubium Sibth.) near Starkville, MS, in March and April. Sclerotia were produced on diseased plants in spring and apothecia developed from them in autumn. Sclerotia and apothecia from parasitized hop clover in the field were smaller than those from berseem and crimson clovers, but ascospore features were characteristic of S. trifoliorum. Isolates of S. trifoliorum from hop clover were significantly more virulent on that host, and less virulent on crimson clover, than were isolates from berseem and crimson clovers. These results document small hop clover as a natural host of S. trifoliorum, apparently for the first time in North America, and indicate that isolates from this host may be partially specialized in virulence. The susceptibility of small hop clover may partly account for the widespread distribution of S. trifoliorum in the southeastern USA.

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EFFECT OF GARLIC EXTRACTS ON GERMINATION OF DISCULA CONIDIA. S. D. McElreath, J. Yao, and F. H. Tainter. Department of Forest Resources, Clemson University, Clemson, South Carolina 29634-1003.

Extracts of garlic (Allium sativum L.) have been reported for a number of years to have antimicrobial properties. Using an agar plate method and saturated paper disks, extracts of garlic cloves as well as aqueous solutions of commercial garlic powder inhibited germination of conidia from 15/15 isolates of Discula sp., the dogwood anthracnose fungus. Garlic oil, commercially available in a softgel capsule, had no effect on 8/9 isolates tested. Germination of one isolate was very slightly inhibited. Further investigations, using a tube dilution technique and eight isolates, showed that the garlic extract and the powder were immediately sporicidal at 5% and 10% concentrations for all eight isolates and sporicidal in 5 hours at 1% for 7/8 isolates.

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DROUGHT STRESS AS A PREDISPOSING FACTOR TO BRANCH DIEBACK IN CALIFORNIA CHAPARRAL PLANT COMMUNITIES. F. Brooks and D. M. Ferrin, Department of Plant Pathology, University of California, Riverside 92521.

Since 1985, incidence of branch dieback, caused by *Dothiorella* sp. (= *Botryosphaeria dothidea*), has increased in the chaparral community of the San Gabriel Mtns. This range forms the northeastern border of the Los Angeles Basin. Drought stress is believed to be a predisposing factor to this disease. Field inoculations of bigberry manzanita (*Arctostaphylos glauca*) with *Dothiorella* sp. were made in September, 1990, and January, 1991. Xylem water potentials were determined in concert with lesion expansion on the branches. A relationship was observed between low xylem water potentials (<-2.0 MPa) and lesion expansion. Current season's growth inoculated in September (<-2.0 MPa) was killed within 2 wk (lesions>150 mm), whereas that inoculated in January (>-2.0 MPa) showed initial necrosis and callus formation but no lesion expansion (lesions<10 mm). However, temperature and summer dormancy may also be factors in lesion development.

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TOXIN PRODUCTION BY GERMINATING SPORES OF PYRICULARIA ORYZAE. S. Arase, Y. Honda, and M. Nozu, Faculty of Agriculture, Shimane University, Matsue, Japan, 690.

The toxin(s) stimulating Pyricularia oryzae infection on rice leaves were detected by using a rice cv. Sekiguchi-asahi as a test plant. The toxins were detected and partially purified from spore germination fluids of virulent isolates of P. oryzae. Spores of Alternaria alternata, which is non-pathogenic to rice plants, formed numerous necrotic spots on rice leaves when inoculated with the toxins, but not on several other non-host plants(e.g., crabgrass and tobacco). The toxins induced a characteristic necrosis on leaves of cv. Sekiguchi-asahi as those on P. oryzae-infected leaves. Leaf necrosis formation by the toxins was light-dependent, and not observed in the dark. Induction of A. alternata infection to rice leaves by the toxins was recognized even in the dark where necrosis formation was suppressed. Such toxins were detected in fluids at 3 hr after spore germination.

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PATHOGENIC VARIABILITY IN *FUSARIUM SOLANI* ISOLATED FROM SOYBEANS WITH SUDDEN DEATH SYNDROME SYMPTOMS. S. M. Lim and H. Jin. USDA-ARS and University of Illinois, Urbana, IL 61801.

Pathogenic variability in isolates of *Fusarium solani* was evaluated by inoculating seedlings of 30 soybean genotypes with each of 12 isolates of the fungus in the greenhouse. Single-conidia isolates, 10 from Illinois, 1 from Mississippi, and 1 from Arkansas, were grown on autoclaved oat grains. Soybean plants were inoculated by placing 3 to 5 colonized oat grains around crown roots of plants grown in sterilized soil (3 pots of each genotype/isolate). Five plants at the V3 stage were in each 15-cm-d clay pot. Responses of soybeans to the individual isolates were evaluated at 3 day intervals after inoculation for 3 weeks based on leaf symptoms; symptomless or mild mottling as resistant and interveinal chlorotic spots and necrosis as susceptible. At least 5 races were identified based on differential responses of 10 genotypes; Hamilton, Ripley, Williams, A3205, A3307, A3427, CM368, D83-4377, PI 520733, and PI 71506.

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ORGANIZATION OF GENETIC AND PATHOTYPE VARIATION IN THE RICE BLAST FUNGUS AT A COLOMBIAN "HOT SPOT". Morris Levy, F. J. Correa-Victoria[1], R. S. Zeigler[1], S. Xu and J. E. Hamer. Dept. of Biological Sciences, Purdue University, West Lafayette, Indiana and [1]C. I. A. T., A. A. 6713, Cali, Colombia.

DNA "fingerprints" (EcoRI restriction fragment length profiles) of a repetitive genomic sequence (MGR) specific to the rice blast fungus were obtained for 140 field isolates collected from 17 rice cultivars at an upland rice blast disease hot spot. Preliminary results indicate that only seven MGR-defined fungal lineages comprise the more than 50 pathotypes (International races) apparent among the isolates. Each lineage is associated with a specific group

of pathotypes; most associations are cultivar-specific. A model is presented that indicates that the pathotype groups within lineages represent series of single-step or quantitative changes in pathogenicity on International differential host testers. MGR-fingerprints can, thus, define the cultivar range of specific rice blast fungal lineages and the paths of pathogenic diversification within lineages under chronic epidemic conditions.

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CONTROL OF EYESPOT WITH TRIADIMENOL SEED TREATMENT AND CULTIVAR RESISTANCE, AND SIDE-EFFECTS ON OTHER FOOT AND CROWN ROT DISEASES. F. Montfort, P. Lucas, N. Cavelier, N. Maurin, A. Cavelier, INRA, SRIV, BP 29, 35650 Le Rheu, France.

Eyespot (Pseudocercosporella herpotrichoides) is the major foot rot disease on winter wheat in France. In order to reduce the number of fungicide foliar applications, field experiments have been carried out since 1986. An eyespot-resistant cultivar controlled the disease but increased the severity of sharp eyespot (Rhizoctonia cerealis) and crown rot (Fusarium "roseum" types and Microdochium nivale). Triadimenol seed treatments reduced the occurrence of both eyespot and sharp eyespot, but caused an increase in the frequency of crown rot. Chemical seed protection in combination with genetic resistance limits the damage from two of the three crown and foot rots. The different modes of action of the involved methods also promise to delay the build-up of fungicide resistance as well as the ability of P. herpotrichoides to overcome host resistance genes. Additional improvements in this disease control system are necessary to avoid the increase in importance of minor pathogens.

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***DISCULA* GROWTH IN APPLES: A BIOASSAY FOR THE ESTIMATION OF ISOLATE PATHOGENICITY AND/OR VIRULENCE?** D.A. Brown, M.T. Windham, and R.N. Trigiano, The University of Tennessee, Knoxville, TN 37901-1071.

Dogwood anthracnose is caused by the imperfect fungus, *Discula* sp. Characteristic symptoms of this disease include an easily recognizable leaf blight and an annual canker. To date, consistent reproduction of this disease under controlled conditions has been difficult and a reliable assay is not available for the estimation of *Discula* pathogenicity and/or virulence to the flowering dogwood. An apple growth bioassay was developed for this purpose. *Discula* isolates were aseptically inoculated to 'Golden Delicious' apples by the transfer of a 7mm plug of the fungus (grown on PDA) to a wound on the apple made with a 7mm cork borer. Inoculation sites on the apples were sealed with clear adhesive tape to prevent dessication and incubated at 17 C for one month in constant darkness. Diameters of brown lesions developing on the fruit surface were measured. *Discula* colony type I isolates from anthracnose-infected flowering and Chinese dogwoods consistently produced larger lesions than type II isolates. Lesions were not observed on control apples inoculated with 7mm PDA plugs. Special care must be taken to avoid contamination of inoculations and apples should be spaced within the growth chamber to avoid contact. This *Discula* growth bioassay may prove useful in preliminary pathogenicity and/or virulence tests on *Discula* isolates, especially during those times of year when dogwood leaf material is not available or until a suitable host inoculation assay has been developed.

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A METHOD FOR ISOLATION OF *Pythium myriotylum* FROM COCOYAM ROOT ROT AFFECTED AND IDLE FIELD SOILS IN CAMEROON. R. P. Pacumbaba, J. G. Wutoh, Sama Anne Eyango, J. T. Tambong, and L. M. Nyochembeng. Department of Plant and Soil Science, Alabama A&M University, Normal, AL., U.S.A., and ROTREP, USAID/GOC, Ekona Res. Centre, Buea, Cameroon.

Pythium myriotylum (Pm) was isolated from the rhizosphere of cocoyam root rot (CRRD) affected plants and from the soil of an experimental field plot temporarily devoid of cocoyams from Mamu, Ekona by the cocoyam leaf disc baits. *Fusarium solani* (Fs) and *Rhizoctonia solani* (Rs) were also isolated from the same soils by the water dilution method and from the roots of CRRD-affected cocoyams but were always associated with growth of Pm. Pathogenicity tests indicated that cocoyam plantlets, 4 months old, inoculated with inoculums containing Pm and in combination with other isolated pathogens, developed symptoms of CRRD within 2-3 days after inoculations. On 7 months old cocoyam plantlets, CRRD symptoms appeared within 7 days after inoculations. Plantlets inoculated with inoculums containing Fs, Rs, Fs + Rs and distilled water did not develop symptoms of CRRD. This indicated that CRRD is not caused by several pathogens but only by Pm. Pm grew significantly faster in 24 hr period at 31 C in petri dishes containing lima bean sucrose agar, V-8 juice sucrose agar, and potato sucrose agar than on potato dextrose agar and 2% water agar. The cocoyam plantlets were raised axenically from tissue culture of explants in the laboratory.

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A METHOD FOR INDUCING MOTILE ZOOSPORES OF COCOYAM ROOT ROT PATHOGEN. R. P. Pacumbaba, J. G. Wutoh, Muyali Mary B. Meboka, and J. T. Tambong. Department of Plant and Soil Science, Alabama A&M University, Normal, AL., U.S.A., and ROTREP, USAID/GOC, Ekona Res. Centre, Buea, Cameroon.

A method has been developed for the first time for inducing motile zoospores of *Pythium myriotylum* (Pm) in the laboratory of the ROTREP at Ekona Research Centre. Flocculent mycelial mats of PM grown on lima bean sucrose medium for 5-7 days was carefully scraped from the agar surface into a sterile disposable petri dish with a sterilized transfer needle. The collected mycelia was then flooded with 30 ml of 0.001 M saccharose (sucrose) in sterile deionized water and adjusted to pH 7.0 with 1 N HCl. The flooded petri dish was carefully shaken in a rotary manner by hand to spread the mycelia mat and incubated at 31 C in continuous fluorescent light for 24 hours to encourage sporangia development. After the 24-hour period, motile zoospores were harvested by pouring the soaking saccharose buffer with the zoospores directly into a beaker without undergoing chilling. The mycelia mat was then reflooded with fresh saccharose buffer and motile zoospores were harvested after 3-4 hr. This process was repeated several times. The zoospores remained motile for more than 48 hr. The number of zoospores collected during the first harvest was 1 x 10^3/ml. Several soaking buffers were tried without success. The significance of inducing motile zoospores is to guard against contamination of the Pm cultures.

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Differentiation of Peronsporales and isolates of Peronospora tabacina by direct sequencing of an internal transcribed spacer (ITS2). M.D. Wiglesworth, C.L. Schardl, W.C. Nesmith, and M.R. Siegel. Department of Plant Pathology, University of Kentucky, Lexington, Ky. 40546.

Differentiation of Peronsporales and isolates of Peronospora tabacina by direct sequencing of an internal transcribed spacer (ITS2). M.D. Wiglesworth, C.L. Schardl, W.C. Nesmith, and M.R. Siegel. Department of Plant Pathology, University of Kentucky, Lexington, Ky. 40546. The source of epidemics of the tobacco blue mold fungus, *Peronospora tabacina* Adam, in North America remains unknown because traditional techniques for differentiation of isolates have been inadequate. Molecular techniques have been used for taxonomy, but rarely for epidemiological purposes. Combining two molecular methods, the amplication of target DNA, via the polymerase chain reaction (PCR) and DNA sequencing, may enable the detection of isolate differences and determine the nature of blue mold epidemics. The purpose of this work was to determine whether this technique could distinguish between different genera and species of plant pathogenic Peronosporales (Oomycetes) and differentate *P. tabacina* isolates. In this study, the region sequenced was an internal transcribed spacer (ITS2) of approximately 450-550 basepairs located between the 5.8S and 26S rRNA genes. Fungal species tested were *Pythium ultimum, Phytothphora parasitica* f.sp. *nicotiana* (race 0 and 1), *Phytophthora infestans, Peronospora trifolium, Peronspora tabacina* (four isolates), *Peronosclerospora sacchari* (two isolates), and *Peronosclerospora maydis*. The ITS2 sequences distinguished genera and species, but not isolates or races. The ITS2 of the *Peronosclerospora* isolates have an insert of 1.5-2.0 kb that limits the ability for complete sequencing, but this region can still be used in differentiation. Phylogenic analysis also suggested that the *Peronospora* isolates may have arisen for a common ancestor. The more variable rRNA intergenic spacer regions (IGS) are being analyzed to determine whether isolates of *P. tabacina* can be differentiated using this method of DNA sequencing.

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PREDICTION OF TERMINAL RICE SHEATH BLIGHT LEVELS AND YIELD LOSS BASED ON EARLY SEASON DISEASE INCIDENCE LEVELS. D. Groth and M. C. Rush,
Rice Research Station and Dept. of Plant Pathology and Crop Physiology, La. Agri. Exp. Stn., L.S.U. Agricultural Center, P.O.Box 1429, Crowley, LA 70527.

Five rice cultivars with a range of susceptibilities to *Rhizoctonia solani* were inoculated with 0 to 150 ml/plot of (5.7 m^2) inoculum at the mid-tillering growth stage. Inoculum was prepared by growing the pathogen on sterile rice grain:rice hull mixture (1:2). Weekly counts of infected tillers were made to determine disease incidence. Sheath blight severity ratings and rice yields were determined at maturity. Yields of susceptible varieties were reduced over 1100 kg/ha when tiller infection levels at the early joint elongation stages exceeded 5-10%. Yields of moderately susceptible and moderately resistant varieties remained stable until early infection levels exceeded 10 to 15% of tillers infected. Disease severity at maturity and reduced yields were highly correlated to early infection levels. Yield reductions of over 2000 kg/ha were observed in susceptible varieties with early infection levels higher than 10%.

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AN ISOLATE OF PHYTOPHTHORA CRYPTOGEA PATHOGENIC ON SOYBEAN SEEDLINGS. P. W. Reeser[1], H. H. Ho[2], and D. H. Scott[1]. [1]Purdue University, West Lafayette, IN 47907 and [2]State University of New York, New Paltz, NY 12561.

During 1990 fields of diseased soybeans in Indiana were surveyed for the presence of P. megasperma f. sp. glycinea (Pmg). Chips of discolored tissue were aseptically removed from the taproots of symptomatic plants and plated on PVP medium. Isolates were maintained on dilute V-8 agar slants. Pathogenicity was tested in 8 soybean cvs. routinely used for Pmg race identification by inoculating the hypocotyls of 8-10 day old seedlings with mycelium of 2-3 wk old oatmeal agar cultures. An isolate identified as P. cryptogea killed 50-100% of plants in each cv. and was re-isolated from diseased plants. The isolate is heterothallic with oospores 29 μm dia. and amphigynous antheridia. Sporangia are non-deciduous, non-papillate, and oval to obpyriform borne terminally on unbranched or often close sympodially branched sporangiophores.

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LEAF DISK ASSAY FOR VIRULENCE OF COLLETOTRICHUM COCCODES ON VELVETLEAF (ABUTILON). N. J. Alexander and T. D. Leathers. National Center for Agricultural Utilization Research, ARS, USDA, 1815 N. University St., Peoria, Illinois 61604.

Velvetleaf (Abutilon theophrasti) causes significant economic impact as a weed in field crops. We selected Colletotrichum coccodes as a potential biocontrol agent. To increase our ability to screen large numbers of fungal strains, we devised a quantitative method for measuring virulence on leaf disks. Leaves were sonicated in a dilute bleach solution, rinsed in sterile water and cut into disks using sterile technique. At least three disks from each leaf served as a control. The remaining disks were placed adaxial side up on sterile water agar plates. Fungal spores were inoculated at 100,000/disk. Total chlorophyll was obtained from methanol-extracted disks following Porra et al. (1989, BBA 975:384). Significant decreases in total chlorophyll from that of the control disks were used as an indication of virulence. The method is reproducible and suitable for selecting highly virulent strains.

790

APPLICATION OF SYSTEMS ANALYSIS TO DESIGNING PLANT AND ANIMAL RISK INFORMATION SYSTEMS. S. D. Cohen, L. W. Chang, J. A. Acree, Planning and Risk Analysis Systems, and M. H. Royer, Biological Assessment and Taxonomic Support, Animal and Plant Health Inspection Service, Hyattsville, Maryland 20782.

Systems analysis is a structured approach to examining the data needs, data relationships, and the users involved in plant and animal risk information systems. System analysis can be used to effectively construct two major models, the essential and the implementation model. Current work on the plant and animal risk information systems is concerned with further development of the essential models. Information needed for systems analysis was acquired by a combination of written questionnaires and/or oral interviews. Questionnaires and interviews specifically addressed the scope of the systems, the events which drive the systems, the data needs, data relationships and the mechanisms for user retrieval of information. We will show how the collected data from the questionnaires and/or interviews will be used to design effective essential models for the risk information systems.

791

AN IMPROVED METHOD TO PRODUCE CONIDIAL SUSPENSIONS OF PYRENOPHORA TRITICI-REPENTIS. C. K. Evans, and R. M. Hunger. Plant Pathology Department, Oklahoma State University, Stillwater, OK 74078-9947.

A technique was developed for the production of conidial suspensions of Pyrenophora tritici-repentis (PTR) nearly free from other infective units (OIU) such as conidiophores and aerial hyphae. Isolates of PTR were grown by shake culture in potato dextrose broth and the resulting mycelial mass was macerated and used to inoculate liquid clarified V-8 juice agar (V8) in petri plates. Conidiophores and conidia were produced over the entire agar surface by alternating photoperiods (24:24 light [3300 LUX], dark). Conidia were washed from the surface of the agar. The average counts of OIU/ml, conidia/ml, and conidia/mm^2 of agar surface area were 36, 1140, and 20, respectively. The advantages of this technique over those described previously include; 1) the production of conidial suspensions nearly free from OIU, and 2) production of large quantities of conidia on fewer petri plates.

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A TECHNIQUE FOR INOCULATING SOYBEANS IN THE GREENHOUSE WITH *FUSARIUM SOLANI*. S. M. Lim, USDA-ARS and Department of Plant Pathology, University of Illinois, Urbana, IL 61801.

An effective inoculation technique using soybean seedlings was developed to evaluate resistance in soybeans and pathogenic variability in isolates of *Fusarium solani*. Oat grains were washed and soaked in tap water for 2-3 hours. One-hundred ml of the soaked grains were placed in 250 ml Erlenmeyer flasks and autoclaved twice for 40 minutes each time at a 24 hour interval and inoculated after cooling by adding 5 ml of the conidial suspensions (10^7 conidia/ml). The cultures were incubated at 24 C in darkness for 2-3 weeks. Three to five colonized oat grains were placed around crown roots of plants at the V3 stage. The epidermal layer around the crown roots of plants grown in sterilized soil (5 plants/15-cm-d clay pot) was gently scraped prior to inoculation. Each inoculation site was then covered with soil. Within a week after inoculation, symptoms of sudden death syndrome developed on the inoculated susceptible plants.

793

A NEW TRIPLE-STAIN USEFUL IN THE DIFFERENTIATION OF FUNGAL PARASITES FROM HOST TISSUES. P. D. Dery and M. M. Kulik. USDA-ARS, Soybean and Alfalfa Research Laboratory, Beltsville Agricultural Research Center, Beltsville, MD 20705.

A new stain combination using thionin, orange G, and fast green FCF was developed for the the purpose of differentiating between fungal pathogens and plant host tissues. In studies using *Puccinia zoysia* on zoysiagrass and *Paederia spp.*, *Puccinia graminis ssp. graminicola* on tall fescue, and *Puccinia graminis ssp. tritici* on wheat, fungal spores stained dark blue, mycelium stained yellow, host tissue stained blue-green, and nuclei stained blue. This clearly delineated internal hyphal structures from host tissues, both inter- and intra-cellular, which was not possible using the double stains of safranin O/fast green FCF or thionin/orange G; and it is simpler and much less time-consuming than Johansen's quadruple stain.

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G. E. Weber & E. Jörg. ERRORS IN DISEASE ASSESSMENT - A SURVEY. G. E. Weber, Giessen University, Tropeninstitut, Schottstr.2, 6300 Giessen, GERMANY. E. Jörg, Landespflanzenschutzamt Rheinland-Pfalz, 6500 Mainz, GERMANY.

A series of standard diagrams of mildew infected leaves was assessed for severity by members of the plant protection service of Rheinland Pfalz. Regression analysis revealed four different types of estimators based on combinations of accuracy and precision. Accuracy was measured by the slope parameter and varied from fourfold overestimation to completely accurate assessment of severity with no observed cases of underassessment. Precision measured by the coefficient of determination varied from 0.7 to 0.95. To improve precision and accuracy and thereby homogeneity of disease assessment, training was undertaken using the program ESTIMATE. This program generates realistic images of diseased cereal leaves and offers randomized leaves, multiple diseases, several standard image sets and regression statistics. Results of the assessment training will be discussed.

795

A SIMPLE METHOD FOR PURIFICATION AND DETECTION OF CERCOSPORIN. R. K. Velicheti, I. S. Bhandal, and J. B. Sinclair, Dept. of Plant Pathology, Univ. of Illinois at Urbana-Champaign, 1102 S. Goodwin Ave., Urbana, IL 61801-4709.

Mycelial mats from 25-day-old potato-dextrose broth cultures of Cercospora kikuchii were extracted with diethyl ether, evaporated, redissolved in chloroform, and adsorbed to 90 g of activated silicic acid. The chloroform was then evaporated and the silicic acid dry packed into a column. A yellow fraction was eluted with chloroform, and the cercosporin with methanol. The method was efficient in partially purifying large quantities of cercosporin. Use of TLC did not show the presence of the yellow fraction nor isocercosporin. Cercosporin was further purified on HPLC using Partisil M9 ODS column (1 x 25 cm) and a methanol: water:phosphoric acid (350:150:1 v/v) solvent at a flow rate of 2 ml/min^{-1}, and monitoring at λ 470 nm. Cercosporin had a retention time of 13.3 min. Cercosporin was redissolved in methylene chloride, washed with water, and purity verified by absorption and mass spectrometry.

796

QUANTIFICATION OF INFECTIOUS UNITS (IFU) OF PYTHIUM SPP. IN WHEAT ROOTS. L.L. Singleton, C.C. Russell, and C.S. Anderson. Plant Path. Dept., Okla. State Univ., Stillwater, OK 74078.

A most probable number (MPN) assay method (Phytopathology 78:1616) has been described. **Calibration:** In infested soils (1250 or 2500 oospores of P. irregulare/g), IFU estimates ranged from 2.8-22.5/g with 0.11- 0.90% infectivity. **Preplant:** Field soil populations of Pythium at Cherokee (**Chk**), Lahoma (**Lah**), and Marshall (**Msh**) were assayed. Mean IFU/g for 2 putative seed treatments (none and metalaxyl), were not significantly different: **Chk**, 4.3 and 3.4; **Lah**, 2.6 and 1.1; and **Msh**, 2.4 and 2.7; with C.V.'s of 44, 55, and 33%, respectively. **Fresh vs. Dry:** Post-plant root samples from two treatments at **Chk**, were washed and divided in half. One was assayed fresh and the other dried and assayed. MPN IFU/g determinations were significantly (0.05) higher for fresh root samples. **Metalaxyl activity:** Metalaxyl, based on fresh roots, significantly ($P \geq 0.07$) reduced Pythium spp. root infection in the field (7.7 vs. 10.8/g for none).

797

EVALUATION OF BIOASSAY TECHNIQUES TO DETERMINE *APHANOMYCES* PEA ROOT ROT POTENTIAL IN SOIL. D. K. Malvick, J. A. Percich, and F. L. Pfleger, Department of Plant Pathology, University of Minnesota, St. Paul, 55108.

Three published methods, a rolled towel assay (RTA), most probable number assay (MPN), and greenhouse pot assay (GPA) were compared for estimation of *Aphanomyces euteiches* inoculum potential (IP). The RTA and MPN assays were tested with sandy loam soil infested artificially with a series of oospore concentrations from 100 to 600 per gram. Both methods gave similar linear relationships ($r=0.97$) between actual oospore numbers in soil and estimated IP. All three bioassays were compared with clay loam soil samples from a field infested with *A. euteiches*. The GPA produced the most consistent results. Estimates of IP from the three bioassays were not correlated. These bioassays can be useful; however, more precise methods are required for detailed studies on the IP and ecology of *A. eutiches*.

798

IDENTIFICATION OF REPEATED SEQUENCES IN *HELMINTHOSPORIUM SOLANI* WITH A MULTI-WELL DOT BLOT PROCEDURE. S. M. Brown, C. L. Merida, and R. Loria. Department of Plant Pathology, Cornell University, Ithaca, NY 14853.

Our objective was to identify repeated sequences for use as probes for *Helminthosporium solani*, the cause of silver scurf on potato. A genomic library from *H. solani* was prepared in pUC18. Bacterial colonies were inoculated individually into 200 µl of LB broth in wells of a sterile 96-well cell culture plate. After growth to stationary phase, 100 µl aliquots of bacterial suspensions were transferred to a nylon membrane via a dot-blot manifold. Cell culture plates were stored at -80C after adding glycerol to each well to a final concentration of 25%. Filters were hybridized at moderate stringency with ^{32}P-labelled random fragments of *H. solani* DNA. Strongly hybridizing clones were recovered from the culture plates. Plasmid DNA was used to probe Southern blots of restriction-digested total DNA of *H. solani*. These repeated sequences are now being used as probes for RFLP analysis of *H. solani*.

799

PHYTOPHTHORA MEGASPERMA F.SP. *MEDICAGINIS* SEXUAL LIFE CYCLE SPAN AND OOSPORE GERMINATION *IN SITU*. J. Jiang and D. C. Erwin, Dept. of Plant Pathology, University of California, Riverside, CA 92521.

Oospores of *Phytophthora megasperma* f.sp. *medicaginis* produced in carrot broth were used to inoculate alfalfa seedlings. The time from inoculation to new oospore formation and germination in plant tissues (including oospore germination, infection, new oospore formation, and new oospore germination in the plant) was 10 days. When the seedlings were incubated in water, oospores formed in roots, hypocotyls, cotyledons, and true leaves. Oospores germinated *in situ*. Oospores germinated well in both susceptible (Moapa 69) and resistant (A77-10B) varieties, but the germination rate was slightly lower in the resistant than in the susceptible variety. After oospores had formed in Moapa 69 seedlings for 10 days, germination (after 1 week) was 85% in true leaves and 96% in cotyledons and roots. When the same age oospores were isolated from roots and cotyledons, only 10% germinated in water.

800

PATHOGENIC AND VIRULENCE VARIABILITY IN Pyricularia grisea IN A "HOT SPOT" SITE FOR RICE BLAST RESISTANCE BREEDING. F. J. Correa Victoria, and R. S. Zeigler. Centro Internacional de Agricultura Tropical, CIAT, A.A. 6713, Cali, Colombia.

The pathogenic diversity of Pyricularia grisea was studied to evaluate a "hot spot" used to breed for blast resistance. Virulence factors were present for all known resistance genes and donor cultivars tested. Virulence frequencies ranged from 0.0 to 0.86, and no cultivar was susceptible to all strains. Accumulation of virulence factors to most known resistance genes was observed in single strains. Certain virulence combinations were not found, suggesting that predictable combinations of resistance genes may be useful. Virulence to the newly released cultivars Oryzica Llanos 5 and Oryzica Llanos 4 developed at this station was not present in the pathogen population, indicating that breeding at this site will yield highly resistant material.

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CHARACTERIZATION OF PROGENY FROM SEXUAL CROSSES BETWEEN STRAINS OF COLLETOTRICHUM GLOEOSPORIOIDES WITH DIFFERENT HOST SPECIFICITIES. C.R. Cisar, D.O. TeBeest, and F.W. Spiegel*, Department of Plant Pathology and *Dept. of Biological Sciences, University of Arkansas, Fayetteville, AR 72701.

Two strains of Colletotrichum gloeosporioides (Clar-5A and B-21) which are virulent on northern jointvetch (Aeschynomene virginica) were crossed with another strain of C. gloeosporioides (CRP-2) originally isolated from pecan (Carya illinoensis). Progeny from these crosses were compared to their parents with respect to culture phenotype, mating ability, rDNA RFLP patterns, pathogenicity on northern jointvetch and on apples, and for benlate resistance (B-21 x CRP-2 only). The F1 progeny exhibit characteristics of each parent as well as some unique characteristics. These results provide evidence for sexual recombination between strains of C. gloeosporioides with very different host specificities and this may be a mechanism for increasing diversity within this species.

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ANALYSIS OF FATTY ACIDS AS AN AID TO DETERMINE SPECIATION IN CERCOSPORA. R. D. Berger, T. A. Davoli, and N. C. Hodge, Plant Pathology Department, Univ. of Florida, Gainesville, FL 32611.

Isolates of >50 Cercospora spp. from 40 species of plants were grown in trypticase soy broth and were then lyophilized. Fatty acids of isolates were converted to their methyl ester derivatives (FAMEs) for gas chromatography and subsequent statistical analysis by the Microbial Identification System. Some species with diverse conidial morphology were more closely related by FAME profiles than were other species that had similar conidial characters. However, C. cornicola and C. liquidambaris were almost identical in their FAME profiles, sporulation habit on leaves, lesion type, and cultural and morphological characteristics. Likewise, the FAME profiles of an isolate (C. arachidicola?) from perennial peanut and C. eucalypti were similar as were the profiles of species from cotton and water hyacinth. Fatty acid analyses can be used to determine the degree of relatedness between species and to identify species that merit further examination for cross-pathogenicity.

803

A System for Modelling Fungus-Fungus Interactions and Fungus-Root Interactions. C.M. Liddell and D.N. Hansen. Department of Entomology, Plant Pathology and Weed Science, New Mexico State University, Las Cruces, NM 88003.

The number of contact points, successful infections or anastomoses and other interaction parameters depend to a large degree on the physical structures of the interacting organism. Models have been developed using techniques derived from fractal geometry, that come closer to capturing dynamic, structural complexities than previous efforts. The dynamics of mycelial branching and interactions of several fungi in culture were studied under different conditions to develop the models. Employing iterative application of a stochastic rule set to model tip directed growth, these models allow a greater understanding of the dynamics of fungal growth and interactions and host-pathogen interactions. Models were evaluated using in vitro interactions between different anastamosis groups of Rhizoctonia.

804

PHYLOGENETIC RELATIONSHIPS AMONG **CERCOSPORA** AND ALLIED GENERA ON BANANA BASED ON RDNA SEQUENCE COMPARISONS. Z. Liu[1], E.L. Stewart[1], and L.J. Szabo[1,2], 1) Department of Plant Pathology, 2) USDA-ARS Cereal Rust Laboratory, University of Minnesota, St. Paul, Minnesota 55108.

To investigate the phylogenetic relationships of **Cercospora** and allied genera on banana, nucleotide sequences from the ITS and 5′ end of the large subunit (F63/635) of rDNA were obtained using polymerase chain reaction amplified DNA from **Cercospora hayi, Paracercospora fijiensis, P. fijiensis** var. **difformis, Pseudocercospora musae** and reference species **Cercospora apii** and **Mycocentrospora acerina**. The ITS regions varied more than the F63/635 among the species examined. In the ITS, the flanking spacer segments varied widely, while the 5.8s rDNA was highly conserved. The 5′ end of the F63/635 region varied moderately. The results indicate that sequence data will be useful for phylogenetic studies in **Cercospora** and its allied genera.

805

MOLECULAR SYSTEMATICS OF *TRICHODERMA* SPECIES BY RESTRICTION ANALYSIS OF PCR-AMPLIFIED RIBOSOMAL DNA FRAGMENTS. R. J. Meyer. USDA, ARS, Nematology Laboratory, B-011A, BARC-West, Beltsville, MD 20705.

A portion of the nuclear ribosomal DNA (rDNA) was examined for characters that would be suitable for species identification in *Trichoderma*. A fragment that extends from the middle of the 5.8S rDNA to the middle of the 25S rDNA was amplified by the polymerase chain reaction and digested with twelve restriction endonucleases. Phenetic cluster analysis (UPGMA) was used to evaluate the restriction patterns. This method was used to examine fifty strains that included isolates of *T. longibrachiatum, T. reesei, T.* (*Gliocladium*) *virens, T. viride* Group I, and *T. viride* Group II. Also included were cultures derived from the sexual states of several *Trichoderma* species. The UPGMA groupings do correspond to some extent with the previously defined morphological groups. However, the molecular data indicated that some of the previously proposed relationships among the *Trichoderma/Hypocrea* species based on morphological data were incorrect. These results will provide a useful framework for further studies on species concepts in *Trichoderma*.

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RELATIONSHIP BETWEEN MICROFLORA AND ODOR IN COMMERCIAL GRAIN SAMPLES. D. B. Sauer and L. M. Seitz, USDA-ARS, U.S. Grain Marketing Research Laboratory, Manhattan, KS 66502.

In a study to identify compounds associated with "off" odors in grain, we examined about 600 commercial samples of wheat, corn, soybeans and grain sorghum. Many off odors result from microbiological spoilage. Samples were evaluated for odor type and intensity by a panel of four persons in our laboratory and by the Federal Grain Inspection Service. Surface-disinfected kernels from each sample were plated on malt salt agar to determine percent of kernels with various fungi. For the four grain types, samples classified as musty had an average of 4 times more kernels with storage fungi, primarily Aspergillus glaucus, than samples classified as "OK". Field fungi such as Alternaria and Fusarium were of highest incidence in OK samples. There was little relationship between fungi and odors classified as sour, insect, and COFO (commercially objectionable foreign odor).

807

CHARACTERIZATION OF ASPERGILLUS PHENOTYPES VARYING IN REGULATION OF AFLATOXIN PRODUCTION. E.H. Gendloff[1], F.S. Chu[2], A.B. Bleecker[1], K. Morrison[1], and T.J. Leonard[1]. [1]Botany Department and [2]Food Research Institute, University of Wisconsin, Madison.

We have previously described three phenotypes varying in regulation of aflatoxin biosynthesis. The classical reg phenotype confers aflatoxin (afl) production only after the log phase of growth on media containing a carbohydrate carbon source, whereas con confers afl production very early in the growth cycle on media with or without carbohydrate, and rep confers lack of afl production on several carbohydrate-contaning media, with the ability to exhibit the reg phenotype after mutagenesis. We have characterized these phenotypes further. The two A. flavus con strains studied made all four common forms of afl, B_1, B_2, G_1, and G_2, but the reg A. flavus strains tested did not. A reg A. parasiticus did, however. Other media have also been found which do not allow the reg, but do allow the con, strains to produce, including a sporulation medium which contains carbohydrate. A commonly used coconut extract medium allowed expression of afl in a rep strain. This medium separated into an upper and lower phase. Growth on the lower phase allowed sporulation and high afl production retaining the rep or con phenotypes, whereas the upper phase supported heavy mycelial growth and little afl production. The influence of ethylene on expression of these phenotypes was also investigated. Contrary to other reports, ethylene was not made by tested con or reg strains at any time through induction of afl production. Added ethylene had no effect on afl expression in these strains.

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UNIQUE DNAs DISTINGUISH ASPERGILLUS FLAVUS AND A. PARASITICUS STRAINS. N. P. Keller, G. A. Payne*, T. E. Cleveland and D. Bhatnagar, USDA/ARS, Southern Regional Research Center, P.O. Box 19687, New Orleans, LA 70179; *Department of Plant Pathology, North Carolina State University, Raleigh, NC 27695.

Aspergillus flavus and A. parasiticus are economically important fungi because of their ability to contaminate food and feed with aflatoxin. Means to distinguish between these and related species, especially on the basis of toxin production, are desired. Genomic clones of A. flavus strain NRRL 3357 were used as probes to map chromosomes of A. flavus and A. parasiticus and to distinguish between these species by restriction fragment length polymorphisms. There was no homology to these probes in A. nidulans and A. niger, species that do not produce aflatoxin. The ability of these clones to differentiate species of the 'Aspergillus flavus group' and serve as an index for aflatoxin producing potential in Aspergillus will be discussed.

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PATHOGENICITY AND POLYGALACTURONASE PRODUCTION BY ALTERNARIA ALTERNATA ISOLATES CAUSING SOFT AND FIRM ROTS ON BLUEBERRY. R.L. Brown, R.A. Cappellini, and J.L. Peterson, Department of Plant Pathology, Rutgers University, New Brunswick, NJ 08903.

Alternaria alternata isolates from soft rots on the cheek and firm rots on the stem scar of New Jersey blueberry (Vaccinium corymbosum) fruit were tested for postharvest pathogenicity and in vitro pectinase production. Cheek isolates caused more severe rots than stem scar isolates when blueberries were inoculated at either the cheek or stem scar. Also, rots initiated at the stem scar were more severe on average than those initiated on the cheek. No major differences in pectinase production between the two isolate-groups were observed. Results suggest that both differences in virulence among isolates and anatomical or physiological differences between loci may be responsible for the originally observed variation in symptoms at the two loci.

810

ASPERGILLUS FLAVUS GROUP AND AFLATOXIN IN PEANUTS WHILE IN INVERTED WINDROWS. D. M. Wilson, J. M. Troeger, B. D. Evans, and R. W. Beaver, Department of Plant Pathology, University of Georgia, and USDA/ARS, Coastal Plain Station, Tifton, GA 31793.

Peanuts were grown under irrigation using normal production practices in 1987, 1988, 1989, and 1990. There were 14 digging dates in 1987, 6 digging dates in 1988, 8 digging dates in 1989, and 9 digging dates in 1990. Peanuts were dug each year beginning in early September through late October. Pod and kernel microflora and aflatoxin were monitored at digging and when the peanuts had field dried. The range and average of the A. flavus group found in the kernels were as follows: 1987 - range 0-1.5%, mean < 0.5%; 1988 - range 0-5%, mean < 1%; 1989 - range 0-5%, mean < 0.5%; and 1990 - range 0-58%, mean > 5%. No increases in A. flavus colonization or aflatoxin content were seen in the windrow in any year regardless of weather conditions. There seems to be little risk for additional aflatoxin contamination in the inverted windrow because the temperatures of peanuts wetted by rain in the windrow are probably not favorable for A. flavus development.

811

CHARACTERIZATION OF A cDNA CLONE INDUCED DURING AFLATOXIN FORMATION. Woloshuk, C.P., G.J. Nystrom, K. Verma and G.A. Payne. Dept of Plant Pathology, North Carolina State University, Raleigh, NC 27695-7616. Aflatoxin production was induced in *Aspergillus flavus* by transfer from a carbohydrate-free to a carbohydrate-rich medium. Aflatoxin appeared in the culture 12 hr after transfer, peaked at 32 hr and then declined. A subtractive hybridization technique was used to clone cDNAs specifically induced during aflatoxin production. A 1.3 kb cDNA clone (CW3) was subsequently isolated and used as a probe to follow the accumulation of its transcript during aflatoxin biosynthesis. On Northern blots, a 1.3 kb band appeared 8 hr after transfer of *A. flavus* to the aflatoxin-inducing medium and remained at a constant level for 40 hr. No transcript was detected in cultures at any time from medium that failed to induce aflatoxin biosynthesis. These data, as well as the sequence of CW3 and a corresponding genomic clone, will be reported.

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SOIL DEPENDENT ANTIBIOSIS AMONG Ggt ANTAGONISTS DETECTED BY AGAR PLATE + STERILE SOIL TECHNIQUE. O. Andrade, D. E. Mathre, and D. C. Sands. Dept. of Plant Pathology, Montana State University, Bozeman, MT 59717.

Expression of antibiosis by bacterial isolates among themselves and against *Gaeumannomyces graminis* var *tritici* (Ggt) was soil dependent when tested in vitro. Soils, collected from wheat producing areas of Montana and southern Chile and characterized by different levels of suppressiveness to take-all disease, were sterilized and placed in wells made in 1/4 strength potato-dextrose agar (PDA). Dilutions of single bacterial isolates obtained from these soils were placed into the soil-containing wells. The bacteria were challenged by all other bacterial isolates to detect hyperantagonists within this group of microorganisms. The same technique was used to detect potential biocontrol agents of Ggt. Enhancement or suppression of the antibiosis, either among bacterial isolates or against Ggt, was consistently soil dependent. These results indicate that abiotic soil factors are involved in the expression of the antagonism. This technique appears to be very useful as a rapid test for detecting antibiosis reactions involving soil microorganisms.

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SUPPRESSION OF *RHIZOCTONIA SOLANI* IN SOIL WITH ANIMAL MANURES. C. A. Martinson and S. M. Al-Rehiayani, Department of Plant Pathology, Iowa State University, Ames, IA 50011

Liquid manures (excrements from hogs and chickens collected in water in enclosed facilities) were added at 0, 18, and 36 MT fresh solids/Ha to two soils in the laboratory that were infested with sclerotia of *Rhizoctonia solani* AG-4 at 1 g/Kg. Activity of *R. solani* was assayed by invasion of baits of sterile red beet seed, conduciveness indices (Henis et al. 1978. Phytopathology 68:900-907) with radish as the suscept, and germinative ability of added sclerotia. Suppressiveness to *R. solani* was detected 10 to 20 days after blending chicken and hog manures into soils with hog manure being the more effective. The pathogen was inactive in manured soils within 30 days and would not invade baits placed in the soil or attack radish seedlings. Suppressiveness to *R. solani* was detected in the soil for at least three months. Germination of *R. solani* sclerotia added to soils was not affected, yet the disease potential of sclerotia in manure amended soils was much less than in unamended soils. Animal manures may be considered a resource for strategic control of soil borne plant pathogens.

814

EFFECT OF POSITION OF INOCULUM OF *GAEUMANNOMYCES GRAMINIS* VAR. *TRITICI* RELATIVE TO THE SEED ON YIELD OF WINTER WHEAT. W. W. Bockus, Department of Plant Pathology, Kansas State University, Manhattan, KS 66506-5502.

Oat grains, colonized by *G. g. tritici*, were introduced (0.5 g/m) into the soil in rows in the field at various distances from the seed furrow. Treatments included a noninoculated check, inoculum placed at seed level, 3.8 cm above the seed, 7.6 cm below, 15.2 cm below, 7.6 cm to the side, and 15.2 cm to the side. Planting and inoculation occurred on 2 October 1989, percentage whiteheads rated at soft and medium dough, and grain yields determined 21 June 1990. Grain yields (kg/ha), followed by mean separation letters ($P=0.05$), were: check = 3820 a; seed level = 1561 d; 3.8 cm above = 2923 bc; 7.6 cm below = 2541 c; 15.2 cm below = 3108 bc; 7.6 cm to the side = 3504 ab; and 15.2 cm to the side = 4048 a. Highest levels of whiteheads and lowest yields occurred when inoculum was placed with the seed. Significant reductions in yield also occurred if inoculum was placed above or below the seed; however, inoculum $\geq$7.6 cm to the side of the seed did not significantly reduce grain yields.

815

INOCULUM DENSITIES OF *POLYMYXA BETAE* AND BEET NECROTIC YELLOW VEIN VIRUS IN CALIFORNIA SUGARBEET FIELD SOILS. J. S. GERIK. USDA, ARS, 1636 E. Alisal St., Salinas, CA 93905.

Soils from five sugarbeet fields in the San Joaquin Valley of California with a history of rhizomania were assayed for number of infecting units (IUs) of *Polymyxa betae* and beet necrotic yellow vein virus (BNYVV) using a most probable number technique. The soils were diluted with a portion of the same soil sample which had been autoclaved. The diluted soils were indexed with bait plants which were assayed visually for *P. betae* and tested with ELISA for infection by BNYVV. The inoculum densities of the soils ranged from 1.2 to 5 IUs/g soil for BNYVV. For *P. betae* the range was from 3.7 to 61 IUs/g soil. Greenhouse experiments were conducted to determine the effects of inoculum density on initiation of infection and disease severity. Sugarbeet seed was planted in sterile soil which had been infested with the pathogens and then serially diluted with more sterile soil. The plants were grown at 25 C for 8 weeks then indexed for infection by each of the pathogens. Infection by both occurred at inoculum concentrations as low as 2 IUs/kg soil. BNYVV inoculum concentrations of 130 IUs/kg soil and greater resulted in significant reduction in plant weight. The data indicate that significant damage can occur at inoculum densities much lower than field levels.

816

EFFECT OF PESTICIDES ON ROOT DISEASES OF ANTHURIUM. L. Y. Guo and W. H. Ko, Department of Plant Pathology, University of Hawaii, Hilo, Hawaii 96720.

Potted anthurium plants were sprayed twice with different pesticides at 1-week intervals. One week after the second spray, plants were removed, washed free of planting medium, inoculated with test fungi and incubated in a moist chamber. Results show that although the binucleate Rhizoctonia sp. was not normally pathogenic to anthurium roots, it was able to cause severe root rot if plants were sprayed with the herbicide diuron before inoculation. Diuron treatment also increased the severity of root rot caused by Pythium vexans, but did not significantly affect root rot caused by Pythium splendens or Calonectria crotalariae. Application of nematicide phenamiphos (Nemacur) to anthurium decreased the susceptibility of roots to P. splendens, but did not affect their susceptibility to P. vexans, C. crotalariae or Rhizoctonia sp. Spraying anthurium plants with miticide fenbutatin oxide before inoculation also decreased susceptibility of roots to C. crotalariae but not to the other three species of fungi tested.

817

FUSARIUM SPECIES ASSOCIATED WITH ROOT LESIONS ON WHITE PINE SEEDLINGS IN A WISCONSIN NURSERY. C.M. Ocamb, J. Juzwik, and K.J. Stolhammer, USDA Forest Service, North Central Forest Experiment Station, 1992 Folwell Avenue, St. Paul, MN 55108.

Fusarium species isolated from lesions on primary and secondary roots of rising 1-year-old white pine seedlings (Pinus strobus) in one field in Wilson State Forest Nursery, Boscobel, WI, between July and October 1990 were identified as F. oxysporum, F. proliferatum, F. solani, F. sporotrichioides, and an unconfirmed species. The latter species produces microconidia in short chains (2-3 spores) as well as false heads; abundant chlamydospores singly, in chains, or clusters; and macroconidia. The isolates of this unknown species closely resemble F. nygamai; if confirmed, this is the first report of this species in North America. Pathogenicity tests will be conducted for all species.

818

INFLUENCE OF SOIL TEMPERATURE AND WATER POTENTIAL ON SUPPRESSION OF SOIL POPULATIONS OF THIELAVIOPSIS BASICOLA BY HAIRY VETCH AMENDMENTS. S.R. Kendig and C.S. Rothrock. Dept. of Plant Pathology, University of Arkansas, Fayetteville, AR. 72701.

The suppression of soil populations of T. basicola by hairy vetch amendment as influenced by soil temperature and water potential were investigated in controlled environments. Nonamended soils and soils amended with 0.25% hairy vetch were maintained at either 20 or 24 C at soil water potentials of -0.1, -0.2, -0.3 and -0.5 bars. Soils were assayed at 3, 7, 14 and 28 days. The soils were then planted to cotton and reassayed after 49 days. Percent infection and plant shoot and root weights were also determined. Temperature did not influence suppression, the ratio of the population in amended to nonamended soils. Suppression of T. basicola in the amended soil was significant at -0.2, -0.3, and -0.5 bars ($P>0.05$), but varied with days after incorporation (DAI). Greatest suppression was observed in soils maintained at -0.3 bars at 7 to 14 DAI. Soil populations and number of infections/g of root tissue were lower and plant shoot and root weights were higher in soils receiving hairy vetch amendments. These data suggest that incorporation of hairy vetch suppresses populations of T. basicola and decreases black root rot of cotton.

819

A METHOD FOR STUDYING SURVIVAL OF FUNGI IN SOIL. M. Q. Yu and W. H. Ko. Department of Plant Pathology, University of Hawaii, Hilo, Hawaii 96720.

Three small soil clumps about 2.5 mg each were placed on the smooth surface of a soil block (40 X 25 X 4 mm) on a glass slide. A predetermined number of spores in 0.2 ul water was added to each soil clump with a Pipetman microliter pipet. The slides were then placed in a moist chamber and incubated at 24 C. To determine the number of spores that remained viable in soil after a certain period, the soil clump was transferred to a drop of rose bengal solution on a glass slide. After mixing, the smear was further mixed with a drop of destaining solution (5.5 M NaOH plus 0.5 M NaCl) to reduce the background color and separate spores from soil particles. For Bipolaris stenospila and B. maydis, more than 90% of conidia added to soil were observed and counted by this method. The recovery rates of chlamydospores of Phytophthora cinnamomi and sporangia of Pythium splendens were 86% and 95%, respectively. The method is useful for studying factors affecting population change of fungi and observing behavior of fungal propagules in soil.

820

Characterization of isolates of Waitea circinata collected from Alaskan agricultural soils. R.H. Leiner and D.E. Carling. University of Alaska, 533 E. Fireweed, Palmer, AK 99645.

A Rhizoctonia similar to R. oryzae and R. zeae occurs commonly in soils in southcentral Alaska. On potato dextrose agar, isolates produced white to pale orange mycelium. Sclerotia, formed in the agar, were irregular in shape (similar to R. oryzae), and were dark orange to brown in color when mature (similar to R. zeae). Hyphal diameter averaged 6.1 um and nuclei/cell averaged 5.4. Growth rate increased from 2.6 mm/da at 11C to 25.5 mm/da at 30C. Most isolates were pathogenic on barley seedlings, although pathogenicity ranged from highly virulent to avirulent. A growth chamber study revealed virulence of isolates generally did not change with temperature. Although not observed in the field, approximately 10% of tested isolates produced teleomorphs, identified as Waitea circinata, on V-8 juice agar or water agar. Although this fungus is not identical to R. oryzae, at this time we believe differences are not extensive enough to warrant a different species name.

821

INFLUENCE OF TEMPERATURE ON THE DEVELOPMENT OF *Pythium splendens*-INDUCED ROOT ROT OF CARAMBOLA (*Averrhoa carambola*). R.C. Ploetz. University of Florida, IFAS, TREC,18905 SW 280th Street, Homestead 33031.

The carambola (*Averrhoa carambola*) has recently become an important tropical fruit in South Florida. A decline syndrome which affects production in the area begins or is worsened by the onset of winter temperatures and is characterized by necrotic root systems and total to partial defoliation of trees. Since *Pythium splendens* was consistently recovered from affected plants during preliminary work, a series of experiments was initiated to investigate the biology and pathology of the fungus on carambola, and the role it and temperature play in the syndrome described above. Growth of carambola (root, shoot, and total dry weight) and *P. splendens* (*in vitro* radial growth) were both positively correlated with temperature and were reduced 2- to 4-fold at 15C, as opposed to 30C. However, *P. splendens* caused significant reductions in host root, shoot, and total dry weight only at $\leq$25C, and these reductions were most consistent at 15 and 20C, temperatures at which colonization of carambola roots by *P. splendens* was greatest. Apparently *P. splendens* is able to cope with cooler temperatures to benefit from reduced host function under these conditions. Studies on the role *P. splendens* plays in the decline syndrome continue.

822

EFFECT OF SOIL TYPE AND ROOTSTOCK ON PHYTOPHTHORA ROOT ROT OF AVOCADO. D. Ferrin, F. Guillemet, S. Campbell, and J. Menge, Dept. of Plant Pathology, Univ. of California, Riverside 92521.

In greenhouse studies, total and infected root lengths of three avocado rootstocks (Borchard, Barr Duke and Thomas) were assessed 12 and 24 wk after planting into three field soils infested with *Phytophthora cinnamomi* or not. In noninfested soils, differences in root lengths due to soil type occurred only at 24 wk. Differences in root lengths due to rootstock occurred at both times. Total root length of Borchard was greater than that of Thomas at both times but was greater than that of Barr Duke only at 24 wk. There was no interaction between soil type and rootstock at either time. In infested soils, differences in root lengths due to soil type and rootstock occurred at 12 wk, but occurred only due to rootstock at 24 wk. Total root length of Thomas was greatest at 12 wk whereas that of Barr Duke was greatest at 24 wk. Differences in infected root lengths due to soil type, but not rootstock, occurred at both times, and no interactions occurred between them at either time.

823

SURVEY OF SOILS SUPPRESSIVE TO THREE SPECIES OF PHYTOPHTHORA. P. J. Ann, Chia-yi Agricultural Experiment station, Chia-yi, Taiwan, ROC.

A total of 466 soil samples collected from various locations throughout the island of Taiwan was tested for ability to suppress sporangial germination of three species of Phytophthora. Sporangia were preincubated in 50% V-8 juice for 1 hr to ensure uniform direct germination and to overcome the general soil fungistasis. The numbers of soil samples supporting germination of sporangia of P. parasitica, P. palmivora and P. capsici at 20% or less were 77, 86 and 46, respectively, and at more than 80% were 110, 66 and 196, respectively. The three species of Phytophthora showed similar pattern of sporangial germination in the same soils. Soils were either suppressive or conducive to all these three fungi. Only eight of the soil samples tested supported low germination rate of one species and high germination rate of the others. The degree of pathogen suppression was not correlated with soil texture, soil color or vegetation. Soils with pH lower than 4.5 were more suppressive than those with higher pH, although a number of soils with pH lower than 4.5 were conducive.

824

COMPLEMENTATION BETWEEN PEANUT CHLOROTIC STREAK VIRUS AND FIGWORT MOSAIC VIRUS (CAULIMOVIRUSES) IN MIXED INFECTION. D. A. Ducasse and R. J. Shepherd. Dept of Plant Pathology, univ. of Kentucky, Lexington, KY 40546.

Peanut chlorotic streak virus (PClSV), a caulimovirus, was found to be temperature dependant in its ability to systemically infect three hosts, *Datura stramonium, D. innoxia* and *Nicotiana tabacum*. Experiments were conducted in growth chambers set at 25° and 33°. Seedlings were mechanically inoculated and the presence of progeny virus in inoculated and tip leaves was evaluated by ELISA 15 and 25 days after inoculation. At 25° PClSV was able to systemically infect only *D. stramonium*. PClSV failed to establish a systemic infection on *D. innoxia* and *N. tabacum* at 25° although the inoculated leaves supported viral replication at this temperature. At 33° PClSV systemically infected all three hosts. When *D. innoxia* seedlings kept at 25° were inoculated with both PClSV and figwort mosaic virus (FMV), another caulimovirus that systemically infects *D. innoxia*, a double systemic infection occured as indicated by finding PClSV in the top leaves of these plants. This indicates that FMV is assisting PClSV to develop a systemic infection. Since PClSV by itself replicates locally in *D. innoxia*, FMV appears to complement PClSV for systemic movement.

825

PARTIAL CHARACTERIZATION OF OAT BLUE DWARF VIRUS. M.C. Edwards and Y. Zheng. USDA-ARS Cereal Crops Research Unit, Northern Crop Science Laboratory, Fargo, ND 58105-5677.

Oat blue dwarf virus (OBDV) has been designated as a member of the relatively new Marafivirus group, but has not been extensively characterized. To facilitate further characterization, we modified a purification procedure described for barley yellow dwarf virus (D'Arcy, et al., Phytopathology 73:755-759). Initial purification steps involved enzymatic digestion of an extract from infected oat plants, clarification with chloroform-butanol, and concentration by PEG precipitation. Further purification was through both differential and sucrose gradient centrifugation. Purified virions were disrupted and electrophoresed on discontinuous SDS gels to determine the coat protein molecular weight. Three major bands were observed of approximately 26.7, 25.8, and 22.6 kd. Molecular weight of virion RNA was estimated to be 2.35×10^6 after denaturing electrophoresis. Virion RNA was further characterized by in vitro translation.

826

A NEW PROCEDURE FOR THE PURIFICATION OF BARLEY YELLOW STREAK MOSAIC VIRUS. J. S. Skaf and T. W. Carroll. Department of Plant Pathology, Montana State University, Bozeman, MT 59717.

In 1982 barley yellow streak mosaic virus (BaYSMV) was first reported to cause a disease of barley and wheat in Montana. The virus is vectored from barley to barley by the brown wheat mite *Petrobia latens* although it can be transmitted from barley of *Nicotiana benthamiana* to *N. benthamiana* via mechanical inoculation. A previous purification procedure was not satisfactory mainly due to the loss of the virus and/or contamination with host material. In this report, we describe a new purification procedure which involves the use of ultrafiltration and centrifugation in Percoll gradients. The new procedure yields highly purified virions which is the first step toward determining the biological, serological, physicochemical, and molecular characteristics of the virus.

827

CHARACTERIZATION OF A FLORIDA BEAN GOLDEN MOSAIC VIRUS (BGMV-F) ISOLATE. Hiebert, E., Wisler, G.C., Purcifull, D.E., Sanchez, G, and Morales, F.J.*. Department of Plant Pathology, University of Florida. Gainesville, FL 32611 and.CIAT, Cali, Colombia*.

A geminivirus found in *Macroptilium lathyroides* from South Florida was purified from mechanically inoculated *Phaseolus vulgaris* "Top Crop". Polyclonal antiserum prepared to the purified virions cross-reacted without spurs in immunodiffusion tests with BGMV previously described by R. M. Goodman (J. of Gen. Virol. 54: 9-21). Four of 5 monoclonal antibodies prepared to BGMV-F reacted efficiently in ELISA (antibody-trapped

indirect) with 7 BGMV isolates at CIAT. One monoclonal showed a differential reaction with various BGMV isolates, indicating that it is possible to serologically differentiate BGMV isolates. Replicative form DNA was isolated from BGMV-F infected tissue and cloned into a pGEMEX vector after restriction digestion with SacI. Sequence analysis mapped the SacI site in the common region of the two DNA genomic components and indicated a high degree of sequence similarity, but not identity, with BGMV.

828

BIOLOGICAL AND MOLECULAR CHARACTERIZATION OF FOUR BROAD BEAN MOTTLE VIRUS STRAINS. J. Romero, Q. Huang and J. J. Bujarski. Plant Molecular Biology Center, Northern Illinois University, DeKalb, IL, 60115.

Four strains of broad bean mottle virus (BBMV) isolated from northern Africa and Asia have been biologically characterized by their symptom response in both legume and non legume plants. Virus replication was proved by a Dot blot hybridization using a radioactive RNA probe complementary to the conserved 3'end of BBMV RNA. Some strains induced different symptoms when inoculated on the host plants. Virus RNA was purified and analyzed by agarose gel electrophoresis followed by northern blots. Gel patterns revealed size differences among the four analyzed strains. We are in the process of cloning and sequencing of the most different genome segments. This will allow us to correlate the RNA changes with the specific host-virus reactions.

829

THE p88 CAPSID PROTEIN OF THE HELMINTHOSPORIUM VICTORIAE 190S VIRUS IS PHOSPHORYLATED. W. M. Havens and S. A. Ghabrial. Department of Plant Pathology, University of Kentucky, Lexington, KY 40546.

The capsids of the 190S virus of H. victoriae contain three closely related proteins p88, p83 and p78. Purified preparations of the 190S virus were separated into two sedimenting components that differ in capsid structure. Whereas the slower-sedimenting component contained p88 and p83 as the major capsid polypeptides, the faster component contained p88 and p78. When radioactive virions were purified from 14-day-old cultures grown in the presence of [^{32}P] phosphate, radioactivity was predominantly incorporated in p88, as determined by SDS-PAGE. There was considerably less radioactivity incorporated in p83 and none in p78. Similar results were obtained in in vitro phosphorylation studies using [γ-^{32}P] ATP and purified virus. The in vitro results suggest that the 190S virions co-purify with a protein kinase activity which catalyzes the transfer of gamma phosphate from ATP to a target protein, presumably p78. We believe that capsid protein phosphorylation/dephoshorylation plays a regulatory role in the transcription/replication of the dsRNA genome of the 190S virus.

830

Corn Thrips [Frankliniella williamsi Hood] a Major Vector Associated with a 1990 Maize Chlorotic Mottle Virus Epiphytotic in Hawaii. X. Q. Jiang[1], L. J. Meinke[2], R. J. Wright[2], D. R. Wilkinson[1], J. E. Campbell[1], and J. A. Berry[1]. [1]Pioneer Hi-Bred International, Inc., P.O. Box 1004, Johnston, IA 50131.; [2]202 Plant Industry Bldg., Dept. of Entomology, University of Nebraska, Lincoln, NE 68583-0816.

Transmission studies to identify the possible arthropod vectors associated with a Maize Chlorotic Mottle Virus (MCMV) epiphytotic in Hawaii were conducted using six arthropod species [Peregrinus maidis (Ashmead), Sardia pluto (Kirkaldy); (Homoptera:Delphacidae), Empoasca solana DeLong; (Homoptera:Cicadellidae), Adoretus sinicus Burmeister; (Coleoptera:Scarabaeidae), Tetranychus sp.; (Acari:Tetranychidae), and Frankliniella williamsi Hood; (Thysanoptera:Thripidae)] found in MCMV infected maize fields. After a 1-3 day acquisition feeding period on known MCMV-infected maize plants, five arthropod species body sap tested positive for MCMV by ELISA, but only the thrips (F. williamsi) was able to transmit MCMV to healthy maize plants based on symptomatology and ELISA. This is the first report of thrips transmission of MCMV.

831

EFFECTS OF SOIL MATRIC POTENTIAL AND WATER INFILTRATION ON INFECTION OF SOFT RED WINTER WHEAT BY SOILBORNE WHEAT MOSAIC VIRUS. P. T. Himmel[1], F. W. Simmons[2], A. D. Hewings[1], and D. A. Glawe[3]. USDA/ARS[1] and Departments of Agronomy[2] and Plant Pathology[3], University of Illinois, Urbana, IL 61801

The importance of soil matric potential and water infiltration to infections of roots of soft red winter wheat by soilborne wheat mosaic virus (SBWMV) was investigated in greenhouse experiments using cores of Drummer silty clay loam field soil naturally infested with SBWMV and the reported fungal vector, *Polymyxa graminis*. Soil cores were packed to a bulk density of 1.2 g cm^{-3} and adjusted to matric potentials of -6.8, -13.0, -17.5, or -20.5 kPa. Soil matric potentials were established over water columns set up at different heights. After 4 weeks, plant roots and shoots were evaluated for the presence of SBWMV antigen by enzyme-linked immunosorbant assay. The incidence of virus antigen in plant roots and shoots was highest at -13.0 kPa, followed by -17.5 kPa, -6.8 kPa, and -20.5 kPa. Saturation of soil cores at -20.5 kPa and equilibration to -13.0 kPa did not increase the incidence of SBWMV over that in plants growing at static water potentials. Both soil aeration and matric potential are thought to affect zoospore movement of the fungal vector and probably the incidence of SBWMV.

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BELLADONNA MOTTLE VIRUS CAUSING A SEVERE DISEASE OF TOMATILLO IN LOUISIANA. F. A. Can, R. A. Valverde, and M. C. Rush. Department of Plant Pathology and Crop Physiology, Louisiana Agricultural Experiment Station, Louisiana State University Agricultural Center, Baton Rouge, 70803.

Tomatillo (Physalis ixocarpa) is being evaluated as a food crop in Louisiana. Preliminary studies indicate that viral diseases will be the major limiting factor to production. A viral disease with symptoms of mosaic and yellow mottle was commonly found affecting plants on experimental plots. The virus causing the disease was identified as an isolate of belladonna mottle virus (BeMV). Tests used for virus identification included host reaction, serology, electron microscopy, and dsRNA analysis. Other viruses occasionally found included cucumber mosaic virus and tomato spotted wilt virus. Studies are underway to determine factors involved in the spread of BeMV in the field.

833

CHEMILUMINESCENT DETECTION OF RNA OF BARLEY YELLOW DWARF VIRUSES IN TOLERANT AND SUSCEPTIBLE SISTER OAT LINES. H. Fouly[1], L. L. Domier[2], and C. J. D'Arcy[1], Department of Plant Pathology[1] and USDA-ARS Crop Protection Research Unit[2], University of Illinois, Urbana, IL 61801.

Purified BYDV-PAV-IL RNA was detected with biotinylated *in vitro* transcripts or cDNA and chemiluminescent substrate on nylon membranes. Signals were detected on x-ray film and quantified by densitometry. The *in vitro* transcript probe had greater sensitivity (1 ng) and better discrimination among higher virus concentrations (300-600 ng/ml). cDNAs of PAV-IL and RPV-Australia (courtesy A. Miller) were used to measure PAV-IL and RPV-NY RNA in two pairs of sister oat lines previously classified as tolerant or susceptible to PAV. Plants were grown in aeroponic culture, inoculated at 15 days old, and sampled (4 plants per oat line) 2-15 days after PAV-IL inoculation or 3-21 days after RPV-NY inoculation. Each plant was cut into a shoot and 3 root segments and analyzed for viral RNA. Only susceptible lines developed symptoms of virus infection. PAV-IL RNA content was higher in shoots of both tolerant lines than in susceptible lines on early sampling dates (2-6 days), but was not different in 41 of 42 root samples. RPV-NY RNA content was higher in one or both tolerant oat lines than in susceptible sister lines in shoot samples 6-15 days after inoculation. In roots, RPV-IL RNA content was generally higher in tolerant lines on early sampling dates (3-12 days) and lower on later dates (15-21 days). These results indicate that replication and/or movement of RPV-NY but not of PAV-IL is affected in the tolerant oat lines.

834

TOBACCO VEIN-BANDING MOSAIC VIRUS: A NEW VIRUS IN NORTH AMERICA. B. B. Reddick[1], R. G. Christie[2], G. V. Gooding, Jr.[3], and M. H. Collins-Shepard[1]. [1]Dept. of Ent. & Pl. Path., Univ. of Tennessee, Knoxville, TN 37901; [2]Agronomy Dept., Univ. of Florida, Gainesville, Fl 32611; [3]Dept. of Pl. Path., N.C. State Univ., Raleigh, N.C. 27695.

The area surrounding Greeneville, TN was the site of a virus survey of burley tobacco in 1990. In one field, 25% of the symptomatic plants tested did not react with antisera of common tobacco viruses of North America. Electron micrographs of partially purified virions suggested a potyvirus was involved. Inclusion bodies of the unknown virus were compared to those of several potyviruses and found to be most similar to those of tobacco vein-banding mosaic virus (TVBMV). SDS-agar double diffusion and ELISA tests revealed a positive reaction with antisera to TVBMV, a virus originally identified in Taiwan in 1966. To our knowledge, this is the first time this virus has been found in the United States.

835

PARTIAL CHARACTERIZATION OF A POTYVIRUS ISOLATED FROM WATERMELON (CITRULLUS LANATUS) IN FLORIDA. D.E. Purcifull, E. Hiebert, M.A. Petersen, G.W. Simone, and M.D. Gooch. Department of Plant Pathology, University of Florida, Gainesville, FL 32611.

Prominent nuclear inclusions in stained leaf epidermal strips implicated a virus as the cause of foliar mosaic in a watermelon plant received for analysis from south Florida in 1990. In greenhouse tests, mechanically inoculated plants of Cucurbita pepo ('Small Sugar' pumpkin and 'Early Prolific Straightneck' squash) and watermelon ('Crimson Sweet') developed mosaic symptoms. Crystalline nuclear inclusions, cytoplasmic amorphous inclusions, and cytoplasmic cylindrical inclusions were detected by light and electron microscopy in leaf tissues of squash and watermelon. Of 159 filamentous particles measured in squash leaf extracts, 86% ranged from 800 nm to 890 nm. The virus was transmitted in a stylet-borne manner by Myzus persicae from squash to squash in two of 3 trials. Immunodiffusion tests with antisera prepared to purified virus or its capsid protein showed that the isolate was different from papaya ringspot virus type W, watermelon mosaic virus 2, and zucchini yellow mosaic virus. The properties of the watermelon isolate indicate that it is distinct from the three potyviruses commonly identified in cucurbits in Florida.

836

VIRUS ELIMINATION FROM HEAT-SENSITIVE POTATO PLANTLETS USING A REDUCED THERAPY PERIOD. G. E. Sánchez, S. A. Slack, H. M. Griffiths and J. H. Dodds*. Department of Plant Pathology, Cornell University, Ithaca, NY, 14853. *International Potato Center, P.O. Box 5969, Lima, Peru.

Ten heat-sensitive virus-infected *Solanum* genotypes (wild spp. and group andigena) were subjected to a 50% reduced thermotherapy period. Plantlets from nodal cuttings were established *in vitro* on basic Murashige-Skoog (MS) medium amended with ribavirin (Can. J. Bot. 68:1515). After 15 days of thermotherapy, plantlets were tested by ELISA and those testing negative (OD_{405} $\leq$0.05) were further propagated on MS medium. Plantlets were again tested by ELISA after both *in vitro* propagation and greenhouse grow-out. PVS and PVX concentrations were notably reduced following therapy with 60% and 87% plantlets testing negative, in contrast to PVY (19%) and PLRV (0%) variable responses. However, the percentage of plantlets still testing free from PVS, PVX and PVY was only 3%, 13% and 2%, respectively, following greenhouse grow-out. In contrast, previous 30-day thermotherapy experiments generally yielded 10-30% more plantlets free from the viruses tested and the percentage of plantlets remaining virus-free upon greenhouse grow-out was >85%. Therefore, heat sensitive genotypes should be held in thermotherapy as close to 30 days as possible to enhance virus elimination efficiency.

837

A NEW GEMINIVIRUS WITH A BROAD HOST RANGE IN THE BRASSICACEAE. J. O. Strandberg, E. Hiebert, G. L. Leibee, and A. Abouzid. University of Florida, IFAS, Central Florida Research and Education Center, 2700 East Celery Ave., Sanford, FL 32771.

A viral infection of cabbage plants produced severe stunting, interveinal chlorosis, distortion of new leaves and reduced internode length. The virus was transmitted by the whitefly, Bemesia tabaci, and produced symptoms on cabbage, collards, cauliflower, mustard, Chinese cabbage, turnip, and radish, Brassica juncea, and other species in the Brassicaceae. The virus was mechanically transmitted with plant sap to several of these species and to Nicotiana edwardsonii. No symptoms were produced on tomato, bean, squash, cucumber, potato, or Vinca minor. Agarose gel electrophoresis of viral DNA extracted from mustard demonstrated ssDNA and replicative forms of approximately 2800 bp. Viral DNA from Southern blots hybridized strongly with molecular probes to BGMV and Florida tomato gemini virus and indicated a possible relationship. However, a new geminivirus with a distinctly different host range in the Brassicaceae is indicated.

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A NEW POTYVIRUS ISOLATED FROW **Cucurbita pepo** IN BRAZIL.J.A. A. Lima, C.D.G.Santos, C.C.Vale & E.W.Kitajima(Lab.Virol.Vegetal, UFC, C.P. 12.168 - Fortaleza, CE, 60.355, Brazil).

A virus isolated from **Cucurbita pepo** in the State of Ceará , Brazil was identified as a new potyvirus on the basis of its particle morphology, aphid transmissibility, cytopathological properties of infected plants, and no reactivity with antisera to other potyviruses that infect cucurbits. The virus was inoculated in 23 plant species to the families:Amarantaceae(1), Chenopodiaceae(3), Cucurbitaceae(10), Leguminosae(5) and Solanaceae(4), but was able to infect only 9 cucurbit species. The virus was transmitted by **Aphis gossypii** in a non - persistent manner and infected plants showed cytoplasmic pinwheel inclusions.Extracts from infected plants did not react with antisera specific to papaya ringspot virus-W, watermelon mosaic virus-2 and zucchini yellow mosaic virus.

840

CONJUGAL TRANSFER OF A POLYGALACTURONASE-ENCODING PLASMID FROM *PSEUDOMONAS CEPACIA*. C. F. Gonzalez and M. E. Angell, Department of Plant Pathology and Microbiology, Texas A&M University, College Station.

Phytopathogenic strains of *Pseudomonas cepacia* produce endopolygalacturonase (pec^+) and bacteriocin PC1 whereas strains of clinical and soil origin do not. Growth of a phytopathogenic strain (PCO25) at elevated temperatures resulted in nonpectolytic (pec^-) derivatives that were either cured of a resident plasmid or contained a plasmid of reduced mass. The resident plasmid (pPEC320) in strain PCO25 was labeled with Tn*5*-Mob. The pPEC320::Tn*5*-Mob (pPEC321) plasmid was mobilized into strain TL249-2, a plasmid-free pec^- soil isolate, at a frequency of $2x10^{-6}$ transconjugants/recipient. Mobilization of pPEC321 from TL249-2(pPEC321/RP4-4) to strain PCO2511, a pec^- derivative of PCO25, and to strain B4648 (*P. cepacia* of clinical origin) occurred at frequencies of $4.2x10^{-5}$ and $7.5x10^{-5}$, respectively. Transconjugants containing pPEC321 expressed polygalacturonase and plant macerating activity. A 5.3 kb fragment from pPEC320 encoding for polygalacturonase activity has been cloned.

841

CHARACTERIZATION OF A PERIPLASMIC PHOSPHATE-BINDING PROTEIN FROM *XANTHOMONAS ORYZAE* PV. *ORYZAE*. C. M. Hopkins, F. F. White, and J. E. Leach. Department of Plant Pathology, Kansas State University, Manhattan, KS 66506.

Under low phosphate conditions, phosphate enters an *Escherichia coli* cell via a phosphate-specific transport (*pst*) system. A critical element of the *pst* system is a periplasmic phosphate-binding protein, PhoS. A 1 kb open reading frame having similarity to phoS, designated *phoX*, was identified by nucleotide sequencing of a 2.5 kb genomic clone from *Xanthomonas oryzae* pv. *oryzae*. *phoX* was cloned into a T7 expression vector, and the protein produced was shown to be processed and have phosphate-binding activity. Specific mutations were introduced into the cloned gene at amino acids thought to be involved in phosphate binding as previously identified by X-ray crystallography of the PhoS protein. Both wild type and mutant protein will be assayed for phosphate binding activity in *E. coli* and *in vitro*.

842

ISOLATION OF A NATURALLY OCCURRING TRANSPOSABLE ELEMENT FROM A NON-PIGMENTED STRAIN OF *XANTHOMONAS CAMPESTRIS* PV. *CAMPESTRIS*. Alan R. Poplawsky Department of P.S.E.S./Division of Plant Pathology, University of Idaho, Moscow ID 83843

Xanthomonads generally produce characteristic yellow pigments known as xanthomonadins. A cluster of genes (*pig*) has been identified which is involved in pigment production. Conjugation of pLAFR3-derived cosmid clones containing *pig* genes into non-pigmented strains of *X. c.* pv. *campestris* usually restored pigment production. This was observed with strain B-122. However, when pigmented transconjugants of strain B-122 were plated on medium selective for retention of the *pig* clone, non-pigmented colonies were observed at a frequency of 1×10^{-4}. This experiment was repeated with similar results. In all cases, restriction endonuclease analysis of plasmid DNA from non-

pigmented isolates revealed a 1.3 kb insertion at the same site within the *pig* gene cluster. Additional studies are being conducted to determine the nature of this element, and to evaluate its potential use as a tool for disease diagnosis or mutagenesis.

843

PILUS-MEDIATED ADSORPTION OF PSEUDOMONAS SYRINGAE TO BEAN LEAVES. M. Romantschuk, E.-L. Nurmiaho-Lassila, and E. Rantala. Department of General Microbiology, University of Helsinki, Mannerheimintie 172, SF-00300 Helsinki, Finland.

Various strains of Pseudomonas syringae adsorb to the leaves of its host plant using its pili. P.s.syringae R32 adsorbs evenly to the surface of bean leaves, whereas pv. phaseolicola adsorbs specifically to the stomata. Non-piliated mutants adsorb with an appr. 70% reduced efficiency compared to the wild type. The mutants were complemented with cosmids from a wild type phaseolicola-library. The complemented strains express pili at approximately three times the wild type levelas measured by ability to adsorb to bean leaf surface, and as measured by ability to adsorb phage that use the pilus as primary receptor. Prethreatment of leaves with complemented piliated bacteria lowered the rate of adsorption of subsequently added piliated bacteria (wild type or complemented mutants), but did not affect the low level adsorption of the non-piliated mutants.

844

THE PECTATE LYASE GENE OF *PSEUDOMONAS VIRIDIFLAVA* STRAIN SJ074 IS ENCODED IN THE PLASMID C. -H Liao, K. Sasaki, G. Nagahashi and K. Hicks, U.S. Department Agriculture, ARS, Eastern Regional Research Center, 600 E. Mermaid Lane, Philadelphia, PA 19118.

We have previously reported the cloning of a *pel* gene in a 3.8-kb *Sph*I fragment from the genome of *P. viridiflava* SJ074. Data obtained from further investigations show that the *pel* structural sequence is in the internal 1.1-kb *Pst*I-*Bgl*II region and the transcription is from a region upstream of the *Pst*I site. By using this 1.1-kb fragment as a probe, we detected *pel* homologs in 11 strains of *P. fluorescens* and *P. viridiflava* and in 4 pathovars of *P. syringae*. Moreover, we found that the *pel* homolog is present both in the chromosome and in the plasmid of strain SJ04. Results from marker-exchange and direct-cloning experiments also indicate that the indigenous plasmid (1.1 MDa) of strain SJ074 contains the *pel* gene.

845

STRUCTURE OF *pel-1* FROM *ERWINIA CAROTOVORA* SUBSP. *CAROTOVORA* (Ecc) STRAIN 71 AND BIOCHEMICAL PROPERTIES OF THE ENCODED PECTATE LYASE. A. Chatterjee, H. Murata, and A.K. Chatterjee; Department of Plant Pathology; 108 Waters Hall; University of Missouri; Columbia, MO 65211.

Pel-1 is one of several pectate lyases produced by Ecc71. The sequence of the corresponding gene, *pel-1*, contains an open reading frame of 1122 base pairs encoding a 374 amino acid polypeptide with a predicted molecular mass of *ca.* 41 kD and a pI of 9.96. The regulatory region containing several putative protein-binding sites was localized within 135 bases upstream of the presumed start codon. A putative signal sequence of 23 amino acid residues was detected. *pel-1* has higher homology with some of the *pel* genes of *E. carotovora* than with *pel* genes of *E. chrysanthemi*. Pel-1, obtained from *Escherichia coli* carrying a *pel-1*$^+$ plasmid, preferred pectate over pectin as a substrate, required Ca^{2+} (0.34 mM) for maximal activity, and had a pH optimum of *ca.* 8.0. Pel-1 caused cell separation, electrolyte loss, and cell death in potato tuber tissue.

846

IDENTIFICATION AND CLONING OF A *TRANS*-ACTING FACTOR REQUIRED FOR COPPER-INDUCIBLE EXPRESSION OF THE *COP* OPERON OF *PSEUDOMONAS SYRINGAE*. D. A. Cooksey and C. A. Jasalavich, Department of Plant Pathology, University of California, Riverside, CA 92521.

The copper-inducible promoter of the copper resistance operon (*cop*) from plasmid pPT23D of *Pseudomonas syringae* pv. *tomato* was previously fused to β-galactosidase (pCOP38) to measure transcriptional activation. No expression from the promoter was observed when pCOP38 was in the copper-sensitive strain PS61 of *P. syringae*, except when pPT23D was also placed in this strain and induced with copper. Therefore, a factor supplied in *trans* from pPT23D was required for activation of this promoter. Transposon mutagenesis was performed on pPT23D, and several insertion mutants were identified that lacked the ability to activate pCOP38 in *trans*. All of these mutations mapped to a region of about 1.6 kilobases immediately 3′ to the *cop* operon. This region was cloned, and it restored copper-inducible gene expression to the mutants when introduced on a plasmid in *trans*.

847

PREVALENCE OF HYDROXAMATE AND CATECHOL SIDEROPHORE PRODUCTION IN ERWINIA CAROTOVORA SUBST. CAROTOVORA AND E. C. SUBSP. ATROSEPTICA. C. A. Ishimaru and A. Van Buren. Department of Plant Pathology and Weed Science, Colorado State University, Fort Collins, CO 80523.

The distribution of catechol and hydroxamate siderophores among several strains of Erwinia carotovora subsp. carotovora and E. c. subsp. atroseptica was assessed. Each of 306 strains of E. carotovora isolated from a variety of sources produced catechol siderophore(s). Hydroxamate production was detected in only seven strains of E. carotovora subsp. carotovora. Prevalence of hydroxamate production varied with source; five of the 150 potato isolates and none of the 115 water isolates produced a hydroxamate. The hydroxamate siderophore is aerobactin, since hydroxamate-producing strains crossfed an indicator strain (LG1522) specific for aerobactin, and contained DNA sequences homologous to the aerobactin biosynthesis genes from E. coli. Aerobactin biosynthesis is an uncommon phenotype in these two subspecies of E. carotovora. Therefore, its role, if any, in their virulence or survival is probably very limited.

848

NUCLEOTIDE SEQUENCE ANALYSIS OF THE IAA BIOSYNTHETIC GENES OF *PSEUDOMONAS SYRINGAE* PV. *SYRINGAE*. M. Mazzola and F.F. White, Dept. Plant Pathology, Kansas State Univ., Manhattan, KS 66506.

Pseudomonas syringae pv. *syringae* is the causal agent of brown spot of bean. Many plant-associated bacteria, including *P. s.* pv. *syringae* have the ability to synthesize indole acetic acid (IAA). A DNA fragment containing the *iaa*M and *iaa*H genes from *P. s.* pv. *savastanoi* previously was used as a probe to identify IAA biosynthetic genes from *P. s.* pv. *syringae*. We have conducted a sequence analysis of the tryptophan-2-monooxygenase (*iaa*M) and indoleacetamide hydrolase (*iaa*H) genes of *P. s.* pv. *syringae*. Both *iaa*M and *iaa*H possess significant nucleic acid sequence similarity with the corresponding genes of *P. s.* pv. *savastanoi*. The predicted amino acid sequence for the *iaa*M and *iaa*H genes of *P. s.* pv. *syringae* have approximately 90% identity with the predicted amino acid sequence of their respective homologues in *P. s.* pv. *savastanoi*. In contrast, the region upstream from the *iaa*M structural gene of *P. s.* pv. *syringae* does not possess significant sequence similarity with the promoter region of the *iaa* operon from *P. s.* pv. *savastanoi*.

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MOLECULAR CHARACTERIZATION OF THE PSEUDOMONAS SYRINGAE PV. SYRINGAE 61 HRMA LOCUS. S. Heu and S.W. Hutcheson, Department of Botany, University of Maryland, College Park, MD 20742

Escherichia coli carrying the P. syringae pv. syringae 61 (Pss61) hrp gene cluster requires the hrmA locus to initiate the hypersensitive response in tobacco, whereas the parental Pss61 strain does not. The nucleotide sequence of the hrmA locus revealed an open reading frame that encodes for a protein of 41,440 Da. Upstream sequences contain putative RpoN and CRP recognition sites that may function in E. coli. The deduced amino acid sequence lacked definitive features. The downstream sequence contains a typical rho-independent termination signal. Hybridization analysis indicates that some, but not all, of the P. syringae strains tested carry a homologous locus.

850

TRANSCRIPTIONAL ORGANIZATION AND EXPRESSION OF THE PSEUDOMONAS SYRINGAE PV. SYRINGAE 61 HRP GENE CLUSTER. Y. Xiao and S.W. Hutcheson, Department of Botany, University of Maryland, College Park, MD 20742

The hrp/hrm cluster of P. syringae pv. syringae 61 (Pss61) consists of at least 12 hrp genes and one hrm gene. A set of chromosomal hrp-uidA fusions was constructed to investigate the transcriptional organization and expression of this cluster. The cluster is organized as at least 6 transcriptional units. The expression of most transcriptional units was enhanced in planta. A representative fusion

(hrpIII::Tn5-gusA1) was induced within 3 h after the mutant was inoculated into tobacco leaves or minimal salts media. Complete amino acid sources, but not gln or NH_4^+, could repress the expression in culture. The results support the hypothesis that Pss61 hrp genes are regulated by nutritional conditions.

851

RESTRICTION MAPPING, DELETION ANALYSIS AND CURING OF THE ENDOGENOUS PLASMID HARORING AVIRULENCE GENE D IN *PSEUDOMONAS SYRINGAE* PV. *TOMATO*. H. Shen, A. Sharma, D. Gerhold, J. Murillo, and N. T. Keen, Univerisity of California, Riverside, CA 92521.

Avirulence gene D (*avrD*), previously cloned from *Pseudomonas syringae* pv. *tomato* resides on the endogenous B plasmid that is about 80 kb. A physical map of this plasmid was constructed with the following restriction enzymes: *Bam*HI, *Not*I, *Pac*I, *Sac*I, and *Xho*I. Various deletions in the sequences adjacent to the *avrD* gene were constructed *in vitro*, and then marker-exchanged into the B plasmid in *P. s.* pv. *tomato*. The B plasmid was also evicted from a marker-exchange mutant which carries a *nptI-sacB-sacR* cartridge as an anti-selection in the presence of sucrose. This strain devoid of the B plasmid did not produce the *avrD* elicitor, but was complemented when the cloned *avrD* gene was introduced on a plasmid. This demonstrated that no other genes on the B plasmid are required for *avrD* elicitor production. Mutants with deletions in open reading frames 3 and 4 downstream from *avrD* gene produced more elicitor, suggesting that these downstream ORFs might be involved in further metabolism of the elicitor.

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CHARACTERIZATION OF AVRRXV: ITS ROLE IN RESISTANCE OF TOMATO TO XANTHOMONAS CAMPESTRIS PV. VESICATORIA. M. Whalen, P. Antall, A. Toms, M. Heiskell, S. Conover, F. Carland, D. Dahlbeck, and B. Staskawicz. Department of Biology, Colby College, Waterville, ME 04901.

Resistance against many plant pathogens occurs when a plant carries a dominant resistance gene that specifically corresponds to a dominant avirulence gene in a pathogen. Isolation and characterization of avirulence genes that serve to induce resistance reactions will help us to understand the molecular basis of disease resistance. Jones and Scott have described the hypersensitive resistance response (HR) of tomato line Hawaii 7998 to Xanthomonas campestris pv. vesicatoria (XcvT) (Plant Dis. 70:337-9, 1986). When carried by a normally virulent XcvT strain, the avirulence gene avrRxv isolated from XcvT 75-3 induces HR in Hawaii 7998. This avrRxv-specific resistance is temperature sensitive. We will discuss results from sequence analysis of avrRxv, and from experiments addressing the regulation of expression of avrRxv.

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FUNCTIONAL ANALYSIS OF A CHLORELLA VIRUS ADENINE METHYL TRANSFERASE GENE PROMOTER IN AGROBACTERIUM AND PLANT CELLS. **N. Rohe, D. Higgins, and A. Department of Plant Pathology, University of Nebraska, Lincoln, NE 68583-0722.**

The promoter of Chlorella virus adenine methyl transferase gene was tested in Agrobacterium and in plant cells by fusing the 861 base pair promoter sequence with Chloramphenicol acetyl transferase coding sequence followed by termination sequence from the T-DNA nopaline synthase gene. The AMT-CAT was subcloned into a binary vector with RK-2 wide host range replicon and was mobilized into virulent and avirulent strains of Agrobacterium as well as in several different plants including monocots. Attempts are currently being made to characterize the cis promoter elements both in Agrobacterium and plant cells in order to determine whether different cis elements are function in Agrobacterium and in plants. This promoter can be useful to express foreign genes in plants and bacteria.

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POLYMERASE CHAIN REACTION (PCR) AMPLIFICATION AND NUCLEOTIDE SEQUENCING OF APPLE SCAR SKIN VIROID. A. Hadidi, X. Yang, and R. W. Hammond. USDA-ARS, Beltsville, MD 20705-2350.

Rapid amplification of apple scar skin viroid (ASSV) cDNA transcribed in vitro in total nucleic acids from infected tissue is now possible using Taq I DNA polymerese and ASSV DNA primers in a reverse transcription-polymerase chain reaction (RT-PCR) (Hadidi, A. and Yang, X. 1990. J. of Virol. Methods 30:261-269). We have cloned and subcloned the RT-PCR amplified product of ASSV cDNA into the vector PUC9 and PGEM, respectively, then determined the nucleotide sequence of the insert in each vector. The sequence is 330 nucleotides in length which can be arranged into the authentic ASSV-specific rod-like structure. These results indicate that RT-PCR assay transcribes and amplifies viroid RNA in nucleic acid extracts of infected tissue with high specificity and fidelity. Thus, reliable cloning and sequencing analyses of ASSV as well as other viroids can now be performed without viroid purification or the need of large amounts of infected tissue.

855

CONSTRUCTION OF TOBACCO MOSAIC TOBAMOVIRUSES ENCODING SWEET POTATO FEATHERY MOTTLE POTYVIRUS COAT PROTEIN
Carlos A. Malpica, Curtis A. Holt, Roger N. Beachy
Washington University. Campus Box 1137. St. Louis MO 63130, U.S.A.

The coat protein (CP) gene of sweet potato feathery mottle potyvirus (SPFMV, obtained from J. Abad & J. Moyer, North Carolina State University) has been isolated by PCR and subsequently cloned. The oligonucleotides used for PCR were designed to modify the 5' and 3' end sequences of the original SPFMV CP coding sequence and introduce a plant consensus initiation codon and a consensus stop codon. The modified SPFMV CP was subsequently introduced into an infectious cDNA clone of tobacco mosaic tobamovirus in place of either the TMV movement protein (MP) gene or the CP gene.

Transcripts derived *in vitro* from both constructs are infectious when inoculated onto transgenic tobacco (*N. tabacum* cvs. Xanthi and Xanthi NN) plants that express a TMV MP gene. Levels of SPFMV CP accumulation are currently being assessed via western blot analysis and ELISA.

The use of a transient gene expression system to express heterologous CP genes from a TMV-derived vector may prove useful in the rapid analysis of CP-mediated resistance.

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CHARACTERIZATION OF RICE YELLOW MOTTLE VIRUS PROTEINS
M. Ngon A Yassi, C. Ritzenthaler, A. de Kochko, C. Fauquet and R. N. Beachy, Department of biology, Washington University, St Louis, MO 63130

Rice yellow mottle virus (RYMV) has been proposed as a member of the sobemovirus group and is recognized as an important endemic rice viral disease in Africa. SDS-PAGE analysis, serological methods and *in vitro* translation of RYMV RNA were used to characterize the virus proteins. The approximate molecular weight (MW) of its coat protein (CP) is 29 kD. The CP can aggregate into a dimer with a molecular weight of 58 kD. Three unique proteins with molecular weights estimated at 29, 17.5 and 13kD are associated with RYMV infection in rice. *In vitro* translation yields five proteins with molecular weights corresponding to 105, 68, 29, 17.5 and 13 kD. The 58, 29, 17.5 and 13kD proteins from purified virus and infected leaf extracts are recognized by the antibodies (raised against purified RYMV) on western blots. The 17.5 and 13 kD produced *in vitro* are also immunoprecipitated by the RYMV antibodies, though the 105 and 68 kD proteins are not. The 29 kD protein (putative CP) is translated *in vitro* in small amounts perhaps due to the CP being synthesized *in vivo* via a subgenomic RNA.

857

CLONING OF PEANUT STRIPE VIRUS (PStV) COAT PROTEIN AND ITS EXPRESSION IN E. COLI. B.G. Cassidy[1], J. Sherwood[2], R.S. Nelson[1]. [1]The Samuel Roberts Noble Foundation, P.O. Box 2180, Ardmore, OK 73402 and [2]Department of Plant Pathology, Oklahoma State University, Stillwater, OK 74078.

The 3' terminus of PStV was cloned and sequenced. The region included the 281 amino acid coat protein (CP) and 256 nucleotides of 3' nontranslated sequence. Clustal program analysis (PC/Gene, Intelligenetics, Inc.) of the CP amino acid sequence indicated a 74% and 73% identity to zucchini yellow mosaic virus and watermelon mosaic virus CP, respectively. The PStV CP and portions of the CP were expressed in E. coli. Viral CP was detected by Western, dot blot, and Elisa with polyclonal and monoclonal antibodies. The antigenicity of different portions of the CP were determined. Transgenic tobacco plants expressing the CP are to be produced and tested for resistance to potyviruses.

858

MUTATIONAL ANALYSIS OF COAT PROTEIN-MEDIATED PROTECTION IN TRANSGENIC TOBACCO PLANTS WHICH EXPRESS ASSEMBLY DEFECTIVE COAT PROTEIN GENES FROM TOBACCO MOSAIC VIRUS. W.G. Clark and R.N. Beachy, Department of Biology, Washington University, St. Louis, Missouri 63130.

Point mutants in the coat protein gene of the U1 strain of TMV were constructed using oligomucleotide mutagenesis and were then expressed in tobacco plants, in

order to test their affect on coat protein-mediated protection. The mutants were based on X-ray diffraction analysis of TMV and upon previously isolated virus mutants that carried coat protein (CP) genes encoding proteins unable to assemble into virus rods. A CP mutant with changes at amino acid 28 and 95 was constructed as well as single mutants with changes at these positions. These mutants were expressed in local lesion and systemic tobacco plant lines. Plant lines expressing the single point mutants exhibited protection against the U1 strain of TMV when compared to the negative control line. Data will be presented showing how the protection observed in the plant lines expressing mutant CP genes compares with that in lines expressing the wild type gene. The data will be discussed with regard to structure-function relationships between CP, viral disassembly and coat protein-mediated protection.

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STUDIES ON RECOMBINATION IN THE COAT PROTEIN CODING REGION OF BROME MOSAIC VIRUS RNA 3. S. Flasinski, J. J. Bujarski, Plant Molecular Biology Center, Northern Illinois University, DeKalb, IL 60115

To study RNA-RNA recombination in BMV coding regions, several frame-shift mutations were introduced in the RNA 3 component using biologically active cDNA clones. One such mutation (designated RNA 3-5) contains a frame shift immediately after the initiation codon of the coat protein gene. The synthesis of an 8 amino acid-deficient coat protein was still possible from the next AUG codon. *Chenopodium hybridum* plants inoculated with a mixture of wt RNA 1 and 2 transcribed from cDNA clones, and RNA 3-5 developed smaller local lesions that emerged later as compared to those obtained with the wt RNA 3. An RT-PCR analysis demonstrated that about 90% of lesions screened were found to contain mutated RNA 3-5. The remaining 10% had both the mutated RNA 3-5 sequence in addition to recombinant wt RNA 3. Only one lesion was found to have exclusively the recombinant wt RNA 3. Since the RNA 3-5 mutant can replicate in *C. hybridum* but has lower infectivity and pathogenicity, it can be used as a convenient vector to study the mechanism of recombination in coding regions of RNA 3.

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DETECTION OF BARLEY YELLOW DWARF AND OTHER LUTEOVIRUSES WITH GROUP-SPECIFIC PRIMERS AND PCR. Roy French and Nancy L. Robertson. USDA, ARS, Department of Plant Pathology, University of Nebraska, Lincoln, Nebraska 68583

Four potentially luteovirus group-specific primers for use in the polymerase chain reaction (PCR) were synthesized based on RNA sequence data of three luteoviruses. One primer pair (designated LU1 and LU4) amplifying ca. 530 bp cDNA fragments spanning the virion capsid protein genes of potato leafroll virus (PLRV), beet western yellows virus (BWYV), New York barley yellow dwarf virus (BYDV) serotype PAV also amplified the corresponding regions of BYDV serotypes MAV, RMV, RPV, and SGV, as well as 28 Nebraskan BYDV field isolates. The different luteoviruses were readily distinguished by restriction analysis of the PCR cDNA products. Virus-specific products were readily detected in stained gels of PCR assays of crude extracts from 5 ng BYDV-infected tissue. To reduce costs, it was found that assays utilizing reaction volumes of 2 µl for reverse transcription and 10 µl for PCR were as effective as larger reaction volumes. This rapid and accurate assay will be useful for epidemiological studies and initial cDNA cloning experiments.

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USE OF THE POLYMERASE CHAIN REACTION TO DISTINGUISH AND CHARACTERIZE THE MT-RMV ISOLATE OF BYDV. S. M. Geske [1], R. French [2], N. L. Robertson [2], and T. W. Carroll [1].
[1]Montana State University, Bozeman, MT 59717
[2]USDA-ARS, University of Nebraska, Lincoln, NE 68583

Use of 1-fold, 2-fold, and 16-fold degenerate primers derived from published sequences of PLRV, BWYV, and BYDV-PAV in polymerase chain reactions generated about 530 bp and 1.4 kb fragments. The smaller fragment encompasses most of the viral coat protein gene. The larger fragment encompasses part of the 3' end of the 60k protein gene, the viral coat protein gene, and the intervening non-coding region. The fragments were compared by restriction enzyme analysis with similarly obtained fragments from BWYV and the five New York isolates of BYDV. Unique banding patterns distinguished the MT-RMV isolate from the others, indicating genome sequence differences. Both fragments were cloned into *E. coli* using pUC119 and are currently being sequenced.

862

SITE-SPECIFIC MUTATIONS IN CODONS OF THE PUTATIVE NTP-BINDING MOTIF OF THE AL1 GENE OF BEAN GOLDEN MOSAIC GEMINIVIRUS ABOLISH INFECTIVITY. S. F. Hanson[1], R. L. Gilbertson[2], P. G. Ahlquist[1], D. R. Russell[3], and D. P. Maxwell[1]. [1]Dept. of Plant Pathology, University of Wisconsin, Madison, WI 53706, [2]Dept. of Plant Pathology, University of California, Davis, CA 95616, and [3]Agracetus, Inc., Middleton, WI 53717.

Bean golden mosaic virus (BGMV) is a whitefly-transmitted, ssDNA geminivirus with a bipartite genome, DNA-A and DNA-B. The product of the AL1 gene of DNA-A of tomato golden mosaic geminivirus is essential for viral DNA replication (Hanley-Bowdoin et al. 1990. PNAS,USA 87:1446). A putative NTP-binding motif, (ß-strand) Glu Asp XXXX Gly Lys Thr (α-helix) X_{32} (ß-strand) Asp Asp, was present in the deduced amino acid sequences of the AL1 protein for nine geminiviruses including BGMV-GA (Guatemala isolate). Infectivity of BGMV-GA was abolished when codons were changed for Glu-221 to Arg and for Lys-228 to His within the putative NTP-binding site, whereas infectivity was retained for the codon change of Ile-190 to Asp at a variable site not associated with the putative NTP-binding site. A trans-dominant interference scheme for virus-derived resistance is being explored using these constructions.

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COMPLETE NUCLEOTIDE SEQUENCES OF THE GENOMES OF SOYBEAN MOSAIC VIRUS STRAINS G7 AND G2
Ch. Jayaram, John H. Hill and Allen Miller, Plant Pathology Department, Iowa State University, Ames, IA 50011.

Several isolates of soybean mosaic virus (SMV) have been distinguished on the basis of their differential ability to infect soybean cultivars (lines). The SMV G7 strain can infect soybean plants containing the *Rsv1* resistance gene whereas SMV G2 cannot. As a first step in identifying the region(s) in the G7 genome which allow it to overcome this resistance, the complete nucleotide sequences of strains G7 and G2 were determined. Proteins encoded by SMV G7 and G2 are 90 to 98% identical, suggesting that a only a few amino acid differences are responsible for the differential ability to overcome resistance. SMV G7 shares 42 to 48% amino acid homology with tobacco vein mottling virus in the coat protein, replicase, protease, VPg and cylindrical inclusion protein regions of the polyprotein. On the other hand, similarities with the proteins encoded at the 5'end of the genome (42K, helper component and putative movement protein) were considerably less.

864

NUCLEOTIDE SEQUENCE OF CARNATION RINGSPOT VIRUS RNA-2. T.L. Kendall and S.A. Lommel, Department of Plant Pathology, North Carolina State University, Raleigh, North Carolina 27695.

The RNA-2 of carnation ringspot virus (CRSV), the type member of the dianthovirus group, has been cDNA cloned and sequenced. RNA-2 is 1428 nucleotides in length and contains a single open reading frame with the capacity to code for a 304 amino acid 33.8 kDa polypeptide. Amino acid sequence alignment of this polypeptide with the cell-to-cell movement proteins encoded by RNA-2 of red clover necrotic mosaic virus (RCNMV) Australian and Czechoslovakian (TpM34) isolates indicates 59.6 % and 55.7 % sequence identity, respectively. The amino terminal 230 amino acids are even more highly conserved with 64.3% and 62.6% sequence identity between CRSV and RCNMV (Aus) and TpM34, respectively. The cell-to-cell movement proteins of the two RCNMV isolates are 82.5% homologous and 91.7% homologous when comparing the amino terminal 230 amino acids. The increased amino acid sequence similarity in the amino two-thirds of the dianthovirus movement proteins and the retention of activity in mutants with the carboxy-terminal 39 amino acids deleted indicates that cell-to-cell movement is not dependent on the carboxy-terminal domain of the protein.

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SEPARATION OF RECOMBINATION AND REPLICATION FUNCTIONS IN THE 3' NONCODING REGION OF BROME MOSAIC VIRUS RNA 3. P. D. Nagy, and J. J. Bujarski, Plant Mol. Biol. Center, Northern Illinois University, DeKalb, Illinois 60115

Previously we have demonstrated that a deletion (designated M4) in the 3' replication promoter of brome mosaic virus (BMV) RNA 3 was repaired in vivo by either legitimate or illegitimate intermolecular recombination with wt RNAs 1 or 2 (Bujarski and Kaesberg, Nature,1986; Bujarski and Dzianott, submitted). We have extended our studies on the mechanism of recombination in BMV, to include RNA 3 constructs that incorporate a duplication of the 3' end promoter. The terminal promoter had all the sequences required for the initiation of RNA synthesis, whereas the internal promoter lacked 6 3' nucleotides and therefore would not be active in replication. This arrangement allowed for a separation of replication and recombination functions. To study the effect of replication on recombination, the M4 deletion was introduced either one of the promoters or in both promoters. All mutants bearing the M4 deletion in the 3' end promoter generated new recombinants during infection. Conversely, when the same mutation was positioned internally no effect on recombination was observed. In a control construct the internal promoter (tRNA-like structure) region was deleted. This stabilized the nonhomologous 3' region. In addition, a construct deficient in the replication promoter generated new recombinants demonstrating that, if copy-choice is the mechanism, recombination occurred during negative strand synthesis.

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DEVELOPMENT OF POTATO LEAFROLL VIRUS RESISTANCE IN RUSSET BURBANK. D.L. Nida, G.S. Anderson, C.L. Hemenway, W.K.

Kaniewski, E.C. Lawson, J.D. Weiss, and N.E. Tumer. Monsanto Company, 700 Chesterfield Village Parkway, St. Louis, MO 63198.

PLRV is a luteovirus that causes an economically important disease on potato. It reduces yield and tuber quality. Thus far, traditional breeding for disease resistance in Russet Burbank (RB) has not been successful; therefore coat protein-mediated protection was pursued to develop virus resistance. Vectors encoding PLRV coat protein (CP) were constructed and transformed into Russet Burbank potato. PLRV CP gene expression was confirmed by northern and western analyses. Transgenic and non-transgenic plants were challenged with viruliferous aphids and later evaluated by ELISA to determine incidence of virus infection and virus titer. Results of gene expression analyses and protection experiments will be discussed.

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VIROID INTERACTION WITH HOST CELL COMPONENTS. Cynthia P. Paul[1], Andrea D. Branch[2], and Hugh D. Robertson[1]. [1]Cornell University Medical College and [2]The Rockefeller University, New York, NY 10021.

Viroids are composed solely of RNA and do not code for any proteins; therefore, they rely on host cell components to complete their life cycle. Host components may be required for several steps in replication and for viroid transport. One particular interaction between viroid and host cell components is suggested by the sequence and structural similarity between the viroid central conserved region and a region of 5S ribosomal RNA (rRNA) (Branch et al. 1985. PNAS 82:6590-6594.). This region of 5S rRNA is the binding site for transcription factor IIIA (TFIIIA). The binding of TFIIIA to 5S ribosomal DNA (rDNA) is required for the transcription of the 5S gene by RNA polymerase III. 5S rRNA acts as a negative regulatory factor, inhibiting transcription of rDNA by binding to TFIIIA itself. The possibility that viroid RNA may interact with TFIIIA as does 5S rRNA is being investigated.

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STRUCTURE AND EXPRESSION OF THE GENOMIC RNAs OF GRAPEVINE FANLEAF NEPOVIRUS. C. Ritzenthaler, M. Viry, M. Pinck, R. Margis, F. Hans and L. Pinck, IBMP, 12, rue du Gal Zimmer 67084 STRASBOURG. FRANCE

The GFLV is responsible for an economically significant disease in vineyards all over the world. Its genome is composed of two single stranded positive-sense polyadenylated RNAs which carry a VPg. RNA1 (7342 nt) and RNA2 (3774 nt) encode 253kDa and 122kDa polyproteins, respectively. The 253kDa polyprotein includes the consensus sequences characteristic of RNA-dependent RNA-polymerase, protease, and nucleotide binding protein. Microsequencing of the VPg and the coat protein as well as search for putative cleavage sites within the 253kDa and 122kDa allow us to propose a genomic organization for GFLV. The 253kDa polyprotein is expected to be cleaved in a 92kDa polymerase, 25kDa protease, 2.9kDa (23 residues) VPg and 133kDa protein containing the NTP binding pattern and a probable protease cofactor. The 122kDa polyprotein is cleaved in a 56kDa coat protein and a 66kDa protein which is probably matured to yield a 44kDa putative movement protein and a protein of unknown function. It is assumed that all these proteins are produced by trypsin-type cleavages after an R residue, except for the N-terminus of the VPg cleaved at a C/S site.

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NUCLEOTIDE SEQUENCE COMPARISON OF THE 3'-TERMINAL REGIONS OF A SEVERE AND A MILD STRAINS OF PAPAYA RINGSPOT VIRUS. **Shyi-Dong Yeh, and Ching-hsien Wang; Dept. Plant Pathology, National Chung Hsing Univ., Taichung, Taiwan, R.O.C.**

The nucleotide sequence of the 3'-terminal region of the genome of a severe strain of papaya ringspot virus, PRV HA (a potyvirus originated from Hawaii), was elucidated by cDNA cloning and sequencing. The 2,561 nucleotide residues covered most of the nuclear inclusion b (NIb) gene, the complete coat protein (CP) gene and the whole 3'-untranslated region. Analysis with the published 3' region of the mild strain PRV HA 5-1, that was obtained from HA by nitrous acid induction and has been widely used for control of PRV by cross protection, indicated that they shared 99.3 % of identity in their 3'-terminal 2,235 nucleotide residues. There were eleven and two nucleotide residues found different in the NIb gene and the CP gene, respectively, resulting in six amino acid changes at the NIb gene and two amino acid changes at the CP gene. The 3'-untranslated regions of both strains were identical except that PRV HA contained two more residues (AG) at the 3' extreme.

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HOST-RELATED PECTIC ENZYME PATTERNS IN DISCULA UMBRINELLA.. L. Toti, A. Chassin du Guerny, O. Viret, and O. Petrini, Microbiology Institute, ETH-Zentrum, CH-8092 Zürich, Switzerland.

Extracellular pectin pectyl hydrolase and polygalacturonase production by Discula umbrinella (Berk. et Br.) Sutton (teleomorph: Apiognomonia errabunda (Rob.) Höhnel) isolated from different hosts has been investigated by polyacrylamide gel electrophoresis. All strains investigated are able to produce both enzymes and the banding is consistent with the origin of the isolates. The pectic zymogram patterns are apparently not dependent on the growth substrate or on the age of the cultures used. Pectinase production in strains inoculated on media supplemented with non-host-tissues is apparently reduced. Pectin pectyl hydrolase is already produced in two to three-days-old cultures. Polygalacturonase bands are visible on the gel only two to three days after detection of pectin pectyl hydrolase bands, thus pointing to a possible induction of polygalacturonase by degradation products of pectin pectyl hydrolase. All D. umbrinella isolates are thus able to produce enzymes that may be crucial in the host penetration and colonization processes. This study demonstrates also that pectic zymogram patterns may be a quick and reliable means of detection of host-specific strain formation within fungal species.

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QUANTIFICATION OF FUNGAL INFECTION USING ERGOSTEROL AS BIOMARKER P. Nilsson, I. Almgren*, M. Gustafsson* & G. Odham. Chemical Ecology, Department of Ecology, Lund University, S-223 62 Lund, Sweden; *The Swedish University of Agricultural Sciences, Department of Plant Breeding, S-268 00 Svalöv, Sweden.

Ergosterol is the major sterol in most fungi. This component is absent or a minor constituent in most higher plants. When fungal tissues can not be separated from a plant tissue, growth of the fungus can be monitored by measuring this specific biomarker. Leaves from barley plants infected by the plant pathogenic fungus B.sorokiniana were extracted with methanol, hydrolyzed by KOH and then quantified by reverse phase HPLC. By using this method, both viable and non-viable fungi can be quantified. Confirmation of ergosterol was carried out with on-line LC/MS using plasmaspray ionization. The ability of the fungus to infect three barley varieties were investigated.

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EFFECTS OF BIPOLARIS SOROKINIANA TOXIN ON ATP LEAKAGE FROM ROOTS AND ON ROOT CORTICAL CELL DEATH OF BARLEY. I. Almgren, E. Liljeroth and M. Gustafsson. The Swedish University of Agricultural Sciences, Department of Plant Breeding, S-268 00 Svalöv, Sweden.

The pathogenic fungus Bipolaris sorokiniana produces toxins. The toxins play a role in the infection process by affecting plant cells in advance of hyphal colonization. The effects of prehelminthosporol, one active component, on ATP leakage from barley roots as well as on root cortical death, were studied. Barley was grown in tubes with nutrient solution. A significant increase in the rate of ATP release from the roots was observed after adding 60 $\mu g\ ml^{-1}$ prehelminthosporol indicating a negative effect on the cell membranes. Further, the rate of cortical cell death of excised barley roots increased after addition of prehelminthosporol. Differential reactions were found among different barley genotypes.

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BIPOLARIS SOROKINIANA, A FUNGAL PATHOGEN OF BARLEY. A MICROSCOPIC STUDY OF ROOT INFECTION. H. Carlson, U. Stenram*, M. Gustafsson* and H-B. Jansson. Microbial Ecology, Department of Ecology, Lund University, S-223 62 Lund, Sweden; *The Swedish University of Agricultural Sciences, Department of Plant Breeding, S-268 00 Svalöv, Sweden.

An electron microscopic investigation of barley roots infected by Bipolaris sorokiniana showed the existence of extracellular polymers on germ tubes and hyphae as well as appressoria attached to the root surface. Growth of the fungus in the epidermis and outer cortex was predominantly intracellular, whereas in the inner cortex the hyphae observed were mainly intercellular. No hyphae could be detected in the stele. A host response to fungal infection was indicated by the development of papilla between the plasma membrane and cell wall of the plant.

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MARKER-INTEGRATION MUTAGENESIS: ANALYSIS OF A HIGHLY CONSERVED PATHOGENICITY GENE IN *XANTHOMONAS*. S. Kamoun, H. V. Kamdar, E. Tola and C. I. Kado. Dept. of Plant Pathology, University of California, Davis, CA 95616

X. campestris pv. campestris (Xcc) contains a plant inducible *hrpXc* gene that control both pathogenicity and hypersensitivity (HR) on nonhost plants (S. Kamoun & C. Kado, J. Bacteriol. 172:5165, 1990). Electroporation of integrative plasmids, which carry small insert fragments internal to the *hrpXc* transcriptional unit into Xcc, generated HrpXc mutants. A collection of subclones and nested-deletion-integrative plasmids allowed the mapping of the *hrpXc* locus to a 1.5 kb region of the Xcc chromosome. Chromosomal transcriptional fusions of *hrpXc* to *gusA* and *cat* reporter genes were also constructed by marker-integration and used to confirm that *hrpXc* is expressed in Xcc only in plants. *hrpXc* is conserved in all Xcc strains and HrpXc mutants were constructed in 8 Xcc of four races with various levels of virulence. All mutants were non-pathogenic and HR. Thus, *hrpXc* is essential for pathogenicity regardless of host-specificity or degree of virulence.

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Cross-reactivities of anti-THRGP antibodies against extensin-like epitopes in various plant pathogen interactions.*S.H.Hippe,*K.H. Marticke, M. Kieliszewski, D. Lamport, and S.C.Somerville.*CAU University of Kiel, Institute of Botany, D-2300 Kiel, Germany; DOE-Plant Research Laboratory, Michigan State University, East Lansing, MI, 48824, USA.

Immunoelectron microscopy was used to study the subcellular cross-reactivity of maize THRGP (threonine hydroxyproline rich glycoprotein) polyclonal antisera during the compatible and incompatible interaction of Erysiphe graminis f.sp.hordei on barley and Puccinia graminis f.sp. tritici on wheat.The IEM analysis was focussed on the localization of extensin-like epitopes at the host parasite interface, the extrahaustorial matrix. High cross-reactivities of anti-THRGP antibodies were examined to the extrahaustorial matrix in both systems. In the incompatible interactions a similar immunogold distribution pattern was found. The results suggest the involvement of extensin structural proteins in the host-pathogen interaction of monocot plants with powdery mildew and rust fungi, respectively.

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INTERACTION BETWEEN MELOIDOGYNE INCOGNITA RACE 3 AND M. ARENARIA ON A M. INCOGNITA-RESISTANT TOBACCO CULTIVAR. T. B. S. Ng'ambi, K. R. Barker, and R. C. Rufty, North Carolina State University, Raleigh, NC 27695-7620.

A greenhouse study was conducted to determine whether a tobacco cultivar resistant to M. incognita race 3 could be predisposed to infection by this race when simultaneously inoculated with M. arenaria. Plants of resistant cultivar Speight G-28 and of susceptible cultivar NC2326 were inoculated with M. incognita or M. arenaria, race 2, or with both species. Treatments were replicated five times in a RCB design. For both cultivars, rates of larval penetration 10 days after inoculation with both species were similar to single inoculations with M. arenaria. Fewer nematodes were detected in roots of the resistant cultivar with mixed inoculation than with M. arenaria alone 30 days after inoculation. In both cultivars root-gall indices and reproduction rates were lower in the two-species condition than in the monospecific. Thus, there appears to be an antagonistic interaction between Melodogyne species.

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